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NSG 2183

REGULATION OF THE ADRENAL CORTEX FUNCTION  
DURING STRESS

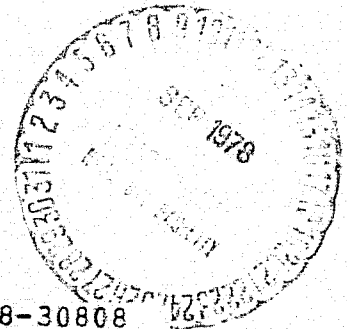
Report and Request for Grant Renewal

from

NASA Ames Research Center  
Human Studies Branch  
Moffett Field, California

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FROM  
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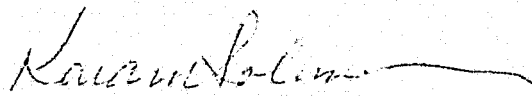
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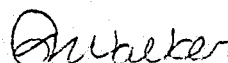
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## REGULATION OF THE ADRENAL CORTEX FUNCTION DURING STRESS

ANNUAL REPORT

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Contract Number: NSG2183

## OBJECTIVES

- a) To determine the role of brain neurotransmitter under stress.
- b) To determine glucocorticoid binding influence on the adrenal function under stress.
- c) To determine the alteration of brain glucocorticoids uptake.
- d) To determine the pharmacokinetics of glucocorticoids under normal and stressful conditions.
- e) To establish the circadian rhythm behavior of both brain amines and glucocorticoids under normal and stressful conditions.

## Progress Report

During the period of September, 1977 to the present several aspects of the research proposal have been examined. Several experiments were designed to examine the rate of glucocorticoid metabolism under stress. In one experiment the uptake of corticosterone was investigated under normal and stressful condition. The establishment of corticosterone circadian rhythm was studied and related to the response to some cholinergic and anticholinergic drugs in the immature rat in another experiment. In a third experiment the role of histamine antagonist agents ( $H_2$  Antagonist) on corticosterone level was investigated under normal and stressful condition. In a fourth experiment the effect of colchicine on corticosterone was investigated under normal and stressful conditions. The following is a summary of each study conducted for this grant:

(1) Stress and Corticosterone Uptake. The uptake of corticosterone was investigated in adult male Sprague-Dawley rats under normal and stressful conditions. In this study 200  $\mu$ ci/kg of  $^3H$ -Corticosterone were injected IV. Tissue and plasma counts of  $^3H$ -Corticosterone were determined. The tissue: plasma ratios of the stressed group were for the liver 3.14; kidney 1.84; spleen .43; Gut 2.36 and adrenal 1.94. The same tissues: plasma ratios for the control animals were: 2.36; 1.97; .41; 2.31 and 1.83 respectively.  $^3H$ -Corticosterone in brain regions: plasma

of the stressed group were: cortex 0.18; midbrain 0.28; cerebellum 0.28; Caudate nucleus 0.29 and Pons 0.27. The same brain regions: plasma ratios for the control animals were 0.23; 0.22, 0.23, 0.25 and 0.26, respectively. Plasma  $^3\text{H}$ -Corticosterone count in stressed group were twice as much as the control animals. The possible influence of epinephrine may also explain the high level of corticosterone obtained when the anticholinestrase physostigmine and neostigmine administered to hypophysectomized rat as well as to rat with pituitary intact. Also low level of corticosterone was observed when atropin or hexamethonium were administered. These results suggest the presence of a direct relationship between the hormone released under stress i.e. epinephrine and the metabolic rate of corticosterone.

(2) Involvement Of The Adrenal Medulla In The Maturation Process Of The Adrenal Cortex. The establishment of corticosterone circadian rhythm was studied and related to the response to some cholinergic and anticholinergic drugs in the immature rat. Three different age groups of Sprague-Dawley rats were placed on 14:10 light dark cycle at a controlled temperature of  $23 \pm 1^\circ\text{C}$ . Litter size was limited to 10 animals per litter. Animals were sacrificed by decapitation at the ages of 12-13, 18-19 and 21-22 days old. Plasma was obtained for corticosterone determination every 4 hours along the 24 hrs. period. The results from the different age groups indicate the absence of diurnal rhythm in this adrenocortical hormone. Following the absences of nyctothermal variation in glucocorticoid secretion, cholinergic study was designed.

Different sets of rats in the age range of 12-14 and 29-30 days old rats were utilized. One group was subjected to cholinergic drug treatment while the other group also received the same treatment but was later exposed to ether stress. Physostigmine (0.20 mg/kg) and neostigmine (0.1 mg/kg) administered did not produce any significant changes in corticosterone level. Neither did 0.1 mg/kg of tetraethylammonium, hexamethonium nor atropine caused any change in the level of corticosterone. Since epinephrine was found to prolong the plasma half-life of corticosterone in this group of immature animals, it may be suggested that the cortex maturation is probably related and regulated by the adrenal medulla.

(3) Involvement of H<sub>2</sub> Receptors In The Regulation Of The Adrenal Gland. The role of histamine antagonist agents (H<sub>2</sub> antagonist) on corticosterone level was investigated in adult male Sprague-Dawley rats under normal and stressful conditions. In this study, Metiamide and Cimetidine were used. Half of the drug treated group was exposed to ether stress. Metiamide administration in low dose (25 mg/kg) caused no significant changes (P .05) in corticosterone level comparing to the control group. H<sub>2</sub> antagonist was found to prevent the stress corticosterone rise. When the same drug administered in higher dose (50 mg/kg) a marked and significant increase (P .01) in plasma corticosterone level was observed over the control rats. However, when the rats exposed to ether stress a slight but no significant increase (P .05) in corticosterone was observed. Meanwhile, Cimetidine administration



(25 mg/kg) inhibit the rise of corticosterone in the plasma. The control animals of the same group showed a significant ( $P < .01$ ) increase in corticosterone level than the preinjected rats. Similar results were obtained when the rats were injected with cimetidine which block the stress induced corticosterone. Results from these experiments suggest the involvement of  $H_2$  receptors in corticosterone rise during stress.

(4) Effect Of Colchicine On The Adrenal Cortical Function Under Normal And Stressful Conditions. The effect of colchicine on corticosterone levels of adult male Sprague-Dawley rats was investigated under normal and stressful conditions. In this study colchicine was injected and 75 min later animals were decapitated. Half of each group were exposed to ether stress for 1 min and 60 min post-injection animals were decapitated 15 min after stress exposure. Colchicine (0.5 mg/kg) administration cause a marked and significant ( $P < .01$ ) increase in plasma corticosterone level comparing to the control group. However, colchicine block the stress induced corticosterone rise. The same results were obtained when higher dose of colchicine (1.0 mg/kg) was used. No significant different ( $P < .05$ ) was observed between the preinjected and stressed animals to those received the drug only. The control rats of this group showed the lowest level. The results of this experiment indicate that the adrenal microtubules may be involved in stress corticosterone rise.

## Publications

### A - Articles

1. Soliman, K. F. A. and C. A. Walker, Effect of estrogen and progesterone on the circadian periodicity of blood cells. *Chronobiology Proc.* XII 239-242 (1977).
2. Clausell, D. C. and K. F. A. Soliman, Ovarian serotonin content in relation to ovulation, *Experientia* 34, 410-411 (1978).
3. Soliman, K. F. A. and T. M. Huston, Inhibitory effect of serotonin on ovulation in the domestic fowl, Gallus Domesticus. *Animal Repr. Sci.* 1, 69-73 (1978).
4. Okonmah, A. D. and K. F. A. Soliman, CNS stimulation effect on the sexual maturation of the female rat. *Experientia* (accepted for publication, 1978).
5. Okonmah, A. D. and K. F. A. Soliman, CNS stimulation effect on the circadian rhythm maturation of the adrenal cortex. *Life Sci.* (submitted for publication, 1978).
6. Kolta, M. G. and K. F. A. Soliman, Cholinergic regulation of the adrenal cortex under normal and stressful conditions. *Life Sci.* (submitted for publication, 1978).

### B - Abstracts

1. Soliman K. F. A., A. D. Okonmah, and C. A. Walker, In vitro response of the avian oviduct to autonomic drugs. *Fed Proc.* (1978).
2. Pinkston, J. N. and K. F. A. Soliman, Glucocorticoids modification of ethanol effect on respiratory depression in the rat. *Fed. Proc.* 37:420 (1978).
3. Kolta, M. G. and K. F. A. Soliman, The role of peripheral cholinergic activities in the regulation of the adrenal cortex. *Fed. Proc.* (1978).
4. Okonmah, A. D., K. F. A. Soliman, and C. A. Walker, CNS stimulation effect on the circadian rhythm maturation of adrenal cortex. XIII Int. Conf. Chronobiology Pavia, Italy (1978).

## Abstracts Cont.

5. Soliman, K. F. A. and M. G. Kolta, Regulation of the adrenal cortex function under stressful condition. The Endocrine Society Annual Meeting--Miami. (1978).
6. Cook, E. E. and K. F. A. Soliman, Glucocorticoids involvement in the testes function of the rat. Amer. Soc. Andrology, Nashville. (1977).
7. Soliman, K. F. A. and M. G. Kolta, Adrenal medulla regulation of glucocorticoids levels in the rat. Int. Symp. Clin. Chronopharmaco. 1:13 (1978).
8. Okonmah, A. D. and K. F. A. Soliman, Biological half-life of corticosterone in the immature rat. Int. Symp. Clin. Chronopharmaco. 1:46 (1978).
9. Pinkston, J. N. and K. F. A. Soliman, Circadian variation of ethanol metabolism. Int. Symp. Clin. Chronopharmaco. 1:48 (1978).
10. Kolta, M. G. and K. F. A. Soliman, Stress and corticosterone uptake. Int. Symp. Clin. Chronopharmaco. 1:43 (1978).
11. Griesser, P., M. G. Kolta, A. D. Okonmah and K. F. A. Soliman, Involvement of  $H_2$  receptors in the regulation of the adrenal cortex. 6th MBS Bio. Med. Symp. 6:105 (1978).
12. Kolta, M. G. and K. F. A. Soliman, Tissue distribution of corticosterone under normal and stressful conditions. 6th MBS Bio. Med. Symp. 6:53 (1978).
13. Pinkston, J. N. and K. F. A. Soliman, Circadian variation of ethanol metabolism. 6th MBS Bio. Med. Symp.
14. Okonmah, A. D. and K. F. A. Soliman, Adrenal cortex maturation. 6th MBS Bio. Med. Symp. 6:52 (1978).
15. Johnson, D., K. F. A. Soliman and M. G. Kolta, Effect of colchicine on the adrenal cortex function under normal and stressful conditions. 6th MBS Bio. Med. Symp. 6:104 (1978).

ABSTRACT

This research is designed to study the function of the adrenal gland in the rat during stress. Under stressful conditions the corticosteroid feedback control of ACTH release is not well defined. The regulation of ACTH was suggested to be mediated by brain neurotransmitters, and the role of glucocorticoid binding has not been fully investigated. During stress, alteration in brain uptake of glucocorticoids might be a possible means of modifying the adrenal-brain feedback regulation. In the proposed project, three different phases of experimentation will be undertaken. The first phase includes establishment of the circadian rhythm behavior of both brain amines and glucocorticoids, under normal conditions and under chronic and acute stressful conditions. The second phase includes the study of the pharmacokinetics of glucocorticoid binding under normal and stress conditions. The third phase includes brain uptake and binding under different experimental conditions. In the outlined experiments brain biogenic amines will be evaluated, adrenal function will be measured and stress effect on those parameters will be studied. It is hoped that this investigation will explain some of the complex relationships between the brain neurotransmitters and adrenal function.

## I. Introduction

The adrenal cortex function, as measured by glucocorticoids output, fluctuates in a daily rhythm as does ACTH release. Circadian rhythms have also been established in levels of brain biogenic amines. A definite relationship between brain biogenic amines and glucocorticoids has yet to be established.

The influence of the adrenal cortex on homeostatic mechanism during stress is well documented. The adrenal cortical hormones, specifically the glucocorticoids, are released during the state of resistance and have a chronic effect. Under stressful conditions the corticosteroid feedback control of ACTH release is not well defined. In some instances it was not possible to demonstrate glucocorticoid inhibition of ACTH release in response to stress; in other instances it was possible to demonstrate inhibition of stress response. The regulation of ACTH release was suggested to be mediated by hypothalamus neurotransmitters. Previous studies in this laboratory indicate the involvement of the neurotransmitters of other brain parts.

The role of glucocorticoid binding has not been fully investigated under stress. Alteration in regional brain uptake of glucocorticoids might be a possible means of modifying the adrenal-brain feedback regulation.

## II. Objectives

The main objectives of this project are to determine:

- (1) the relationship between brain biogenic amines and adrenal glucocorticoids
- (2) the role of negative feedback in the regulation of circadian rhythm of glucocorticoids
- (3) the role of brain biogenic amines in the regulation of adrenal cortex function under stress
- (4) the role of glucocorticoids binding in regulation of the adrenal/brain axis
- (5) regional brain uptake of glucocorticoids as a possible means of adrenal-brain feedback.

## III. Review of Literature

### A. Role of brain biogenic amines in ACTH release

In recent years, a considerable amount of data has accumulated suggesting that systems of monoaminergic neurons, particularly within the hypothalamus, are involved in some of the mechanisms which control the secretion of anterior pituitary hormones. However, interpretation of the physiological role of these neurons in neuroendocrine regulation raises important theoretical and practical difficulties. Changes in monoamine metabolism in discrete parts of the brain, recorded concomitantly with variations in hormonal activity, can be the consequence as well as the cause of shifts in central neuroendocrine regulating levels. Furthermore, experimental alterations of monoaminergic transmission in synapses can theoretically

affect adenohipophyseal control in many ways: it may control the transfer of sensory or somesthetic information to neuroendocrine integrating centers, affect directly the activity of such centers themselves or their sensitivity to hormonal feedback; it can also interfere with biosynthesis, axonal transport or release of the neurohormones known as hypophysiotropic regulator hormones. In most cases, moreover, and particularly in long term experiments, all these sequential events are likely to be affected simultaneously by pharmacological modulation of synaptic transmission, so that the endocrine responses assumed to result from a punctual intervention may only reflect an overall modification of all factors involved in their regulation.

Finally, drugs known to inhibit biosynthesis of monoamines from their original precursor for a prolonged period of time may affect synaptic release of the considered amine only in a very transient way, because transmitters stored in neurons before synthesis inhibition may still be released extraneuronally under certain conditions and may exhibit physiological activity (Kordon and Glowinski, 1969; Kordon, 1970; Thierry et al., 1971).

B. Determination of aminergic system involved in ACTH control

The secretion of the anterior pituitary gland has been shown to be under the control of peptide releasing and inhibiting hormones, arriving at the pituitary via a portal blood vessel system originating in the medial basal hypothalamus (McCann et al., 1968; Blackwell and Guillemin, 1973). Evidence suggests that the release of these

factors is regulated at least in part by the action of several neurotransmitter pathways known to be present in the hypothalamus. In addition, the influence of other areas such as midbrain and limbic system on hormonal secretion is believed to be exerted through excitatory and inhibitory inputs to the hypothalamus, which may be mediated by one or more transmitters (Fuxe, 1969). Among the biogenic amines that have been proposed as influencing hormone secretion by the anterior pituitary are serotonin (5-HT), norepinephrine (NE) and dopamine (DA) (Simon and George, 1975).

Daily rhythms in basal plasma concentration of anterior pituitary hormones have been reported (Rubin et al., 1973) and daily cycles of adrenal corticoids are well established (Solleberger, 1965), which may be related to the variation in brain NE, DA and 5-HT. Plasma corticosterone was found recently (Simon and George, 1975) to be inversely correlated with striatal and cortical DA and with 5-HT of amygdala.

The content of 5 hydroxytryptamine in the whole brain and in parts of the brain in mice, cats, hamsters and rats, exhibits a diurnal fluctuation (Albercht et al., 1956; Matussek and Patschke, 1963; Montanaro et al., 1964; Dixit and Buckley, 1967; Scheving et al., 1968; Reis et al., 1969). In rats, diurnal variations have been found in a number of brain regions (Quay, 1964; Friedman and Walker, 1968). Daily variation in the pattern of adrenocortical activity has been well documented in rats and many other animal-species (Critchlow, 1963; Chiefetz et al., 1968). Several investigators have attempted



to relate the daily fluctuation of brain 5-HT to the daily rhythm of plasma corticosteroids (Albrecht et al., 1956; Dixit and Buckley, 1967; Friedman and Walker, 1968).

Diurnal fluctuation of corticosterone was found to be parallel to the daily fluctuation of the amygdala and hippocampus serotonin content in the rat (Scapagnini et al., 1971).

Circadian variation in the activity of the pituitary-adrenal axis is well documented in animals (Critchlow, 1963; Guillemin et al., 1959; Halberg et al., 1959; Mason et al., 1957) as well as in man (Krieger and Krieger, 1966; Migeon et al., 1966; Oppenheimer et al., 1961).

Since the secretion of ACTH is believed to be under the control of corticotropin releasing factor (Guillemin, 1964, 1967; McCann and Dharwal, 1966; McCann et al., 1968) a similar change in CRF activity was postulated, but few attempts designed to evidence this were successful. For example, Retiene et al., 1968, could not demonstrate a circadian variation in CRF activity in the rat hypothalamus, although they observed a typical daily change in the pituitary ACTH contents as well as in the plasma ACTH level. Ungar, 1964, in an in vitro experiment, succeeded in showing a circadian rhythm not only in the response of the adrenal to ACTH, but also in the ACTH activity of the isolated pituitary as well as CRF activity in the mouse hypothalamus.

In a more recent report by Hiroshige and Sakakura (1971), it was found that CRF activity in the rat hypothalamus showed a definite circadian variation, having a peak value at 6:00 PM and the minimum at 8:00 AM. A close temporal relationship with a definite phase shift was observed between CRF activity and plasma corticosterone level. These findings support the concept that the circadian rhythm of the pituitary-adrenal axis is a direct reflection of the rhythmicity of CRF activity in the median eminence. Furthermore, the persistent periodicity observed in the CRF activity in the absence of circulating corticosterone, suggests that the dominating mechanism for control of the circadian rhythm of CRF activity is of neural origin, being independent of the negative feedback mechanism.

Evidence has been obtained indicating that norepinephrine (NE) in the hypothalamus exerts an inhibitory effect upon CRF-ACTH secretion (Ganong, 1974; Van Loon, 1973; Scapagnini, 1974).

Administration of the drug:  $\alpha$ -M-P-tyrosine, which inhibits tyrosine hydroxylase activity (Corrodi et al., 1966), produces a decrease in hypothalamic NE accompanied 9 hours later by a remarkable increase in plasma corticosterone (Scapagnini et al., 1970). More recently, with the availability of a new sensitive radioimmunoassay, Van Loon (1973) has shown that, in a similar condition, plasma ACTH levels were increased. However, no information is available about the dynamics of this phenomenon.

Recently Scapagnini et al. (1975) indicated that  $\alpha$ -methyl-P-tyrosine produced a rapid increase in plasma ACTH levels, whereas the hypothalamic norepinephrine concentration and plasma corticosterone changed significantly only 6 hours after the administration of the inhibitor. Their data suggests that the NE inhibitory tonus of CRF-ACTH secretion depends on the availability of a rapidly utilized pool of transmitter affected in a very short time by  $\alpha$ -methyl-P-tyrosine.

C. Hypothalamus-pituitary-adrenal feedback system

Feedback control of ACTH secretion by circulating corticosteroids has been demonstrated both by the chronic increase in ACTH secretion which follows adrenalectomy and by the inhibition of ACTH after corticosteroid treatment. Under stressful conditions, the corticosteroid feedback control of ACTH release is not yet well defined. On one hand it has not been possible to demonstrate inhibitory effect of glucocorticoids on ACTH during stressful conditions. On the other hand, it has been possible to demonstrate inhibition of stress response using low physiological doses. However, it has been repeatedly demonstrated that treatment with corticosteroids will not inhibit the high intensity response within the first few hours of treatment. Dallman and Jones (1973) suggested that there are two temporally discrete periods of ACTH inhibition effected by steroids. The first, a fast, rate-sensitive inhibition, can be demonstrated to occur as plasma corticosterone levels are rising in response to treatment. The second can be demonstrated a few hours

after treatment with glucocorticoids, and is independent of plasma corticosterone concentrations.

During the early post-natal period in the rat, the secretion of ACTH generally does not occur in response to a wide variety of stimuli. It is doubtful whether the small, but statistically significant increase that does occur in response to certain severe stresses, is of physiological significance. The basis for this neonatal neuroendocrine quiescence is unclear. In contrast to the immaturity of ACTH secretion in response to external stresses, the steroid feedback mechanism is functioning during the first post-natal week as judged by the adrenal atrophy caused by exogenous corticoid administration and compensatory adrenal hypertrophy. The apparent increased sensitivity of this feedback mechanism could be due to longer effective persistence of the hormone in the circulation and/or its greater accessibility to CNS sites involved in the feedback regulation.

Glucocorticoid feedback inhibition of ACTH secretion may be exerted on both the brain and pituitary. However, the primary site of steroid action remains controversial (see Review of Kendall, 1971). A number of in vitro observations favor a direct action of corticosteroids on the adenohypophysis. These steroids have been shown to prevent the ACTH secretion induced by either vasopressin (Fleischer and Vale, 1968), or crude CRF extract (Arimura et al., 1969; Kriacer and Mulligan, 1970; Sayers and Portanova, 1974). In addition, it has been

shown that dexamethasone's blockade of ACTH release might involve a DNA-dependent RNA synthesis, thus suggesting a genomic level of action of the steroid. This idea was further supported by the identification of dexamethasone receptors within the nuclear fraction of the pituitary (Koch et al., 1974). Recent investigation by Koch et al., 1975, indicated an inverse relationship between plasma corticosterone titer and the capacity of receptors to combine 3H-dexamethasone. Their results suggest that glucocorticoid binding to the pituitary may be correlated with the physiological action of the steroids on ACTH release.

The effects of glucocorticoid feedback seem to change with age. It has been suggested that this may be due to changes in the nature of extrahypothalamic input to the adrenal control system (Goldman et al., 1973); there is little information on the development of input to the hypothalamus. It is our intention to investigate this point in relation to stress in our proposal.

It was suggested by Schapiro et al. (1971) that in the infant rat the central components of the steroid feedback mechanism (cortico-stat) are more sensitive to corticoids than they are in the adult. A similar suggestion was also presented by Ramirez and McCann (1963) of a hypothetical "gonadostat" to circulating gonadal hormones. Alternative explanations, however, suggest that a slow rate of metabolism can prolong the steroid feedback effect.

It is also possible that the blood-brain barrier is more permeable to corticosterone and this permits a higher effective corticoid titer to reach feedback centers. The work of Woolley and Timiras (1968) with radioactive estrogen would support this suggestion.

There is a distinct diurnal variation in serum corticosterone at day 30-32 (Allen and Kendall, 1967) and at 21-25 days of age (Ader, 1969) and between one and three years of age in humans (Frank, 1967).

#### D. Adrenal function under Stress

Previous data indicated that the adrenal system is of the closed loop type: there is at least one closed informational loop in this system, i.e., the free glucocorticoid concentration of the blood is monitored by the hypothalamus and/or pituitary. However, the exact mode of controlling action (e.g., proportional, integral derivative) taken when deviations of the corticoid level from a "preset" value occur, is not yet clearly established.

Adaptation to stress when measured by glucocorticoids assay showed that rats are able to adapt to the stress of chronic or repeated adverse stimuli (Yuwiller, 1971). This adaptation was shown not to be synonymous with normalization since rats that had been adapted to various stressors and exposed to a second stress stimulus had detectable response at an earlier post stimulus time than in control animals (Sakellaris and Vernikos-Danellis, 1974).

In an experiment in the rat to investigate this phenomenon, Dallman and Jones concluded that corticosterone does act to inhibit ACTH secretion but that stress causes a prolonged period of hyper-responsiveness in either CNS or anterior pituitary component of the adrenocortical system.

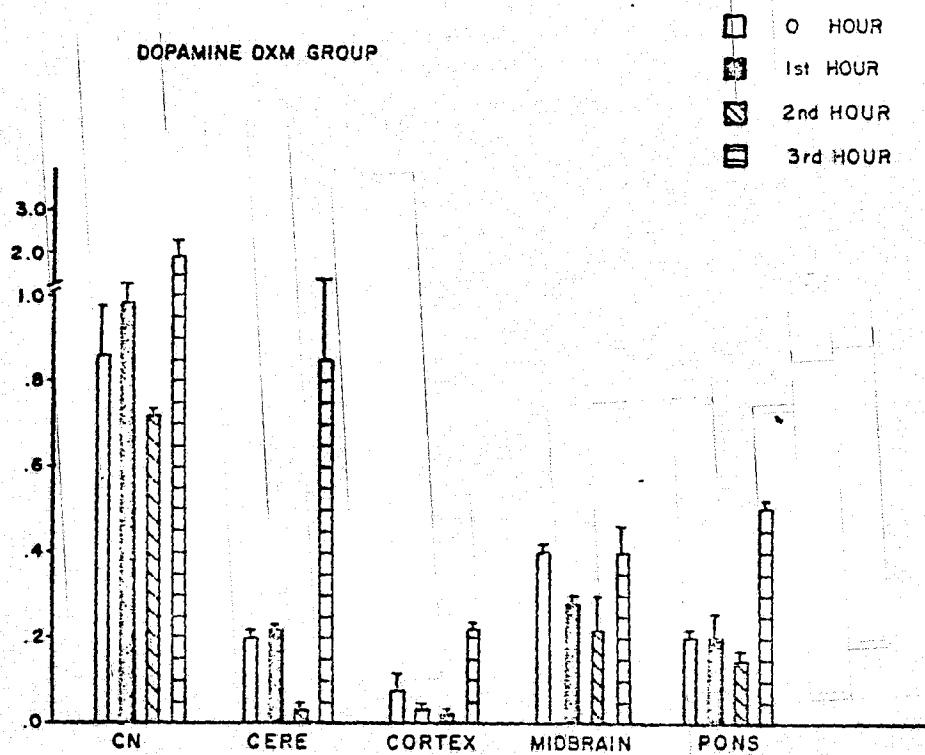
The term adaptation has long been used to refer to that stage during continuous or repeated exposure to a stressful stimulus which is characterized by a return toward pre-stress level of circulating corticosteroids and other indices of pituitary adrenal activation (Sakellaris and Vernikos-Dannellis, 1975).

In recent studies by Sakellaris and Vernikos-Dannellis (1975) on the adrenal response of animals under chronic stress, it was found that during a period of adaptation when plasma corticosterone concentration returned toward pre-stress level, despite continued exposure to the stressor, the animal responded to the different type of stress with a faster increase in plasma corticosterone than controls. They indicated that this responsiveness was not limited to the adrenal, since plasma ACTH showed a greater increase than controls.

Considerable evidence suggests that certain stressful stimuli are capable of activating the pituitary-adrenal system in rats with surgically interrupted central neural connections of the medial basal hypothalamus (MBH). Thus, rats with chronically isolated MBH show significant increases in plasma corticosterone levels following exposure to a variety of stressful stimuli (Halasz *et al.*, 1967;

Feldman et al., 1968, 1970; Palka et al., 1969; Greer et al., 1970; Makara et al., 1970). Results from acute studies utilizing partial forebrain removal consistently indicate that the isolated MBH-pituitary unit can support pituitary-adrenal responses to combined ether and leg break (Matsuda et al., 1963), ether-blood withdrawal or immobilization (Dunn and Critchlow, 1969). Hypothalamic structures, exclusive of those contained in the median eminence, may not be required for such responses to some stimuli. Matsuda et al. (1964) reported that rats with median eminence-pituitary islands (MEPI) showed elevated corticosterone levels following ether-leg break stress and suggested that the median eminence-pituitary unit constituted the minimal substrate for this response. Similarly, Makara et al. (1970) reported that rats with MEPI showed corticosterone response to i.p. injections of histamine and insulin. The median eminence, however, does not appear essential in all situations. Ablation of the medial hypothalamus, including median eminence, is compatible with acute corticosterone responses to injection of E. coli endotoxin or large doses of formaldehyde (Stark et al., 1973, 1974), despite pituitary infarction. Furthermore, Witorsch and Brodish (1972) report that removal of the entire forebrain, including median eminence, does not block corticosterone responses to certain combined stress procedures. These findings suggest that central neural pathways to the MBH, and perhaps the MBH itself, are not essential to the triggering of ACTH secretion by some noxious stimuli. The mechanisms involved in such pituitary activation are unknown, and





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Figure 1. Effect of dexamethasone administration on brain dopamine levels.

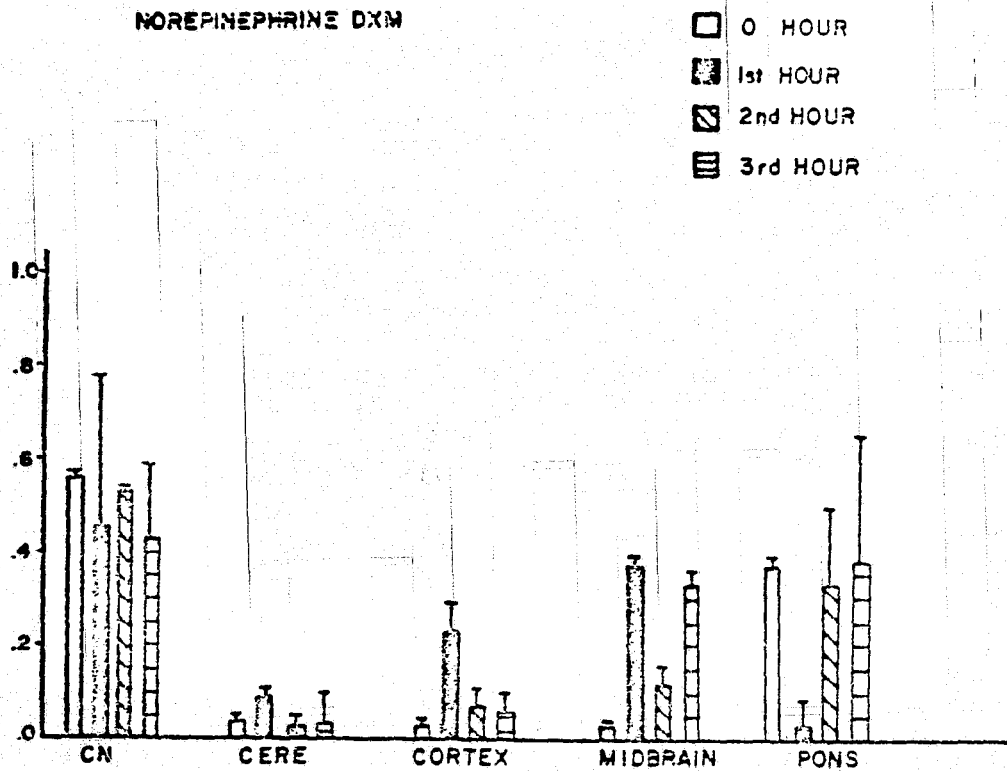
their importance and role in the physiological control of pituitary-adrenal function is undetermined.

It was not possible to elicit plasma corticosterone responses to ether or immobilization stress from pituitary islands in rats subjected to total forebrain removal (Dunn and Critchlow, 1969). There is evidence suggesting that acute corticosterone responses to immobilization-blood withdrawal persisted when the median eminence was retained in connection with the pituitary; the magnitude of such responses was similar to that seen earlier in island preparations containing basal hypothalamic tissue (Dunn and Critchlow, 1969).

#### IV. Preliminary Work

##### Glucocorticoids Effect on Brain Biogenic Amines Levels:

The levels of norepinephrine (NE), serotonin (5-hydroxy-tryptamine, 5HT), dopamine (DA) and 5-hydroxyindoleacetic acid (5HIAA) were studied in the cerebral hemisphere, cerebellum, midbrain, caudate nucleus and pons, in relation to the administration of glucocorticoids. Male Sprague Dawley rats were maintained under controlled light (12D:12L) and temperature ( $23 \pm 1^\circ\text{C}$ ). The glucocorticoid dexamethasone (9 $\alpha$ -fluoro-16 $\alpha$ -methylprednisolone) was injected (2.0 mg/kg) and the animals were sacrificed at 1-hour intervals for 4 hours. DEX administration resulted in a significant change in biogenic amines content of the different parts of the brain studied. Dopamine concentration increased at least fourfold in the cerebellum and threefold in the pons by the third hour (Fig. 1). In the midbrain, both NE (Fig. 2) and 5HT (Fig. 3) increased significantly ( $P \leq .01$ ) by the second hour. Significant increase ( $P \leq .01$ ) was noticed in the cortex NE level (Fig. 2) by the first hour and DA level by the third hour. In the caudate nucleus, there was no significant ( $P \leq .05$ ) difference in the levels of any of the biogenic amines. The result of this experiment indicates that dexamethasone has a definite effect on the biogenic amine level of the different parts of the brain studied, except the caudate nucleus. The data indicate that glucocorticoids' effect on the body might be mediated by their effect on brain biogenic amine levels.



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Figure 2. Effect of dexamethasone administration on brain norepinephrine levels.

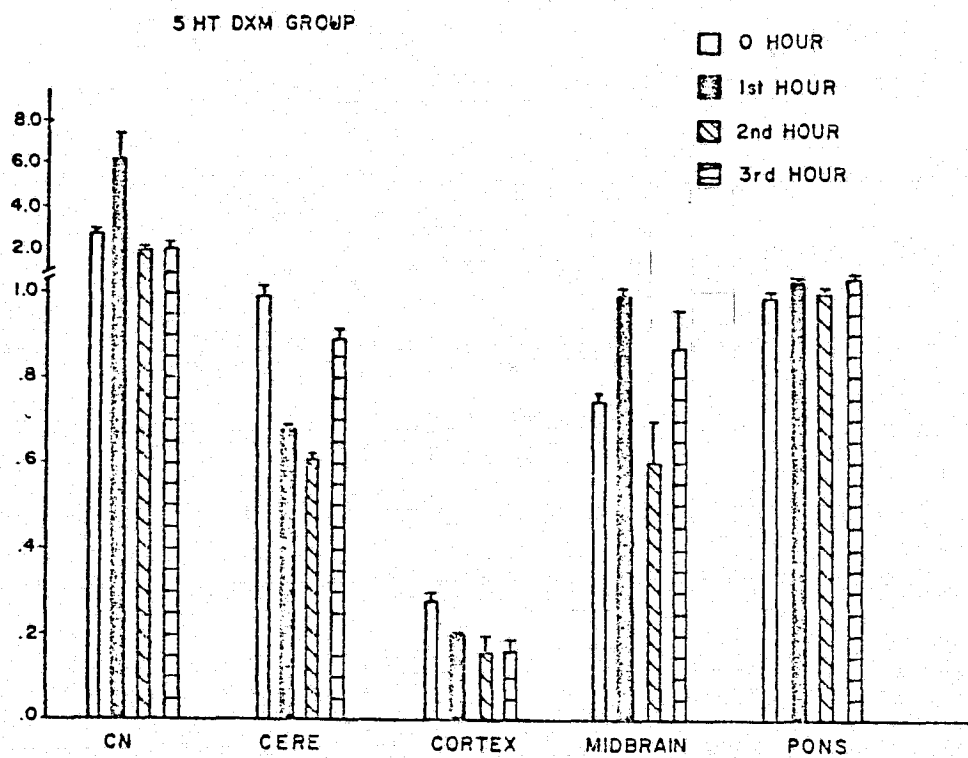


Figure 3. Effect of dexamethasone administration on brain 5-hydroxy tryptamine levels.

## V. Methods of Procedure

A. General: This section describes assay procedures for the different hormones and biogenic amines, the animal conditions and environment. This section applies to all experimentation unless otherwise indicated.

### 1. Animals and environmental conditions.

Female Wistar rats will be used for all experiments. They will be housed in a controlled temperature, humidity and light room. The light schedule will be 14 hours light and 10 hours dark cycle. The rooms are completely insulated and controlled. Temperature will be controlled at  $23 \pm 0.2^{\circ}\text{C}$ . Feed (Purina lab chow) and water will be provided ad libitum. All intravenous injections will be through the tail vein.

### 2. Dissection of the brain.

The different parts of the brain will be dissected according to Kato and Vिलlee (1967 a & b). Parts to be dissected include: cortex, cerebellum, caudate nucleus and hypothalamus. The different parts will be weighed using torsion balance.

### 3. Biogenic amines assay.

The determination of biogenic amines in the rat brain is routinely conducted in this laboratory. The method used is based on the procedures of Shellenberger and Gordon (1971).

### 4. ACTH.

ACTH will be measured by radioimmunoassay (Dallman et al., 1974; Grizzle et al., 1974; Rees et al., 1971).

5. Corticosterone assay.

Serum corticosterone (Compound B) in 100  $\mu$ l of the samples will be determined using a modified method of Silber et al. (1958) by Ramaley (1972). In this procedure, serum samples are mixed with 1.90 ml isooctane and mixed on a Vortex mixer for 30 seconds. Samples will be centrifuged for 5 minutes at 5000 rpm at 4°C. The aqueous defatted sample will be extracted with 6 cc methylen chloride, spun for 1 minute, and centrifuged for 5 minutes. Then 5 ml of the solution will be added to 0.5 ml of 70% ethanolic sulfuric acid. Exactly after 95 minutes, fluorescence will be measured on Aminco-Bowman spectrophotometer.

6. Statistical analysis.

Using Monroe 1665 Computer, we developed permanent computer programs for F and T analysis of variance. Duncan's Multiple Range test and other statistical manubulation such as regression analysis and co-variance analysis will be conducted as described by Steel and Torrie, 1965.

## VI. Phases of Experimentation

1. Circadian variation in brain biogenic amines in relation to the adrenal function:

The intent of these studies will be to observe the level of brain biogenic amines in specific parts of the rat brain under different adrenal function conditions. In these experiments, mature male rats will be used. They will be adapted for at least 3 weeks under controlled light (12D:12L) and temperature ( $23 \pm 1^\circ\text{C}$ ) conditions. Rats will be assigned in different groups of 6 animals per group. Groups in these studies include (a) control animals, (b) adrenalectomized animals, (c) adrenalectomized animals treated with glucocorticoids, (d) intact animals treated with glucocorticoids, (e) sham operated animals. Adrenalectomized animals will be used after 7 days from completing the operation. They will be kept on .9% saline throughout the experiment.

Drug treatment will be daily for one week. Drug or vehicle administration will take place at a specific hour (0900). At the end of the experiment, animals will be sacrificed at 4 hour intervals during a 24 hour period. The brains will be dissected to separate the different brain regions, including cerebral hemisphere, cerebellum, caudate nucleus, pons and hypothalamus. Brain biogenic amines will be determined using the method mentioned above. Results of this experiment will determine 1) the effect of glucocorticoids on the circadian variations of brain biogenic amines, 2) the involvement of glucocorticoids on brain function through alteration of neurotransmitter concentrations, 3) the permissive effect of glucocorticoids on the brain.



2. Effect of stress on brain/pituitary-adrenal axis.

In this experiment, similar groups as in phase 1 will be used. Animals in the same conditions as described above will be used and each group will be further divided into two subgroups. The first subgroup will be maintained under cold stress (4°C) for a period of 3 weeks. The other subgroup will be used to study the effect of acute exposure to ether inhalation. At the end of experimentation, animals will be sacrificed; trunk blood will be collected and brains will be dissected. Brain parts will be analyzed for their biogenic amines content. ACTH and corticosterone will be measured as indicated above.

3. Effect of stress on plasma corticosterone half-life.

The metabolic half-life of corticosterone in rats under different physiological conditions has not been studied in detail. The factors underlying changes in corticosterone half-life are not clear. The major contributing factor might be due to corticoid binding globulin (CBG) and the rate of metabolism. It is known that high CBG was at one time believed to protect cortical hormones from enzymatic degradation (Yates, 1962). Therefore, in this experiment, we will differentiate free corticosterone from bound. This will require specific methodology in the determination of corticosterone from its metabolic byproduct. Ultrafiltration (molecular filtration) technique will be utilized in the determination of percent corticosteroid binding to globulin. Although many experiments have been carried out, they have employed equilibrium dialysis technique, where the buffer and ionic conditions can affect equilibrium

dialysis method. We have evidence that the ultrafiltration technique does not have these disadvantages because we have been employing for the past several months whole blood in the determination of percent drug bound into protein albumin, without disturbing physiological condition of the blood. Molecular filtration is a rapid, gentle, easy-to-use technique for separating macromolecules on the basis of size. The free corticosterone from eluent of molecular filtration technique will be assayed by isotropic dilution and mass fragmentography technique.

The assay technique in the rat will be administered by carbon-14-labeled corticosterone. The carbon-14-labeled corticosterone from non-radioactive corticosterone will cause a shift in a value of 2 m/e in the mass spectra. Therefore the endogenous corticosteroid will not interfere with the labeled corticosteroid. The mass fragmentographic technique of labeled corticosteroids will be used (Hammer et al., 1968).

The plasma profile of corticosteroid may change from one compartment open to two compartments open model or to non-linear pharmacokinetic model. It has been shown that the specific assaying technique significantly affects pharmacokinetic modeling (Finn and Sadee, 1975).

In this project we will employ Nonauton program for analyzing concentration vs. time data described by pharmacokinetic models involving first order and/or Michaelis-Menten kinetics. This

program is used in conjunction with the nonlinear regression program, NONLIN (Sedman and Wagner, 1974).

4. Brain uptake of labeled glucocorticoid.

Several reports have indicated that there is less sensitivity to feedback from steroids produced by the adrenal under stressful conditions. An experiment will be designed to study the uptake of labeled corticosterone by the different brain regions. In this experiment, <sup>14</sup>C corticosterone will be used in different doses. The brain will be dissected to its different parts and the uptake of the steroid will be determined. This experimental design will enable us to explain the role of brain steroid uptake in the regulation of feedback mechanism.

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#### VIII. Facilities Available

The Endocrinology Laboratory at the School of Pharmacy is well equipped. Equipment includes Perkin-Elmer UV-Vis Spectrophotometer, Ultracentrifuge, Hewlett-Packard gas chromatograph, Beckman Dynograph with four transducers, microscopes, Aminco-Bowman spectrofluorometer, a Packard Tricarb liquid scintillation counter, and three controlled environmental rooms. The laboratory also is provided with all conventional instruments, e.g., microbalances, water bath, oven, pH meter, refrigerated centrifuges and refrigerator. The pharmacokinetics laboratory within the School has a gas chromatograph/mass spectrometer/computer system. Staff and laboratories are available in the School area for use and consultation in connection with this project. They include: Tallahassee Memorial Hospital, Florida State University, various departments, and several departments at Florida A & M University.



BIOGRAPHICAL OUTLINE FOR KARAM F.A. SOLIMAN

Birth Place: [REDACTED] [REDACTED]  
Date of Birth: [REDACTED] [REDACTED]  
Citizenship: Egyptian, U.S. Permanent Residence  
Marital Status: Married and one son  
Social Security No.: [REDACTED]  
Telephone Numbers: Home - (904) 575-2419  
Office - (904) 599-3849

Academic Background

1960-64 B.Sc., Cairo University,  
Cairo, Egypt  
1966-68 Graduate Studies, Physiology,  
Cairo University  
Cairo, Egypt  
1968-71 M.S., Environmental Physiology,  
University of Georgia,  
Athens, Georgia, 30602  
1971-72 Ph.D., Endocrinology,  
University of Georgia  
Athens, Georgia, 30602

Academic Employment

1968-71 Research Assistant, University of Georgia  
Athens, Georgia, 30602  
1971-72 Teaching Assistant, University of Georgia  
Athens, Georgia, 30602  
1972-75 Assistant Professor of Physiology and  
Pharmacology, School of Veterinary  
Medicine, Tuskegee Institute,  
Alabama, 36088  
1973-75 Graduate Faculty, Graduate School  
Tuskegee Institute, Alabama 36088

1975-Present

Associate Professor Physiology and Pharmacology, School of Pharmacy Florida A & M University, Tallahassee, Florida 32307

Research Experience

1966-68

Research on the reproductive performance of the Meriono Sheep. (Cairo University)

1968-71

Research on the cardiovascular system and embryonic circulation. (University of Georgia)

1971-72

Research on the ovulation cycle and the involvement of the adrenal gland and serotonin in ovulation regulation (university of Georgia)

1972-75

Research in the area of circadian rhythm of alcohol metabolism and toxicity in relation to the brain biogenic amines levels. (Tuskegee Institute)

Research on enzyme induction in the ovary in relation to ovulation. (Tuskegee Institute)

1975-Present

Research in the area of circadian rhythm of ethanol toxicity. (FAMU)

Research on ethanol effects of brain biogenic amines metabolism and circadian rhythm. (FAMU)

Research on sympathetic relation to  $T_3$  and  $T_4$  levels and thyroid. (FAMU)

Research on the oviduct motility and its adrenergic receptors. (FAMU)

Research on ovarian biogenic amines and ovulation. (FAMU)

Research on adrenal medullary regulation of glucocorticoids levels. (FAMU)

Research on adrenal cortex maturation. (FAMU)

Training Program

- Summer 1973                      Vanderbilt University, School of  
Medicine, Training on Cyclic AMP,  
Dr. Robert Taylor Laboratory
- Summer 1974                      Wayne State University, School of  
Medicine, Training on Reproductive  
Physiology, Dr. E.S.E. Hafez Laboratory
- Fall 1975                         Miami University, School of Medicine  
Continuing Education Conference in  
Clinical Pharmacology (12 credit  
hours)
- Summer 1975                      NASA Ames Research Center  
Moffett Field, California,  
Human Research Laboratory

Collaborated Research Work

- 1976 - Present                    The suprachiasmatic nucleus  
involvement in the regulation of  
different hormonal balance - with  
Dr. C.M. Winget, NASA, Moffett field,  
California
- 1977 - Present                    Ovarian Serotonin role in pathogenesis  
of ovarian diseases in collaboration  
with Dr. Paul McDonough, the Medical  
College of Georgia, Augusta, Ga Dept  
of Obstetrics & Gynecology

Teaching Experience

- Physiology (University of Georgia): Undergraduate Course, 380.  
Instructed the laboratory classes--Introductory course in Systemic  
Physiology.
- Physiology 601 (University of Georgia): Graduate Course. Instructed  
laboratory class--mostly physiology of different systems of the  
body. Lecturing in gonads physiology.
- Physiology 801 (University of Georgia): Graduate Course. Instructed  
laboratory classes--Reproductive Physiology. Lecturing Neuro-  
endocrinology.
- Human Physiology (Tuskegee Institute, School of Nursing): Under-  
graduate Course, 373, for human physiology. Lecturing the course  
and instructing the laboratory.
- Physiological Chemistry (Tuskegee Institute, School of Veterinary  
Medicine): Course offered for freshman veterinary students, 250;  
lecturing the course and instructing the laboratory.
- Mammalian Physiology (Tuskegee Institute, School of Veterinary  
Medicine): Undergraduate course, 251, offered for freshmen veterinary

students; lecturing the course and instructing the laboratory/

Endocrinology (Tuskegee Institute, School of Veterinary Medicine): Course number 520 offered to junior veterinary students and to graduate students; lecturing the course.

Experimental Endocrinology (Tuskegee Institute, School of Veterinary Medicine): Course offered to sophomore veterinary students (elective) 522; lecturing the course and instructing the laboratory.

Pharmacology 251 (Tuskegee Institute, School of Veterinary Medicine): Course offered to sophomore veterinary students. Participated in lecturing the course.

Pharmacology 352 (Tuskegee Institute, School of Veterinary Medicine): Continuation of Pharmacology 251. Participated in lecturing the course.

Toxicology 353 (Tuskegee Institute, School of Veterinary Medicine): Course offered to junior veterinary students. Participated in lecturing the course.

Pathophysiology (PMC 300) (Florida A & M University): A course offered to third year pharmacy students. Lecturing the course and instructing the laboratory.

Pathophysiology (PMC 301) (Florida A & M University): A course offered to third year pharmacy students. Lecturing the course and instructing the laboratory.

Endocrine Pharmacology (PMC 614) (Florida A & M University): A course offered to graduate students in pharmacology developing and lecturing the course and instructing the laboratory.

Neuropharmacology (PMC 611) (Florida A & M University): A course offered to graduate students in pharmacology lecturing in the course.

Clinical Pharmacology (PMC 502-503) (Florida A & M University): A course offered to medical students & graduate students covered participating in teaching the courses.

#### Graduate Students Advisory:

1. Supervised 8 graduate students towards M.S. in Physiology and Pharmacology; the following is their thesis titles:
  1. Brain Catecholamines and Sexual Maturity in the Rat. (Tuskegee Institute)
  2. Neural Control of Testosterone Release (Tuskegee Institute)
  3. CNS and the Adrenal Cortex Maturation (FAMU)

4. Regulation of the Adrenal Function Under Stress (FAMU)
5. Glucocorticoid Modification of Ethanol Effect (FAMU)
6. Adrenergic Control of Glucocorticoids Release and Metabolism (FAMU)
7. Control of the Circadian Rhythm of Glucocorticoids (FAMU)
8. Stress and Drug Metabolism (FAMU)

Membership in Professional Organizations:

Endocrine Society  
Society for the Study of Reproduction  
International Society for Chronobiology  
International Associations of Fertility Societies  
American Fertility Society  
American Physiology Society (Associate Memeber)  
Poultry Science Association  
Neuroscience Society

Scholarships and Awards:

1962-64	Tution Scholarship and Monthly Assistantship
1969	Member of the Honor Society, Gamma Sigma Delta
1972	Recipient of the best Masters Candidate Award (University of Georgia)
1973	Recipient of the best Doctorate Award (University of Georgia)
1975	Recipient of Lederal Research Achievement Award

Grants and Supporting Funds:

1. Supervising a grant from the Office of Naval Research (N0014-73-A-0329 entitled Circadian Rhythm in the Toxicity of CNS Drugs. The amount of the grant is \$84,519 and the period of support is from 4/1/73 to 3/31/75/ The grant was awarded to Dr. C.A. Walker who moved from Tuskegee Institute, Alabama, to Florida A & M University, Tallahassee, as Dean of the School of Pharmacy.
2. Co-Principal Investigator for a grant from the NIH (RR-8091-02 entitled Circadian Rhythm of Genital Tract Activity. The total amount of the grant was \$128,479 for the period of 1/1/73 to 12/31/77. This was granted to Dr. C. A. Walker.
3. Principal investigator of Research Project entitled Biogenic Amines. The amount of fund provided by NIH is \$92,612 for the period July 15, 1976 through 6/30/78 (Grant # RR 0811)
4. Principal investigator of Research Project entitled "Regulation of the Adrenal Cortex Function During Stress". The amounts of funds provided by NASA is \$59,789 (Grant # NSG 2183)

School & University Activities:School

Search Committee for an Associate Professor of Pharmacology (Member)  
 Research Grants and Proposals (Chairperson)  
 Graduate Program (Co-Chairperson)  
 Proposals and Grants (Member)  
 Publications and Public Relations (Asst. Editor, Research News and Reviews)  
 Syllabi Review Committee (Member)  
 Bi-Racial Committee (Member)  
 Dean's Advisory Council (Member)  
 Symposium Committee (Organizer)

University

Self-Study Research Committee (Chairman)  
 University Research Committee (Member)  
 University Management Committee (Member)

Appointments

Research Programs Director (School)  
 Health Advisory Council (Member)

Other Activities

Reviewing editor for practical clinical pharmacy C.A. Walker, J.R. Foxx, R. Trottier and K.F.A. Soliman Symposia Specialist Medical Books Miami 1977.

Organizer - International Symposium on Chronopharmacology Feb, 1978, Tallahassee, Florida.

Publications

1. Soliman, K. F. A. and T. M. Huston, The Photoelectric Plethysmography technique for recording heart rate in chick embryos, Poultry Sci. 51, 651-654, (1972).
2. Soliman, K. F. A. and T. M. Huston, Effect of environmental temperature on the life span of red blood cells in domestic fowl, Poultry Sci. 51, 1198-1201, (1972).
3. Soliman, K. F. A. and T. M. Huston, Effect of dietary protein and fat on prothrombin time of chickens under different environmental temperatures, Poultry Sci. 51, 1984-1989, (1972).
4. Soliman, K. F. A. and T. M. Huston, Effect of dietary protein and fat on the hematocrit and plasma cholesterol of chickens at different environmental temperatures, Poultry Sci. 53, 161-165, (1974).
5. Soliman, K. F. A. and T. M. Huston, Involvement of the adrenal gland in ovulation of the fowl, Poultry Sci. 53, 1664-1667, (1974).
6. Soliman, K. F. A., The influence of different audio frequencies on the pulse rate of the domestic fowl, IRCS Med. Sci. 3, 550, (1975).
7. Soliman, K. F. A. and C. A. Walker, Avian Post-ovulatory follicle homogenate effect on the genital tract motility, Hormones Res. 6: 357-365 (1975).
8. Soliman, K. F. A. and T. M. Huston, Effect of serotonin on ovulation in the fowl, Poultry Science 55: 1051-1076, (1976).
9. Soliman, K. F. A. and C. A. Walker, In vitro motility of the avian ovarian follicle, Gen. Comp. Endocrinol 30, 372-377, (1976).
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12 MONTH BUDGET REQUEST

	% of Time and Effort	NASA
1. Personnel		
A. Principal Investigator	15	\$ -0-
B. Lab Technician	100	10,000.00
C. Graduate Student (two)	100	8,000.00
D. Fringe Benefits	100	1,946.00
Total Salaries and Fringe Benefits		<u>\$19,946.00</u>
2. Equipment		7,382.00
3. Supplies		
Animals (\$3,248.00)		
Feed and Bedding (1,624.00)		
Drugs and Chemicals (\$2,510.00)		
4. Miscellaneous		500.00
5. Travel		2,000.00
6. Indirect Cost - 51.0%		
Salary and Wages		10,172.00
Social Security		
Retirement		
 Total of Request from NASA		 40,000.00

### Budget Justification

- (1) The amount of time required for this work is extensive. Funds are requested for a full time technician. The technician will help in all phases of experimentation.
- (2) The graduate students help will also be required for conducting the experiments, for laboratory help and assist in the different phases of experimentation.
- (3) The wages include social security (6.50%) and retirement (9%) per DHEW agreement. In the staff benefits (Item B in the budget) a workmen compensation (0.6% for undergraduate students and unemployment compensation of (0.6%) and health insurance (\$9.98/mo.) for permanent employees are included.
- (4) Travel funds in the amount of \$2,000.00 are required for attending a scientific meeting to present information obtained from this research project. Attending scientific meetings is essential in the initiation of a new research area.
- (5) The amount of funds for supplies, \$3,500, should be enough to cover the expenses which include laboratory animals, chemical, feed, bedding etc.