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FUNCTIONAL CHANGES IN THE ADRENAL CORTEX OF THE AGING LABORATORY RAT

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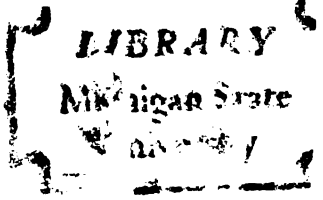
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ABSTRACT

FUNCTIONAL CHANGES IN THE
ADRENAL CORTEX OF THE AGING
LABORATORY RAT

by Gerald D. Hess

Studies were undertaken to determine if adrenocortical corticosterone producing ability decreases in the aging laboratory rat. These studies were conducted in male and female Long-Evans rats averaging 4, 13, and 23 months of age. The primary index of adrenocortical function measured was increased plasma corticosterone concentration following maximal ACTH and ether stress stimuli. Plasma corticosterone concentrations were determined by a fluorometric assay procedure. Plasma corticosterone concentration after maximal ACTH stimulation (1 U ACTH/100 g) decreased with increasing age in both male and female rats. The age-related decrease in plasma corticosterone levels after ACTH stimulation was verified by a similar study conducted in the female rats 1 month after the initial ACTH stimulation. Average plasma corticosterone concentrations were also lower in old female rats than in young female rats following a 10 minute ether stress. ACTH responsiveness was not influenced by the stage of the reproductive cycle or the lack of normal cyclic reproductive activities in aged female rats. Histological sections were prepared from the adrenals of a representative group of the animals included in this study. Adrenal histology revealed a decrease in zona fasciculata width with increasing age.

FUNCTIONAL CHANGES IN THE ADRENAL CORTEX OF THE AGING LABORATORY RAT

By

Gerald D. Hess

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REVIEW OF THE LITERATURE

Introduction

Man's search for the "fountain of youth" is undoubtedly as old as man himself. Every man at some time in his life is faced with the fact that he is a victim of aging. One might imagine that 19th century advances in medical science resulted in serious thought being given to investigation of the aging process. Unfortunately the notion of revitalization was exploited by all sorts of charlatans whose supposed "remedies" caused all studies of aging to be eyed with suspicion. Only in recent decades have scientists become concerned with the problems of aging and re-examined them with modern experimental methods.

The science of aging, which includes both the knowledge of old age and the processes which produce senility in organisms, is known as gerontology (Verzár, 1963). Experimental gerontology seeks answers to the questions, why is aging inevitable and what are the changes which normally occur in the different organs of the body with increasing age. According to Verzár, answers to such questions could provide a basic knowledge of the causes of arthritis, rheumatism, arteriosclerosis and other diseases typical of old age. Many studies in experimental gerontology have been directed toward the elucidation of a theory of aging. Bjorksten (1963, 1968) maintained that cross-linking is the primary factor involved in aging. Since experimental

proof that increased cross-linking occurred with age was derived from studies of collagen fibers alone, Verzár (1963) stated that cross-linking of proteins was unsatisfactory as a general hypothesis for aging. He concluded that aging studies should center in three specific systems: the connective tissue, the musculature and the central nervous system. In Verzár's opinion, aging of the endocrine organs is not the cause but a consequence of aging. Although there is presently no experimental evidence that endocrine changes are causative agents in the aging process, there is evidence to suggest that not all endocrine changes are merely the consequence of aging (Kitay, 1963 a and b; Riegle and Nellor, 1967).

History of Adrenal Endocrinology

Claude Bernard's analyses of adrenal and thyroid extracts were responsible for the introduction of the term "internal secretion" around 1855, according to Fulton (1966), while Turner (1966) credited Thomas Addison with calling attention to the human syndrome of adrenal insufficiency in 1855. Oliver and Schäfer (1895) reported that the "suprarenal capsules" (adrenals) were ductless secretory glands. The medullary portion of the gland was the origin of a material whose physiological activities were upon muscle tissue, the heart and the arteries according to these workers. They concluded that the adrenals produced a material which by its direct action upon the various muscle tissues aided in producing the degree of tonic contraction essential to the physiological activity of muscle. Oliver and Schäfer implied in their discussion that Addison's

disease amounted to some deficiency of the adrenal medulla. Rogoff and Stewart (1927) reported an increase in the survival time of adrenalectomized dogs injected with an extract of the adrenal cortex compared to non-treated adrenalectomized dogs. By demonstrating that injections of epinephrine were not effective in saving the lives of adrenalectomized animals, Rogoff and Stewart distinguished the hormones of the adrenal cortex from those of the medulla. Approximately 30 different steroids were isolated from the adrenal cortices of slaughterhouse animals between 1936 and 1942 (Turner, 1966). Six of these were found to be biologically active.

Throughout the following discussion the term 17-ketosteroid will refer to the C_{19} steroid hormones (of adrenocortical and gonadal origin) and their metabolites, while 17-hydroxycorticosteroid will refer to glucocorticoid hormones and their metabolites. Included in this latter group are the hormones cortisol and corticosterone and such metabolites as tetrahydrocortisol, 3 α-allotetrahydrocortisol, tetrahydrocortisone and β-cortolone.

Development of Assay Procedures

More extensive studies of the adrenal cortex became possible as techniques for detecting and quantitating adrenal steroids were developed. Porter and Silber (1950) reported the development of a colorimetric test specific for 17-21-dihydroxy-20-ketosteroids. Cortisone extracted from blood plasma or urine could be quantitated colorimetrically with an accuracy of 85 to 90%. Nelson and Samuels

(1952) modified the technique of Porter and Silber so that it could be used to measure human plasma cortisol levels clinically. However, the procedures developed originally were cumbersome and time consuming. Silber and Porter (1954) reported a further modification of their procedure which shortened it so that it could be completed in one day. Using this method, they were able to detect cortisol concentrations as low as 6 μg% with 95% confidence limits of ± 3.0 μg%. A colorimetric procedure was developed by Chen and Tewell (1951) which depended upon the reduction of blue tetrazolium salt to the blue formazan by α-ketols (20-keto, 21-hydroxy). Corticosterone and cortisol (hydrocortisone) are adrenal steroids containing α-ketol groups. Chen and Tewell's (1951) procedure when used in conjunction with the Porter-Silber method allowed investigators to distinguish between corticosterone and cortisol, both of which are secreted by the human adrenal cortex.

Colorimetric assay procedures were adequately sensitive to detect the large amount of steroids present in 24 hour urine samples or plasma samples obtained from human subjects. However, they lacked the sensitivity to detect adrenal steroid levels present in individual samples of rat plasma. Therefore, use of the rat for studies of adrenocortical function required the development of more sensitive assays for corticosterone, the predominate glucocorticoid found in rat plasma. Sweat and Farrell (1952) reported a method for fluorometric quantitation of cortisol in sulfuric acid. The procedure of Sweat and Farrell consisted of

chloroform extraction of plasma steroids, a petroleum ether-ethanol partition for the removal of excess lipids, and chromatographic separation (silica-gel) of the steroids prior to fluorometric analysis. Sweat (1955) used the fluorometric technique to measure concentrations of corticosterone and cortisol in human venous blood. Zenker and Bernstein (1958) simplified the technique of Sweat (1955) by using an alkali wash of the chloroform extract in place of the silica-gel column for purification of the adrenal steroids. The method of Zenker and Bernstein (1958) consisted of a chloroform extraction, an alkali wash and extraction into sulfuric acid fluorescing reagent (2.4 vol. H_2SO_4 added to 1.0 vol. 50% v/v aqueous ethanol) prior to development and fluorometric analysis. Their method was sufficiently sensitive to detect the steroid concentration in 0.2 ml of plasma. The specificity of Zenker and Bernstein's procedure for corticosterone was evident from the low fluorescence reading obtained for plasma samples from bi-laterally adrenalectomized rats. Studies conducted by Guilleman et al. (1958) established the specificity of the fluorometric method for studies of the pituitary-adrenal axis of animals secreting mainly corticosterone. They were able to achieve essentially complete recovery of corticosterone added to plasma or water using their fluorometric assay technique. Guilleman et al. (1958) measured resting and stress-stimulated levels of plasma corticosterone. Moncloa et al. (1959) demonstrated that fluorometric assay of the corticosterone content of rat adrenals provided results comparable

to those obtained by paper chromatographic separation and the blue tetrazolium reaction.

Aging of the Endocrine System

Studies of age related changes in the endocrine glands are complicated by the complex interactions of the endocrine system. McGavack (1963) believed that aging is more than a passive, degenerative wearing out of an organism. He further stated that for many years physicians considered the aging of the endocrines to be a passive, degenerative "wearing out" of functional units rather than a positive, active process. Although McGavack found changes in endocrine tissues with increasing age, he concluded that their direct relationship to the aging process was unclear. He suggested that the aging of endocrine structures, which is an active rather than a passive process, may have its beginning soon after or even before the birth of the individual.

Blumenthal (1955) investigated the relationship of pituitary activity to aging changes in the thyroid and adrenals of various strains of mice. He classified endocrine deficiencies in old age as those arising from decreased productivity of the pituitary, those due to a decreased response of the target gland and those arising from inability of peripheral tissues to respond to hormone. Since a progressive decrease in mitotic activity with increasing age is a basic effect of the aging process upon cell proliferation, Blumenthal (1955) suggested that changes in adrenal and thyroid mitotic activity with age may be independent of pitui-

tary influence. Blumenthal observed that a large portion of the degeneration in the endocrine glands of aging animals resulted from encroachment by fibrous tissue. Pittman (1962) reported a reduction in thyroid function with increasing age but questioned its involvement as a causative factor in the aging process. A relative state of estrogen deficiency and decreased adrenal androgen (17-keto-steroid excretion) production following menopause in the human female and decreased testicular androgen production in later life in the human male was reported by Shipley (1962). There is also evidence that the pituitary may play a role in regulating the aging process (Everitt, 1964).

With the aid of multiple regression analysis McGavack and Hock (1965) attempted without success to find a single age-related variable which would indicate the physiological status of an individual. They attempted to make simultaneous observations on changing parameters of pituitary, thyroid, gonadal and adrenal function in a single series of individuals so that correlations could be made between the functional interactions of these endocrine glands. This type of study may prove important in elucidating the extent of endocrine involvement in the aging process in future studies.

Wallach and Reineke (1949) reported that size and secretory activity (based on adrenal ascorbic acid depletion) of the rat adrenal cortex are influenced by the level of thyroid hormone in the body. Flamboe and Reineke (1959) observed a progressive decline

in thyroid secretion rate in aging goats. The suggestion by Hickler (1962) that decreased adrenocortical function in old age could result from reduced thyroid function is supported by these findings. Studies in the rat (Steinetz and Beach, 1963) have shown that corticosterone distribution volumes vary inversely with the level of thyroid activity.

Sex differences in adrenal secretory capacity and half-time for corticosterone disappearance from plasma of the rat have been reported by Kitay (1961) and Glenister and Yates (1961). Further studies by Kitay (1963a, 1963b) suggested that gonadal hormones are responsible for these changes. Although spayed female rats showed consistently lower plasma corticosteroid concentrations than intact controls, castrated males showed no differences from intact males. Gonadectomy was shown to have different effects upon the peripheral metabolism of corticosterone among the two sexes. Steroid half-life was decreased and liver metabolism was increased in castrated males while the opposite effect was noted in spayed females. Kitay reported that estrogen therapy increased steroidogenesis in male rats but decreased it in female rats. In view of decreased estrogen production in aging females (Shipley, 1962) it has been inferred that age-related changes in gonadal function may be responsible for changes in adrenal function.

Recent studies on the effect of adrenal cortical hormones on lysosome function have stimulated considerable interest. Lysosome function may be related to atherosclerosis, rheumatoid arthritis

and aging (de Duve and Wattiaux, 1966). Lysosomes are small proteinaceous sacs, containing hydrolytic enzymes, that electron microscopists have discovered in the cytoplasm of cells. The hydrolytic enzymes are though to digest foreign proteins which find their way into the cell cytoplasm (Grundman, 1966). Recent studies by Weissman and Thomas (1962, 1963, 1964) and Weissman (1964) have shown that glucocorticoids are effective in increasing the integrity of the lysosomal membrane. These findings suggested that the lack of glucocorticoids at the tissue level in old age may account for decreased lysosomal membrane integrity and thus increased tissue degeneration resulting from the actions of lysosome hydrolytic enzymes.

Aging and the Pituitary-Adrenal Axis

The concept of age-related changes in adrenal cortical function originated with Jackson's (1919) studies on the postnatal development of the suprarenal gland in the albino rat. There was evidence of a general increase in the suprarenal parenchymal cell size from one to ten weeks of age with little change thereafter.

Crooke and Gilmour (1938) described the histological changes in adrenal glands resulting from the hypophysectomy of a growing rat. They observed the disappearance of large numbers of cells in the "inner zone" of the cortex following hypophysectomy. These findings suggested that the functional state of the pituitary gland may be related to degenerative changes which occur in the aging

adrenal cortex. The work reported by Blumenthal (1945) indicated that there is a gradual diminution in the number of mitoses occurring in the adrenal gland of the guinea pig with increasing age, while Payne (1949) reported deterioration of cortical and medullary cells of fowl with advancing age. Other studies (Dribben and Wolfe, 1947; Jayne, 1953) have provided evidence of changes in connective tissue structure of the adrenal glands of aging rats. Jayne (1953) investigated adrenal gland cytology in the rat at different ages. Studies of the adrenal glands of 52 male rats ranging in age from 17 to 900 days showed definite changes in the parenchymal cells of the cortex as well as the medulla which he attributed to advancing age. The adrenal capsule was extremely cellular in 17-day rats. Capsule thickness increased and it became more hyaline and fibrous in appearance with increasing age. Occasional observations of regions showing evidence of shrinkage and degeneration occurred in capsules of old rats. Degenerating cells were found in all zones of the cortex of rats over 180 days old. There was a variable amount of cellular deterioration in all age groups with older rats showing a higher percentage and more advanced stages of degeneration than younger rats. As a general rule, the adrenal cortex was affected more by this degeneration than the medulla. Jayne's (1957) studies on histochemical and degenerative changes in the adrenal cortex of the aging rat suggested that cellular degeneration may cause a substantial decrease in the steroid hormone producing ability of the parenchymal cells of the adrenal cortex.

Investigations by Lever (1955) with the electron microscope increased the available information on the structure of the adrenal cortex. He described the general topography of the cortical zones and the cortical cellular-vascular relationship in humans, rats, mice, hamsters, and rabbits. Pauly (1957) described the normal morphology of the adrenal cortex of the laboratory rat from birth to senility. This study showed that every cell in the three zones of the adrenal cortex contacts a blood vessel on at least one surface, suggesting the importance of a functional blood vascular system to optimal adrenal cortical function. The concept of hormone inadequacies facilitating the aging process was supported by studies (Friedman et al., 1965) showing that administration of adrenal and neurohypophyseal hormones significantly prolonged the life span of 24 month old rats and visibly improved general body condition.

Investigation of the functional changes implied by the anatomical studies dates back to the work of Hamburger (1948) who reported that 17-ketosteroid output of both adrenal and gonadal origin was dependent upon the age of the individual (human). Hamilton and Hamilton (1948) reported a progressively decreasing average daily excretion of ketosteroids with increasing age, the predominant cause being a decrease in α-ketosteroids. These compounds are metabolites of androgens and not corticosteroids. Analyses by Salter, Cahen and Sappington (1946) suggested that the α-ketosteroids are of gonadal origin while the β-ketosteroids arise from

the adrenal cortex. The work of Pincus (1955) indicated that the age-related changes in the output of each of the three major groups of urinary steroids were different. Neutral reducing lipids (glucocorticoid metabolites) showed the least degree of change, non-ketonic steroids (estrogen metabolites) showed some degree of change while 17-ketosteroid (androgen metabolites) excretion was changed to the greatest degree. Though an important index of adrenal cortical function, 17-ketosteroid excretion was related to adrenal androstane production rather than to pregnane production.

Samuels (1957) found that the functional capacity of the liver, severe renal damage, thyroid function, pituitary function and Addison's or Cushing's syndrome in the adrenals, influenced the rate of cortisol production and removal as well as the apparent distribution volume of the hormone.

Using multiple regression analysis, Borth, Linder and Riondel (1957) showed that there are definite correlations between urinary excretion of both 17-ketosteroids and 17-hydroxycorticosteroids and age as well as body weight and height. They concluded that 17-ketosteroid excretion was more dependent upon age than was 17-hydroxycorticosteroid excretion, but that the reverse was true for the joint effect of body weight and height.

The work of Borth et al. (1957) suggested that glucocorticoid production by the adrenal cortex does change with advancing age, but Romanoff, Wolf and Pincus (1957) reported that neither age, sex nor 50 IU of ACTH produced any change in the urinary

excretion of the more characteristic corticosteroids, among these tetrahydrocortisone, tetrahydrocortisol, cortisone and cortisol. In all they reported the excretion of thirty or more metabolites found in glucuronidase-hydrolyzed urine extracts which have steroid color reactions on paper chromatograms. Romanoff et al. (1957) reported a 10% decrease in the number of detectable 17-ketosteroids in the urine of elderly subjects as compared to young subjects, following ACTH administration.

Romanoff et al. (1958) determined excretory levels of tetrahydrocortisol (THF), 3 α-allotetrahydrocortisol (ATHF) and tetrahydrocortisone (THE) in the urine of young and old men and women. The mean quantitative excretory levels of the three metabolites were significantly lower in young and old women and in old men than in the young men. They found that the differences in quantitative excretion of these metabolites disappeared when urinary excretion was expressed as a function of creatinine excretion. Further investigation (Romanoff et al., 1959) showed that β-cortolone, a regularly occurring metabolite found in the urine of young and old subjects of both sexes, also shows an age related decline in excretory levels when expressed as mg/24 hours. However, expressing excretion of this metabolite in terms of mg/g creatinine excretion eliminated the differences.

In order to confirm their previous findings, Romanoff et al. (1961) determined the resting adrenal secretory rates of eight young and eight old men. The 14-hour secretion rate of cortisol in

old men was only 75% of that in young men, whether the determination was based on THF, ATHF, THE or cortolone excretion. However, the secretion rates were the same for young and old subjects when expressed as mg/g creatinine/24 hours. This study (Romanoff et al., 1961) confirmed previous findings that apparently neither cortisol metabolism nor cortisol secretion by the adrenal differed with age when the muscle mass of the subject was considered.

The adrenal cortex secretes glucocorticoids in direct response to ACTH stimulation. Assuming that pituitary ACTH secretory capacity remains unchanges with increasing age, decreased adrenal responsiveness to ACTH might account for the decreased urinary excretion of steroids in old age. Tyler et al. (1955) compared plasma 17-hydroxycorticosteroid levels of young adults and geriatric patients following a six hour ACTH infusion. These studies showed that adrenal response to ACTH and hepatic metabolism of 17-hydroxycorticosteroids did not change with increasing age although 17-hydroxcorticosteroid distribution volumes were decreased.

West et al. (1961) investigated the possible relationship between impaired function of the liver, kidney or thyroid gland and decreased cortisol utilization in aged men. A progessive decrease in the rate of cortisol removal from the circulation following a standard intravenous infusion was evident with increasing age, while there was no statistically significant difference in the apparent distribution volume of the infused cortisol. An equal response in 8 A.M. plasma 17-hydroxcorticosteroid levels observed

in young and old men following ACTH stimulation suggested a decreased responsiveness of the adrenal in view of the slower rate of cortisol utilization in the aged. The significant decrease in 17-hydroxycorticosteroid excretion along with normal resting plasma levels of 17-hydroxycorticosteroids implied a slower rate of cortisol production under resting conditions. They were unable to satisfactorily explain the decrease in cortisol utilization in old age from experimental results. No evidence of thyroid involvement in decreased cortisol utilization was obtained in these studies. Elevated blood urea nitrogen levels, decreased endogenous creatinine clearances and decreased 17-hydroxycorticosteroid excretion in old subjects inferred that a renal mechanismsmight explain cortisol utilization differences. Renal mechanism could also explain the decreased clearance of 17-hydroxycorticosteroids from the plasma following cortisol infusion and decreased urinary 17-hydroxycorticosteroid excretion observed by these workers. However, the fact that all age groups showed comparable maximal levels of 17-hydroxycorticosteroids after cortisol infusion suggested that renal disorders could only have an effect on 17-hydroxycorticosteroid excretion in conjunction with metabolic changes elsewhere in the body. These workers postulated that the liver must be the site of this metabolism but could offer no substantial experimental evidence to support their theory that impaired liver function was responsible for decreased cortisol removal.

Samuels (1956) demonstrated a decreased disappearance rate

of cortisol from the circulation of individuals over 65 years of age compared to young subjects. Since cortisol is filtered from the plasma as a conjugate, the increased biological half-time in older subjects probably resulted from a decreased rate of cortisol metabolism. Furthermore, the initial apparent distribution volume (found by extrapolation of disappearance curves to zero time) was 60% of body mass in old subjects compared to 90% of body mass in younger subjects. Only 23.5 μg/kg/hr of cortisol would need to be produced to maintain an average concentration of 15.6 μg/100 ml of plasma compared to 43.3 μg/kg/hr required to maintain an average concentration of 12.6 μg/100 ml of plasma in the younger group. Therefore the adrenals of older persons would not need to be as highly functional as those of younger persons to maintain resting plasma levels of cortisol. These workers also found that achievement of equilibrium between plasma and body tissue cortisol, measured as distribution volume, occurred more slowly in the older subjects, possibly reflecting an increase in noncellular connective tissue and in density of ground substance. Samuels compared the results of cortisol removal and ACTH infusion tests in cirrhotic and aged subjects, and found that decreases in hepatic function do not account for the apparent changes of adrenal function with aging.

In view of increased biological half-life and decreased distribution volume of cortisol in old subjects, a reduction of ACTH release from the pituitary and subsequent atrophy of the adrenal cortex would be expected. Calculations of maximal pro-

duction of cortisol for a 24 hour period in a 70 kg man in response to a 25 IU ACTH infusion over a six hour period showed a response of 259 mg in young subjects compared to 202 mg in old subjects. This data strongly suggested that the adrenal's capacity to respond to a given amount of ACTH was decreased in the old subjects.

Samuels (1956) concluded from these studies of 17-hydroxycorticosteroid metabolism that the age-related changes in adrenal function are the result and not the cause of other changes in body tissues. Data reported by Moncloa, Gómez and Pretell (1963) showed that 17-hydroxycorticosteroid excretion/24 hours decreased progressively with increasing age in healthy men ranging from 20 to 83 years of age. In light of previous discussion, these values indicated a decrease in glucocorticoid production with increasing age. Moncloa et al. concluded that decreased production and increased biological half-life of cortisol accounted for these changes. These workers reported that standard tests for liver function performed on their subjects indicated no abnormalities, implying that decreased hepatic metabolism was not the reason for increased half-life. Moncloa et al. also showed that 17-hydroxycorticosteroid production was lower in old subjects than in young subjects in response to multiple levels of ACTH infusion. These results supported the conclusions of Samuels that the adrenal's ability to respond to a given amount of ACTH decreases with increasing age.

The findings of Samuels and Moncloa et al. regarding ACTH responsiveness are further supported by those of Riegle and Nellor

(1963, 1965, 1967). Plasma levels of glucocorticoids in male cattle following ACTH infusion showed a marked and progressive decrease with age indicative of a decrease in the adrenal's ability to respond to ACTH. Animals above three years of age had significantly higher levels of plasma adrenocorticotropic activity than younger animals, in contrast to the decreased levels of ACTH with increasing age implied by the studies of Samuels (1956) and Moncloa et al. (1963). Histological studies revealed varying degrees of cortical degeneration with age which were positively correlated with the relative insensitivity to exogenous ACTH infusions. Degenerating portions of the cortex (fasciculata) appeared to be replaced by the connective tissue, with the remaining functional tissue often existing in "nodule-like" islands. Riegle and Nellor suggested that increased levels of plasma adrenocorticotropic activity were required in older animals to maintain normal plasma glucocorticoid levels from the deteriorating glands. In cases where ACTH infusion produced no increase in plasma glucocorticoid levels the remaining functional tissue was operating at its maximal capacity under normal conditions.

Statement of Purpose

A decrement in glucocorticoid producing ability of the adrenal cortex with increasing age has been reported in human subjects and in cattle. Further investigation is necessary before the relationship between these changes and the aging process can be understood. The rat is a likely experimental animal for such studies, because

of its availability and its relatively short life-span. Although some anatomical studies of age-related changes in the adrenal cortex of the rat have been reported, no studies dealing with glucocorticoid producing ability as a function of age have been reported. The data reported in this study results from preliminary studies undertaken to determine if adrenocortical corticosterone producing ability decreases in the aging rat. The parameters studied were: plasma corticosterone concentration following maximal stimulation of the adrenal cortex with ACTH, plasma corticosterone concentration following an ether stress and the histology of adrenal glands from representative animals in the different age groups.

MATERIALS AND METHODS:

Experimental Subjects

Male and female Long-Evans rats ranging in age from 3 to 27 months were used in this study. The rats were maintained at the Endocrine Research Unit in a temperature controlled room with 12 hours of light and 12 hours of darkness daily. All the rats had been maintained on MSU regular diet fed ad libitum from weaning (see appendix).

Six experimental groups were established on the basis of sex and chronological age. Male and female animals were chosen at random and separated into three age groups. The youngest age group consisted of animals 3 to 4 months old at the start of the experiments. The next oldest group included animals 12 to 14 months old. The oldest group included animals 19 to 27 months old at the start of the experiments.

Potential reproductive capability of the female rats in this study was ascertained by vaginal smears. Vaginal smears were obtained for at least five and not more than nine consecutive days, with the tests of adrenocortical responsiveness normally made on the last day. For the sake of uniformity, smears were obtained during early to mid-afternoon. The fresh smears were fixed by immersion in 95% ethanol:ether (1:1) (technique described by Montakahabolayaleh, 1964) or with an aerosol spray ("Spray-

Cyte", Clay-Adams, Inc., New York 10, New York) and stained by the technique of Papanicolau (1942).

Administration of ACTH

The procedure most frequently used by investigators to study the functional capacity of the adrenal cortex is subcutaneous injection of ACTH. Moncloa et al. (1959) reported a maximum response to (4 unit) subcutaneous injection of ACTH in 165 gram male rats one hour after injection. In this study 1 unit of ACTH per 100 gram body weight injected subcutaneously in the lower abdominal region was found to be sufficient to produce maximal levels of circulating corticosterone in rats 45 minutes after injection.

Either a 5 unit per ml solution of crystalline ACTH (Upjohn Co., Lot 5354-JO-150, 39.4 IU/mg) or a 20 U/ml solution of Depo-ACTH (Upjohn Co., repository corticotropin injection, 80 USP Units/ml) was used in these experiments. ACTH preparations were refrigerated and used within 48 hours after dilution.

Anesthetization and Blood Collection

Prior to bleeding animals were anesthetized (intraperitoneal injection) with sodium pentabarbital (3% solution in distilled water) with females receiving 33 mg/kg and males receiving 39 mg/kg.

Blood samples were collected 50 to 60 minutes after ACTH administration by the orbital sinus bleeding technique of Stone (1954). This technique was developed into a routine laboratory procedure, providing ready access to a supply of venous blood.

Blood samples of approximately 1 ml were collected at each sampling, using 4 cm "bleeding tubes" prepared from heparinized hematocrit tubes, 1 mm I.D. Blood was collected into heparinized 30 ml beakers. As rapidly as possible, the blood samples were transferred from the collection beakers to conical centrifuge tubes and centrifuged for 5 minutes. Plasma samples that were not extracted following centrifugation were stored at -15°C.

A Calculated Ether Stress

A comparison of ACTH responsiveness and stress-induced responsiveness of the adrenal cortex was included in this study. Pilot studies confirmed the fact that diethyl ether (hereafter referred to as ether) is an effective stressing agent in the rat. Plasma glucocorticoid levels following exposure to ether were definitely elevated, as reported by Zimmerman and Critchlow (1967).

Rats were placed in an ether chamber into which a constant flow of ether vapors was pumped. Attempts were made to quantitate the amount of ether administered to each animal, but such efforts were impractical and were thus abandoned. Instead, duration of ether exposure was employed as the standardizing criterion. Maximal adrenocortical stimulation, as determined by levels of plasma corticosterone, were uniformly achieved following a 10 minute exposure to ether.

Fluorometric Assay

Corticosterone concentration in rat plasma was measured by

the method of Guilleman et al. (1958). A Turner model 110 fluorometer, equipped with filters providing narrow band excitation light @ 470 mμ and emission @ 530 mμ was used in these determinations. The high sensitivity of this procedure was required to determine hormone content in the 0.1 and 0.2 ml duplicate plasma samples assayed.

Five ml of redistilled methylene chloride was propipetted into 15 ml ground glass stoppered centrifuge tubes. Plasma samples were pipetted into the tubes and brought to a volume of 1 ml using distilled water or physiological saline. Tubes were subjected to a vigorous back and forth motion for a 3 minute extraction period.

The hormone-free aqueous layer was then aspirated and discarded. Partial extract purification was obtained by subjecting it to a 0.1 N NaOH wash, which removed excess lipids and acids including estrogens (Dorfman, 1962). Four ml of the original 5 ml of methylene chloride was transferred to a clean centrifuge tube with a volumetric pipette. Five ml of fluorescing reagent was added to the methylene chloride extract. Fluorescing reagent was prepared as needed by combining concentrated sulfuric acid and absolute ethanol. ((Ingredients were volume:volume (3:1) mixture of sulfuric acid (J.T. Baker Chem. Co., Phillipsburg, N.J. - 96.4%, 1.84 sp gr) and ethanol ("Gold Shield Alcohol"--200 proof, Commercial Solvents Corp., Terre Haute, Indiana)). This mixture was cooled and allowed to stand at room temperature for several hours before

use. Corticosterone was extracted into the fluorescing reagent by shaking the tubes vigorously for 1 minute after which the methylene chloride layer was discarded. The reagent-hormone mixture was allowed to develop for 15 minutes before fluorometric determinations were read (See Figure 1). Separation of solvent layers after extraction, purification and fluorescing reagent addition was facilitated by centrifuging the tubes at high speed for 3 minutes in an International Clinical Centrifuge.

Plasma corticosterone concentrations were determined by comparing fluorometer readings for experimental samples with readings obtained from standards of known concentration (See Figure 2). Due to variations in fluorescence intensity at least one standard was run daily. The standard was an aqueous solution of corticosterone, 0.1 μg/ml, which was pipetted into tubes containing 5 ml of methylene chloride and carried through the assay procedure as were plasma samples. A reagent blank, consisting of 1 ml of distilled water (or saline) was carried through the entire assay procedure daily. This served as a correction factor for background fluorescence.

Anatomical Considerations

Histological sections were prepared from the adrenals of a representative group of the animals included in this study. Adrenals and pituitaries, and in several cases ovaries, were removed at autopsy. Excess fat and connective tissue were trimmed off, tissues were weighed and fixed in Carnoy's solution for a minimum of one

FIGURE 1: FLUORESCENCE AS A FUNCTION OF TIME

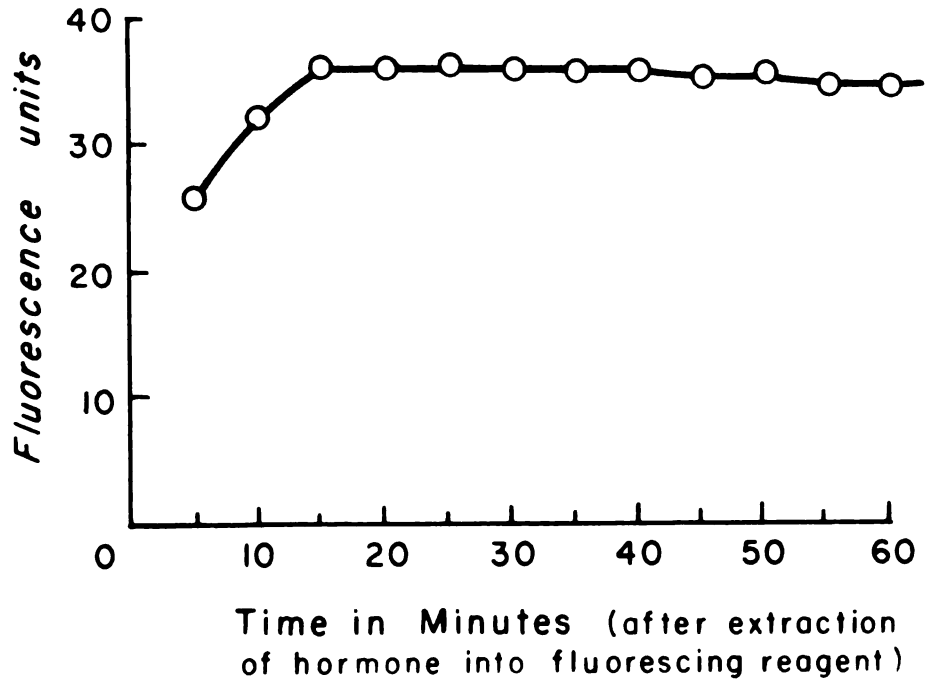
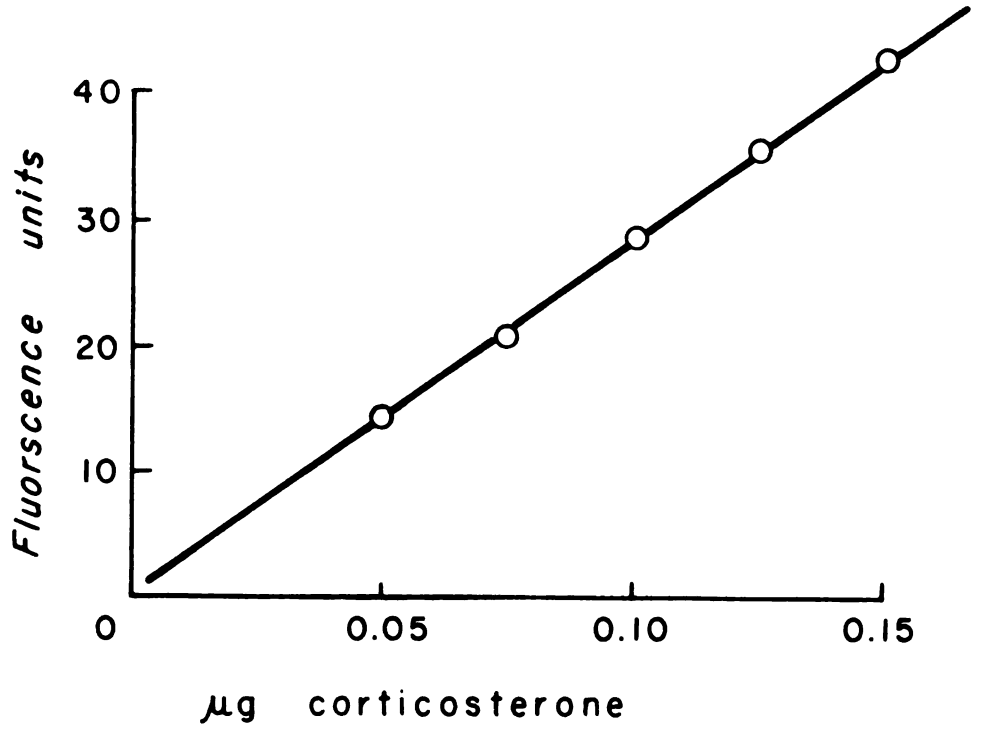


FIGURE 2: DOSE - RESPONSE CURVE



week before embedding. Tissues were embedded in paraffin with the aid of a "Technicon" tissue embedder and sectioned at 6 microns with a rotary microtome.

These tissues were stained by the technique of Papanicolau (1942) as well as hematoxylin and eosin procedure (Armed Forces Institute of Pathology, 1957) for observation of gross anatomical structure. Mallory's aniline blue collagen stain (Mallory, 1942) was used for determining connective tissue differences in the glands studied.

Statistical Considerations

The statistical significance of plasma corticosterone level differences between age groups was determined by the Analysis of Variance F-test (One-way Classification) (Steel and Torrie, 1960). In cases where homogeneity of variance between groups could not be shown by Cochran's test (Eisenhart, Hastay and Wallis, 1949), the approximate F-test of Box (1954) was used. Experimental groups exhibiting differences were further tested with Duncan's new multiple range test (Duncan, 1955). Student's t-test for two means (Steel and Torrie, 1960) was used in determining the statistical significance of other differences reported in this study.

RESULTS

To verify the accuracy of the fluorometric technique for corticosterone quantitation in rat plasma, the corticosterone concentration of a pooled plasma supply was quantitated by both the fluorometric assay and the colorimetric blue tetrazolium reaction of Elliott, et al. (1954), following chromatographic separation. For the blue tetrazolium reaction, plasma samples of 10 and 20 ml were extracted with methylene chloride. The methylene chloride extract was washed with cold 0.1 N NaOH and reduced to dryness in a vacuum evaporator. The residue was defatted and low polar compounds were removed by a 70% methanol:N-hexane partition. The aqueous methanol layer was reduced to dryness in a vacuum evaporator and transferred with ethanol to washed filter paper. The equilibrated toluene:75% methanol paper chromatographic system of Bush and Mahesh (1959) was used to isolate corticosterone from the aqueous methanol residue. Corticosterone zones were eluted and measured by the blue tetrazolium reaction of Elliott et al. (1954). Known quantities of 4-C^{14} corticosterone were added to the samples before extraction to serve as a reference in correcting for hormone loss during extraction, purification and separation. Nearly identical results were obtained by the two methods (μg corticosterone/100 ml plasma).

Fluorometric Assay	Blue-Tetrazolium reaction
32.4 ± .63 μg/100 ml*	32 μg/100 ml
*mean ± standard error	mean of two observations
n = 19	

The accuracy of the fluorometric assay and the apparent lack of interfering fluorogens in rat plasma was substantiated by the results of this experiment.

The validity of a quantitative measurement rests not only upon its accuracy but also upon its precision. A total of 21 samples (0.2 ml) from a pooled plasma supply were assayed fluorometrically with a variation (standard error of the mean) of 0.40 for the series (58.1 ± .40 μg/100 ml). Similarly a series of 10 aqueous corticosterone standards (0.1 μg corticosterone in 1 ml of water) showed a variation of only 0.22 fluorescing units (30.6 ± .22 fluorescing units).

An experiment was designed to test the assumption that hormone extraction from plasma was complete using this procedure. A 10 microliter sample of 4-C^{14} corticosterone was added to 0.2 ml plasma samples prior to methylene chloride extraction. The radioactive counts in a 100 microliter (1/50 of original vol.) sample of methylene chloride following extraction were determined with a proportional counter. The ratio of these counts (total cpm of original volume) to those of a reference standard provided an estimation of the percentage hormone recovery from plasma possible with this technique. Another 100 microliter methylene chloride sample was counted following extraction of hormone into fluorescing reagent. Counts (corrected to total cpm of original volume) di-

vided by reference cpm estimated the completeness of hormone extraction from methylene chloride. A 1 ml aqueous solution of corticosterone (0.1 μg/ml) carried through the same procedure provided an estimation of corticosterone extraction from water. The results of this experiment are listed in the following tables.

Table 1: Extraction of Hormone from Plasma

Source	Total Counts*	% Recovery**
plasma	5660	97
plasma	6080	104
plasma	5495	94
water + cmpd B	5810	100

* Total counts = cpm x 50

** Reference standard = 5826

Table 2: Extraction of Hormone from Methylene Chloride

Source	Total Counts*	% Remaining	% Extracted**
plasma	0	0	100
plasma	155	3	97
plasma	155	3	97
water + cmpd B	60	1	99

* Total counts = cpm x 50

** % of total extracted into fluorescing reagent

In view of a normal operating error of 5% in the proportional counter used in determining cpm of these samples, it is evident

that hormone extraction from plasma and from methylene chloride is essentially complete under standard operating conditions.

The validity of using 1 U ACTH/100 gram body weight to achieve maximal stimulation of the rat adrenal cortex was verified in rats (see Table 3) receiving 1 U and 2 U ACTH/100 grams body weight.

Table 3: Adrenocortical Responsiveness in Male Rats to Variable Levels of ACTH Stimulation

Treatment	No.	Plasma Corticosterone Concentration*
1 U/100 gram	8	54.4 ± 3.1 μg/100 ml
2 U/100 gram	7	54.0 ± 3.2 μg/100 ml

* Average ± s.e.

On this basis, 1 U ACTH/100 grams was used to achieve maximal stimulation of the adrenal cortex.

Since barbiturate anesthesia is known to depress CNS activity (Goodman and Gilman, 1965), reduced levels of ACTH secretion from the pituitary would be expected following anesthetization with sodium pentobarbital. Experimental results have shown that plasma corticosterone concentrations following administration of exogenous ACTH are not affected by sodium pentobarbital anesthesia. Plasma corticosterone concentrations in samples collected following sodium pentobarbital anesthesia were comparable with those in samples collected by rapid decapitation.

Sodium Pentobarbital	Rapid Decapitation
$\bar{x}_1 = 123 \pm 3.0$ μg/100 ml	$\bar{x}_2 = 130 \pm 8.7$ μg/100 ml
$n = 7$	$n = 5$

no statistically significant difference at 95% confidence level using Student's t-test.

Figure 3. Comparison of Plasma Corticosterone Levels Following Stimulation of the Adrenal Cortex.

In the first experiment, the adrenal cortex was stimulated with ACTH (1 U/100 gram). Plasma samples were obtained 50-60 minutes after ACTH injection. The second experiment also involved ACTH stimulation (1 U/100 gram) of the adrenal cortex. This experiment was conducted one month after the first experiment. Plasma samples were obtained 50-60 minutes after ACTH injection. In the third experiment, the rats were subjected to a 10 minute ether stress prior to collection of blood samples.

All of these experiments were conducted during the course of one summer, thereby eliminating seasonal differences as a source of variation.

Plasma corticosterone concentrations reported were determined by fluorometric assay procedures. The values reported are means $\pm$ s.e.

FIGURE 3:

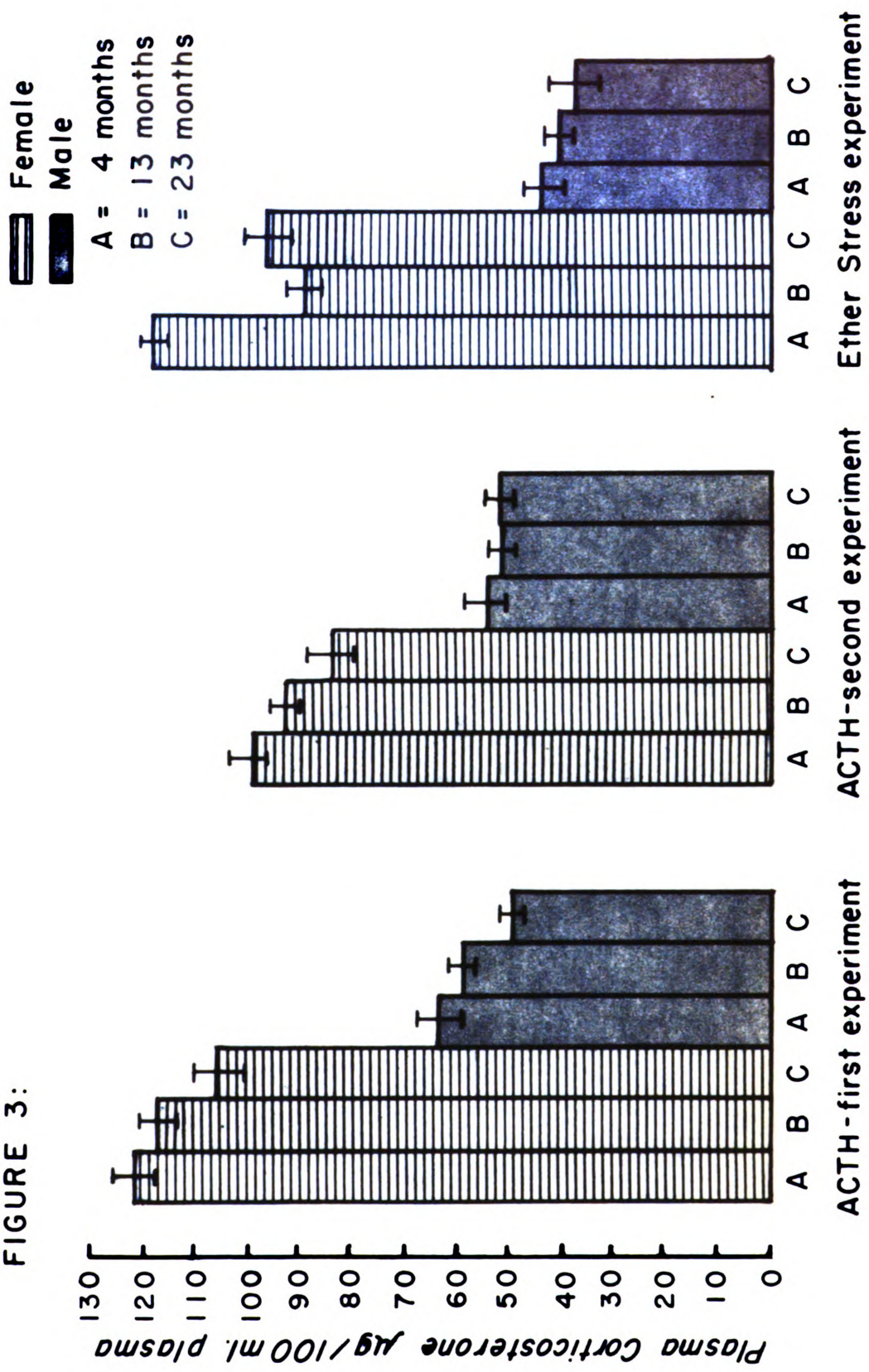

Female
Male
A = 4 months
B = 13 months
C = 23 months
Plasma Corticosterone µg/100 ml. plasma
130
120
110
100
90
80
70
60
50
40
30
20
10
0
A B C A B C
ACTH-first experiment
A B C A B C
ACTH-second experiment
A B C A B C
Ether Stress experiment

Table 4: ACTH Responsiveness - first experiment

Group	Plasma Cmpd B - female*	N	Plasma Cmpd B - male*	N
4 month	122.2 ± 3.9	21	63.3 ± 4.4	10
13 month	115.6 ± 3.7	24	58.7 ± 2.4	10
23 month	106.2 ± 4.8	22	50.1 ± 1.7	10

* Plasma corticosterone concentration (μg/100 ml).
(values reported are mean ± s.e.)
N = number of animals per group

Statistical Significance:

Analysis of Variance + Duncan's Multiple Range test

	female	difference	Rp	male	difference	Rp
4 month vs 13 month	n.s.	6.6	11.7	n.s.	4.9	8.83
13 month vs 23 month	n.s.	9.4	12.0	n.s.	8.7	8.83
4 month vs 23 month	$p < .05$	16.0	12.2	$p < .05$	13.6	9.30

Table 5: ACTH Responsiveness - second experiment

Group	Plasma Cmpd B - female*	N	Plasma Cmpd B - male*	N
4 month	100.4 ± 3.0	21	55.0 ± 3.6	8
13 month	93.7 ± 2.8	21	51.9 ± 2.3	9
23 month	85.0 ± 4.5	22	52.3 ± 2.3	20

* Plasma corticosterone concentration (μg/100 ml).
(values reported are mean ± s.e.)
N = number of animals per group

Statistical Significance:

Analysis of Variance + Duncan's Multiple Range test

	female	difference	Rp	male	difference	Rp
4 month vs 13 month	n.s.	6.7	11.1	n.s.	3.1	-
13 month vs 23 month	n.s	8.7	10.8	n.s.	0.4	-
4 month vs 23 month	$p < .05$	15.4	11.4	n.s.	2.7	-

Table 6: Ether Stress Experiment

Group	Plasma Cmpd B - female*	N	Plasma Cmpd B - male*	N
4 month	119.2 ± 2.4	6	44.3 ± 3.8	5
13 month	90.0 ± 3.5	13	41.4 ± 2.6	9
23 month	97.2 ± 4.6	13	38.1 ± 4.8	7

* Plasma corticosterone concentration (μg/100 ml).
(values reported are mean ± s.e.)
N = number of animals per group

Statistical Significance:

Analysis of Variance + Duncan's Multiple Range test

	female	difference	Rp	male	difference	Rp
4 month vs 13 month	$p < .01$	29.2	19.1	n.s.	2.9	-
13 month vs 23 month	n.s.	7.2	14.5	n.s.	3.3	-
4 month vs 23 month	$p < .01$	22.0	18.3	n.s.	6.2	-

The first set of experiments in this study involved the quantitation of plasma corticosterone levels following maximal stimulation of the adrenal gland by exogenous ACTH. Both male and female animals showed a decrease in plasma corticosterone levels with increasing age, as shown in Figure 3. Differences in plasma corticosterone levels between young animals and old animals were statistically significant as shown in Table 4. The second set of experiments was intended to be identical to the first, with the exception that one month had elapsed between the two experiments. Only female animals showed a statistically significant decrease in plasma glucocorticoid levels with increasing age in this series of experiments (see Table 5).

The most striking result of this second series of experiments was the reduced levels of plasma corticosterone relative to those of the first experiment (see Figure 3). An experiment was designed to test the possibility that the experimental procedure had in some way altered the responsiveness of the adrenal cortex to exogenous ACTH. A group of 13 young female rats which had not been used previously for experimental purposes were subjected to the previously described procedure. Plasma samples were obtained subsequent to maximal stimulation of the adrenal cortex with ACTH. These animals were subjected to an identical experimental procedure, four weeks after obtaining the first blood sample. Plasma samples from both the first and second bleeding were stored at -15^{o}C. All samples were assayed in one day at the

end of the experiment to minimize any possible differences due to day to day variations in the assay technique. To further eliminate possible bias, samples from both first and second bleeding were interspersed, to eliminate any variation in solutions or technique throughout the course of the day. It was not possible to show any statistical difference between the first and second plasma samples at the 95% confidence level.

$\bar{x}_1 = 83.0$ $\qquad$ $t = \bar{d}/S_{\bar{d}} = 1.9/3.83 = 0.496$

$\bar{x}_1 = 81.1$ $\qquad$ c.v. $= t(.05, 12) = \pm 2.179$

A third series of experiments was conducted to measure pituitary-adrenal responsiveness to an ether stress. Young female rats had plasma corticosterone levels that were significantly higher than those of year old or old groups of rats in response to an ether stress (Table 6). Male rats exposed to an ether stress showed no statistically significant difference between any of the three age groups. The plasma corticosterone levels observed in the first ACTH experiment and those observed in the ether stress experiment showed no statistically significant differences for 4 month and 23 month old females.

Vaginal smears of the female rats were obtained in the first two experiments in order to determine the reproductive status of the animals at the time of bleeding as well as the stage of the estrous cycle for animals which were cycling. In the first experimental series, rats were smeared for three consecutive days prior to bleeding. This did not provide adequate information for

determining if an animal was cycling normally. For this reason vaginal smears were obtained for at least 5 days and some to 9 days in the second series of experiments, in order to determine if the animals were cycling. Only 64% of the young females were cycling normally compared to 63% of the year old rats and 58% of the old rats. No relationship between stage of estrous cycle and adrenal responsiveness to ACTH was evident. Some of these rats were acyclic, showing signs of constant estrous, while other rats showed evidence of normal cycling followed by constant estrous several days prior to bleeding.

Histological sections of adrenal glands obtained from representative animals were also studied (see Table 7).

Table 7: Adrencocortical Zone Widths and Adrenal Weights

	4 months	13 months	23 months
Number in Group:	3	4	9
Glomerulosa	.065 ± .005 mm	.058 ± .007 mm	.058 ± .011 mm
Fasciculata	.452 ± .031 mm[a]	.473 ± .057 mm[b]	.262 ± .034 mm[a,b]
Reticularis	.419 ± .046 mm	.490 ± .033 mm	.373 ± .038 mm
Weight/100 g*	18.0 ± 3.4 mg	18.4 ± 2.4 mg	22.2 ± 6.5 mg

Each value reported is mean ± s.e.

* Adrenal weight (pair)/100 gram body weight.

a = statistically significant difference ($p<.05$).

b = statistically significant difference ($p<.01$).

Adrenocortical zone widths reported (Table 7) are averages based on four measurements per animal. Only the zona fasciculata showed

evidence of statistically significant differences in zone width between the different groups. Differences in zona reticularis and zona glomerulosa widths were not statistically significant. The columnar arrangement of the zona fasciculata cells was more prominent in young rats than in old. An increase in the connective tissue content of the zona reticularis was also evident with increasing age.

DISCUSSION

The age-related differences in stimulated plasma glucocorticoid levels are not as drastic for the rats studied as those which have been reported in aging cattle and goats. Riegle and Neller (1967) reported a 6.3 μg/100 ml increase in plasma cortisol levels following a 10 U/100 lb ACTH infusion in a young bull (2 year) compared to no increase in an old bull (15 year) subjected to similar treatment (9.65 to 15.95 μg/100 ml in young, 3.45 to 3.55 μg/100 ml in old). Young goats (1 year) showed an average increase of 4.05 μg/100 ml in plasma cortisol compared to a 0.60 μg/100 ml increase in old goats (9 year) following a 30 U ACTH infusion (4.10 to 8.15 μg/100 ml in young, 4.60 to 5.20 μg/100 ml in old) (Riegle, Przekop, and Nellor, 1968). The changes observed in rats in this study may be more like those reported in humans than those observed in cattle and goats.

Tyler *et al*. (1955) have reported higher plasma cortisol levels in aged humans than in younger humans following ACTH stimulation. However, aged humans have also been shown to have increased biological half-life and decreased distribution volumes of cortisol in comparison to younger humans (Tyler, *et al*., 1955; Samuels, 1956, 1957). This evidence suggests that the secretory capacity of the stimulated adrenal cortex is actually reduced in aged humans. The observations of Moncloa *et al*. (1963) that urinary

excretion of glucocorticoid metabolites was lower in aged humans than in younger humans following ACTH stimulation supports this hypothesis. There is good evidence that both hepatic metabolism of glucocorticoids and renal excretion of glucocorticoid metabolites decrease with increasing age in man (Shock, 1960; Moncloa et al., 1963; West et al., 1961). However, there is no substantial evidence to support the theory that such changes are of sufficient magnitude to account for the decrease in adrenal functional capacity with increasing age.

Similar age-related changes in hepatic (Barrows, Yiengst and Shock, 1958) and renal function (Arataki, 1926; Friedman and Friedman, 1957; Warren and Pruett, 1957; and Dicker and Nunn, 1958) have been observed in studies on the rat. Although it is possible that these functional alterations affect corticosterone metabolism and excretion, none of these workers have studied this parameter. It is probable that the aging rat is subject to similar changes in biological half-life, distribution volume and secretory rate of glucocorticoids as those that have been reported in human subjects. The experiments included in this study did not include measures of these functional parameters. Therefore it is conceivable that there are age related differences in other parameters of adrenocortical function in the rat that are larger than those indicated by plasma corticosterone concentrations following ether stress or ACTH injection.

Although levels of plasma corticosterone following ACTH

stimulation in the rat have been reported by several investigators, none of these workers studied age related changes of adrenocortical function. Moncloa et al. found plasma corticosterone levels of 53.1 ± 10.1 μg/100 ml in young male rats following 4 U ACTH compared to 20.2 ± 7.3 μg/100 ml for controls (unstimulated). It is doubtful that plasma corticosterone levels determined by Moncloa et al. (53.1 ± 10.1 μg/100 ml) are significantly different from those determined for young male rats in this study (63.3 ± 4.4 μg/100 ml). The young male animals included in this study weighed an average of 280 grams compared to 165 grams in the experiments of Moncloa et al., indicating that the former animals were older than the latter.

Another variable to be considered in making such comparisons is strain differences. Moncloa et al. (1959) used Sherman (albino) rats while Long-Evans (hooded) rats were used in these studies. Preliminary evidence from three Sprague-Dawley (albino) rats showed lower plasma concentrations of corticosterone following ACTH stimulation than did Long-Evans rats of the same age and sex (75.0 μg/100 ml versus 115.6 μg/100 ml).

Plasma corticosterone levels were lower following the second ACTH stimulation than after the first ACTH stimulation. This suggested that the procedures of the first experiment had somehow altered adrenal responsiveness to ACTH. However, this theory was not supported by the results of subsequent ether stress experiments or ACTH stimulation experiments on another group of rats.

The adrenocortical histological observations in this study are in agreement with those observed by Dribben and Wolfe (1947) and Jayne (1953, 1957) in the aging rat. Since the zona fasciculata is considered to be the primary source of glucocorticoid hormones (Paschkis et al., 1967), the reduction in zona fasciculata width observed in this study implies that corticosterone producing ability was reduced in the old group of rats. Increasing amounts of connective tissue were observed in the reticular zone of the rat adrenals with increasing age. The anatomical changes observed in the adrenal cortex of aging rats were much less extensive than those observed in aging cattle (Riegle and Neller, 1965). This finding is in agreement with functional evidence of age-related changes occurring in the adrenal cortex of these species.

It is conceivable that adrenal cortical responsiveness might be affected by the stage of the estrous cycle in the female rat. Critchlow et al. (1963) have reported resting plasma glucocorticoid levels as a function of the estrous cycle in the rat. Their studies indicated that proestrous plasma glucocorticoid concentrations were twice as high as those of diestrous, while intermediate levels were observed during estrous. Since the adrenal cortex secretes glucocorticoids in direct response to ACTH stimulation and does not store them, it is apparent that changes in ACTH secretion accounted for these differences in plasma glucocorticoid levels. Maximal stimulation of the adrenal cortex with exogenous ACTH would be expected to supersede estrous cycle re-

lated variations in resting levels. In this study no correlation between stage of estrous cycle and adrenal responsiveness was evident following ACTH stimulation. It is possible, though unlikely, that such differences would be observed following ether stress stimulation. However, no smears were taken from the rats at the time of the ether stress experiments.

The variation among animals of the same age group noted in these experiments gives evidence of the differences between chronological and biological age in these animals. Following ether stress or ACTH stimulation, one group of old female rats showed plasma corticosterone levels comparable to those of young animals. On the basis of histological evidence (adrenal) and body weight changes throughout the course of the experiment, this group appeared to be old rats. Reproductive status, as determined by vaginal cytology as well as adrenal functional capacity suggested that the endocrine systems of this group of old rats were unusually responsive.

SUMMARY

Age related changes in adrenocortical function were studied in male and female Long-Evans rats. The primary index of adrenocortical function measured was increased plasma corticosterone concentration following maximal ACTH and ether stress stimuli, in animals 4, 13 and 23 months of age. Plasma corticosterone concentration after maximal ACTH stimulation decreased with increasing age in both male and female rats (average corticosterone concentrations were 122 μg/100 ml in 4 month female rats, 115 μg/100 ml in 13 month female rats, 106 μg/100 ml in 23 month female rats, 64 μg/100 ml in 4 month male rats, 59 μg/100 ml in 13 month male rats and 52 μg/100 ml in 23 month male rats). The age related decrease in plasma corticosterone levels after ACTH stimulation was verified by a similar study conducted in female rats 1 month after the initial ACTH stimulation. Average plasma corticosterone concentrations after a 10 minute ether stress were also lower in old female rats compared to younger subjects (average corticosterone concentrations were 119 μg/100 ml in 4 month female rats, 90 μg/100 ml in 13 month female rats, 97 μg/100 ml in 23 month female rats, 44 μg/100 ml in 4 month male rats, 41 μg/100 ml in 13 month male rats, and 38 μg/100 ml in 23 month male rats).

ACTH responsiveness was not influenced by the stage of the reproductive cycle or the lack of normal cyclic reproductive

activities in aged female rats. Histological studies of the adrenal revealed a decrease in zona fasciculata width and an increase in the connective tissue content of the zona reticularis with increasing age.

The age related changes observed in adrenocortical function of rats in this study are much less extensive than those which have been observed in aging cattle and goats. This may indicate that the changes occurring in the adrenocortical function of the aging laboratory rat are of a different nature than those occurring in the aging domestic ruminant.

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APPENDIX

MSU Regular Rat Diet

ingredient	amount (lbs.)
Ground Shelled Corn	484.75
Soy Bean Oil Mean (49% protein)	175.00
Fish Meal	100.00
Alfalfa Meal (17% protein, dehydrated)	50.00
Dried Skimmed Milk	100.00
Beet Sugar	50.00
Corn Oil	30.00
Regular Trace Mineralized Salt	5.00
Dawes 4 B - Vitamin	1.00
Vitamin A and D Premix	0.50
Dawes B-12 Vitamin Mix	3.75
	1000.00

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