THE EFFECTS OF AGING ON THYROXINE AND CORTISOL RESPONSES TO LOW AMBIENT TEMPERATURES AND ON CIRCADIAN RHYTHM OF CORTISOL IN THE DOG.

by

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INTRODUCTION

Aging brings about a number of changes in the physiological functions of the body. The neuroendocrine system is one of the main targets of these changes. These changes can occur at all levels: the brain, especially the hypothalamus, the pituitary and the target endocrine glands. One result of the effects of aging is that it alters the secretion of hormones at one or more levels of the hypothalamic-pituitary-target endocrine gland axes. These alterations then lead to disturbances in the negative feedback system, which under normal conditions keep the secretion of hormones under control. These disturbances in the negative feedback system further aggrevate the situation. The modifications in the circulating levels of hormones in turn lead to profound changes in the physiological functions of the body. Probably the most well-known change that accompanies aging is the change in the reproductive system. The reproductive cycles cease, ovulation does not occur, and the animals stop reproducing. Aging produces a number of other changes which, although not as well-known, are nevertheless just as important. These changes include severe alterations in the secretion of hormones, not only under basal conditions, but also in response to various inhibitory and stimulatory influences, which in many situations make the animal more susceptible to various metabolic diseases.

Research during the last few years has shown that by understanding the nature of these changes in the

hypothalamic-pituitary-target endocrine gland axes, we can not only delay, but in certain situations reverse the aging associated with neuroendocrine change. Perhaps the best example of this is the reinitiation of reproductive cycles in laboratory rats by changing neurotransmitter metabolism in the hypothalamus (Quadri et al., 1973).

The effects of aging on the neuroendocrine system can be studied in many ways. Two of these ways include the measurement of circulating hormones under basal conditions, and in response to various stimuli. These methods have been used in this thesis to determine the effects of aging on the functions of the thyroid and adrenal cortex.

There is no consensus on the effects of aging on serum thyroxine levels. Increases, decreases and no change have been reported in blood thyroxine concentrations in old age. Similar controversial results have been reported on the effects of aging on cortisol secretion. The objectives of the first experiment in this thesis was to determine if there is a circadian rhythm in serum cortisol levels in the dog and how this rhythm is affected by aging. The second experiment was conducted to determine the effects of aging on basal levels of plasma thyroxine and cortisol and their responses to the stress of cold. In response to the stimulus of low ambient temperatures, the two systems which are the first to go into action to help the body cope with this stressful situation are the nervous system and the neuroendocrine system. The nervous system, especially the sympathetico-adrenomedullary system, and the various hormones of

the neuroendocrine system, especially the thyroid hormones and the glucocorticoids, are believed to be involved with the initiation and maintenance of various processes which help the body to cope with the stress of low ambient temperatures. These two systems help the animal to meet the increased metabolic needs for the maintenance of homeothermy (Gale, 1973). With aging, the responses to the stimulus of low ambient temperatures are affected, as is reflected by a reduced ability of older animals to maintain the metabolic processes required for homeothermy.

LITERATURE REVIEW

Control of Thyroid and Adrenocortical Secretion

Control of thyroid and adrenal secretion is a complicated matter involving regulation by the central nervous system. In the early 1900's there were a few speculations about the possible involvement of the nervous system in endocrine function, but it was not until 1948 that an English endocrinologist named Geoffery Harris postulated the chemotransmitter hypothesis (Harris, 1955), which implied that the brain plays an important role in the control of the endocrine system. This hypothesis started a revolution in the field of endocrinology. Up until that time, the endocrine system was viewed as a neatly packaged entity, separate from all other systems. But today, it is without question that a neuroendocrinologist accepts the fact that the hypothalamus influences the endocrine system through regulation of the pituitary. The pituitary in turn controls the functions of the target endocrine glands. Only in the past 20 yrs have these neuroendocrine axes of the hypothalamus, pituitary and target endocrine glands been elucidated. It is such axes that regulate the secretions of the thyroid and adrenal glands (Bennett and Whitehead, 1983; Martini and Besser, 1977).

The thyroid gland is responsible for the secretion of thyroxine and triiodothyronine. Histologically, the thyroid is a highly vascularized organ which is composed of numerous follicles containing a gelatinous colloid. Within the colloid there can be found a large

protein called thyroglobulin, which is the source of the thyroid hormone precursors. Circulating iodide is actively pumped into the colloid and oxidized into iodine by the enzyme peroxidase. The iodine is then transferred to the tyrosyl residues of thyroglobulin to form the precursors monoiodotyrosine (MIT) and diiodotyrosine (DIT).

These modified tyrosine residues, while still attached to the thyroglobulin molecule, have the ability to couple and form the hormones triiodothyronine and thyroxine, of which thyroxine far exceeds the production of triiodothyronine. These thyroid hormones are stored within the colloid linked to thyroglobulin and are liberated into the capillary network when the metabolic need requires their release. Release of the thyroid hormones is facilitated by hydrolytic enzymes which break the peptide bond between thyroglobulin and triiodothyronine, thyroxine, MIT and DIT. The thyroid hormones are consequently released into the plasma where approximately 99% is noncovalently bound to carrier proteins. There are three carrier proteins involved in the binding of thyroid hormones in plasma. Thyroxine binding protein (TBG) comprises about 60% of the total binding, thyroxine binding pre-albumin (TBPA) comprises about 30% and albumin makes up the remaining 10%. Since the thyroid hormones are bound noncovalently, there is a dynamic equilibrium between free and bound thyroid hormones of which only the free hormone is metabolically active. The remaining MIT and DIT are immediately deiodinated within the thyroid gland and the iodine formed is reincorporated into the new thyroglobulin molecules (Turakulov et al., 1975).

Metabolically, triiodothyronine is more potent than thyroxine, but their actions are very similar. The metabolic actions of the thyroid hormones include a calorigenic effect, whereby the basal metabolic rate (BMR) is increased by elevating the rate of glucose and fatty acid oxidation. The thyroid hormones are also protein anabolic and play a prominent role in growth processes, especially of the nervous system. The lack of thyroxine in early life can lead to hinderence in body size, deformation and severe mental deficiencies. Inactivation of the free thyroid hormones is accomplished mainly in the liver, kidney, brain and muscle tissues. The broken down iodide is either excreted in the urine or recirculated to the thyroid to be used in synthesizing more thyroid hormones (Oppenheimer, 1979).

The release of the thyroid hormones is influenced by another tropic hormone called thyroid-stimulating hormone (TSH). TSH is a glycoprotein of molecular weight 28,000. It is secreted by the thyrotropic cells of the anterior pituitary and released into the blood. One stimulus for the release of TSH is the reduction in the plasma levels of triiodothyronine and thyroxine. TSH is able to enhance the synthesis of thyroglobulin and increase the active transport of iodide in order to restore circulating levels of thyroid hormone to normal. Once the normal levels of triiodothyronine and thyroxine have been restored, the thyroid hormones feed back to the anterior pituitary to inhibit the production of TSH. Another way to stimulate the release of TSH is mediated by a hypothalamic hormone called thyrotropin releasing hormone (TRH) (Reichlin et al., 1978).

TRH is a tripeptide neurohormone synthesized in the neurons of the tuberoinfundibular system, located just beneath the median eminence of the hypothalamus. This tripeptide is released into the hypopheseal portal system overlaying the pituitary stalk and is shuttled to the anterior pituitary where it stimulates the release of TSH. The stimulation of TSH by TRH is a rapid process and TRH is quickly inactivated by several peptidase enzymes (Griffiths and Bennett, 1982).

The effect of thyroid hormone on the hypothalamus is a subject of controversy. Originally it was thought that triiodothyronine and thyroxine had a negative feedback effect on TRH (Kajihare and Kendall, 1969), but new evidence suggests that triiodothyronine and thyroxine may exert a positive feedback effect on TRH (Mitnick and Reichlin, 1972; Reichlin et al., 1972). If this is the case, it is interesting to note that the thyroid hormones can activate the secretion of TRH, while at the same time it provides a negative feedback at the level of the anterior pituitary to hinder the secretion of TSH. Such a mechanism suggests that TRH and the thyroid hormones are in competition to regulate the secretion of TSH. A shortloop feedback also exists in which TSH acts on the hypothalamus to inhibit TRH release.

The release of TRH is mediated through neurotransmitters liberated at the synaptic junctions of pre-TRH releasing neurons. These neurotransmitters include acetylcholine, the catecholamines

(norepinephrine and dopamine) and serotonin. Norepinephrine has been known to have a stimulatory effect on TSH by facilitating the release of TRH. Dopamine also shows a stimulatory effect on TSH via the TRH pathway. However, at the anterior pituitary level, dopamine can cause an inhibitory effect by acting directly on TSH secreting cells. Acetylcholine can also inhibit TSH at the pituitary level. The studies on the effects of serotonin on TRH release have produced contradictory results: it has been shown to both stimulate and inhibit TRH release (Griffiths and Bennett, 1982; Reichlin et al., 1978).

The glucocorticoids are steroid hormones secreted by the adrenal cortex. These steroid compounds are produced in the zona fasciculata of the adrenal cortex. Of the many glucocorticoids produced by the adrenal gland, cortisol and corticosterone are the main secretory products. The ratio of cortisol to corticosterone varies from species to species. In the dog, as well as in humans, cortisol is the major glucocorticoid secreted. The difference between cortisol and corticosterone is that cortisol has an additional hydroxyl group, otherwise their actions are similar.

Glucocorticoid synthesis is an involved process where acetyl coenzyme A is converted to the cholesterol precursor via mevalonic acid and squalene. Cholesterol is stored in the adrenal cortex and in turn is converted to pregnenolone, the immediate precursor to all steroid compounds. All glucocorticoids are 21 carbon compounds containing three hexangonal and one pentagonal rings with two methyl

groups and one ethyl side chain. The individual glucocorticoids can be identified by the position of specific double bonds, hydroxyl and ketone groups (Samuels and Uchikawa, 1967).

Glucocorticoids are released into the blood and carried to the specific target cells where they produce their effects. Only a small portion of the glucocorticoids circulate free in the plasma. Most of the steroids are bound to an alpha-globulin carrier protein called transcortin. The free glucocorticoids are active. The bound glucocorticoids are not as easily broken down due to the protective nature of transcortin (Daughaday, 1967).

The metabolic actions of the glucocorticoids are widespread. These actions have profound effects on carbohydrate, lipid and protein metabolism. These metabolic actions include the inhibition of glucose utilization and the stimulation of glucose formation from tissue proteins. Protein synthesis is decreased under the effects of these steroid compounds and there is an increase in the mobilization of fats. Other effects of the glucocorticoids include some mineralcorticoid activity due to structural similarities with mineralcorticoids. They also have an anti-inflammatory effect and are involved in stress responses. During stress, levels of cortisol and corticosterone have been observed to display a many fold increase in plasma levels and have been used as an index of stress. Inactivated free glucocorticoids and their steroid analogues are finally excreted in the urine (Bennett and Whitehead, 1983).

The glucocorticoids are released by the adrenal gland under the

influence of adrenalcorticotrophin (ACTH). ACTH is a polypeptide hormone consisting of 39 amino acids. It is secreted from the corticotrophin cells of the anterior pituitary and is released into the plasma. At the level of the adrenal cortex, ACTH stimulates the synthesis and release of the glucocorticoids (McKenna et al., 1979). High levels of glucocorticoids in the plasma will inhibit the release of ACTH, while low levels of glucocorticoids will stimulate the secretion of ACTH (Keller-Wood and Dallman, 1984). At the hypothalamic level, corticotrophin releasing factor (CRF) can also stimulate the release of ACTH.

CRF was the very first hypothalamic hormone to be identified, yet this 41 amino acid polypeptide has only just recently been chemically characterized. The location of CRF releasing neurons have their highest concentration in the median eminence region of the hypothalamus. From here, CRF is released into the hypopheseal portal vessels and is carried to the anterior pituitary. At the anterior pituitary, CRF exerts its action at the corticotrophic cells, stimulating the secretion of ACTH. Just as ACTH is stimulated by low levels of glucocorticoids circulating in the blood, a reduction in the levels of glucocorticoids exhibits a similar effect on the release of CRF at the hypothalamus. In contrast, high levels of glucocorticoids in the plasma inhibit CRF. Additional inhibition is provided by a short-loop feedback in which high levels of ACTH in the circulation will retard the secretion of CRF (Jones et al., 1979; Jones, 1978).

The release of CRF is influenced by the action of the

neurotransmitters at the hypothalamus. The effects of these neural chemicals have not been completely resolved. However, from the existing body of experimental data it would seem likely that both acetylcholine and serotonin play a stimulatory role on the release of CRF (Hillhouse et al., 1975; Fuller, 1981), while the catecholamines seem to produce inhibitory effects on the release of CRF (Vanloon, et al., 1971).

Effects of Aging on Thyroxine Secretion

The aging process is associated with many physiological changes. Some examples of these changes, attributed to the aging phenomenon, are as follows: 1) a progressive decline in the basal metabolic rate (BMR), 2) an increase in serum cholesterol and lipoprotein concentrations, 3) an increased dryness of skin and a number of other epidermological disorders, 4) a reduction in motor function and 5) an intolerance to cold. It is interesting to note that all the above changes that occur in the aging individual are also well established symptoms for hypothyroidism (Ingbar, 1976; Pittman, 1962), indicating that levels of thyroxine might be decreased. Further support for a decline in thyroid function with aging comes from the morphological changes in the aging thyroid gland (Ingbar, 1978). Pathological changes that occur in the aging thyroid include a decrease in glandular weight, an increase in interfollicular connective tissue and a decrease in the amount of colloid and the size of follicles. Such evidence may be suggestive of an age-dependent decrease in the levels

of thyroxine, but the evidence is not conclusive. The effects of aging on thyroxine secretion are not at all well understood, and the data which exists is confusing and contradictory. In man, there are as many studies which say that thyroxine secretion increases with age. Still others insist that levels of thyroxine do not change with age. In laboratory animals, such as the rat, there is somewhat more agreement that thyroxine secretion diminishes with age.

Herrmann et al. (1974) measured thyroxine concentrations in two age groups. Group I consisted of younger persons ranging in age from 20 to 64 years. Group II consisted of euthyroid older subjects ranging in age from 65 to 92 years. Both total and free thyroxine concentrations were significantly lower in the elderly. These results were confirmed by Hesch et al. (1976). They found total thyroxine concentrations in elderly male and female subjects to be significantly lower than those in middle-aged subjects. In a recent study however, Wilke (1983) found that both total and free thyroxine concentrations gradually decline with age in men, but not in women. Ohara et al. (1974) conducted a study in which free thyroxine concentrations in the blood were significantly decreased with age, but no change occurred in the total thyroxine concentrations of aged persons.

A number of earlier metabolic studies hinted toward a decreased thyroid function with aging. Gregerman et al. (1962) found that the functional turnover rate and degradation of thyroxine in humans decreased with age. The turnover rate and degradation of protein bound iodine (PBI), which is used as an index for thyroxine, decreased

by approximately 50%. Since the turnover rate and the degradation of thyroid hormone were equal, the net result, they concluded, was a decrease in thyroid function. Beckers et al. (1966) determined from their own data and the data found in the literature that the secretion and degradation of thyroid hormone during adolescence was intermediate between children and adults. They concluded that there is a progressive decrease in thyroid function with age. However, a decrease in thyroid function is not the same as a decrease in serum thyroxine concentrations. Both Gregerman et al. (1962) and Beckers et al. (1966) found no significant changes in the levels of PBI with aging.

Gaffney et al. (1960) measured serum PBI in euthyroid men ranging from 18 to 94 years of age. They were unable to find evidence of an age-dependant change in thyroxine levels. Similar results were reported by Braverman et al. (1966). They found no evidence that levels of PBI in euthyroid men ranging in age from 18 to 94 years were altered with age. Olsen et al. (1978) performed a study in which they measured serum thyroxine directly in a group of young adult persons, and compared them to serum thyroxine values measured in healthy elderly subjects living at home, healthy elderly subjects living in nursing homes and elderly subjects that were hospitalized. They were not able to detect any differences in serum thyroxine concentrations between young adult persons and the healthy elderly subjects living at home or in nursing homes. However, serum thyroxine levels in the hospitalized elderly group were significantly lower when compared to

the young adult group. Olsen and his group concluded that a decrease in circulating thyroxine is not due to aging per se, but rather, depends on the clinical status of the individual subject. Caplan et al. (1981) agree with Olsen et al. (1978) in advising a more rigorous screening procedure when selecting elderly subjects for thyroid function tests.

Other investigators have also found no change in levels of circulating thyroxine with aging, yet have reported that triiodothyronine decreases significantly with aging. Sawin et al. (1979) reported that age alone was unable to alter levels of thyroxine, but that levels of triiodothyronine were slightly lower in men over 60 years and women over 80 years. Similar results were obtained by Rubenstein et al. (1973), Shigemasa et al. (1981) and Caplan et al. (1981). The results of Snyder and Utiger (1972a) are comparable, but an age-dependant decrease in levels of triiodothyronine was observed in men and not in women.

In contrast to the above studies, levels of thyroxine have been reported to increase in the blood of elderly persons. In measuring levels of thyroxine, Burrows et al. (1975) was able to show an age-related increase. Evidence for elevated levels of circulating thyroxine is supported by Britton et al. (1975) and Burrows et al. (1977) who also showed an increase in the free thyroxine concentrations with aging.

In laboratory animals, such as the rat, most investigators agree that levels of circulating thyroxine decrease with age. Eleftheriou

(1975) reported a significant decrease in levels of PBI for older mice compared to younger mice. This decline was also shown to be strain dependant where the decrease in DBA/2J mice was significantly greater than in C57BL/6J mice. The effects of aging on basal serum thyroxine in female rats were studied by Chen and Walfish (1978). They found a significant decrease in concentrations of total thyroxine, free thyroxine and free triiodothyronine in aged female rats, but no change in concentrations of total triiodothyronine was noted. A similar study by Chen and Walfish (1979), in male rats, revealed significantly lower serum concentrations of total thyroxine, total triiodothyronine and free triiodothyronine in aged male rats, but no change in levels of free thyroxine concentrations. This data is supported by Frolkis and Valueva (1978). In a study by Pekary et al. (1983), an age-dependant decline in concentrations of both total and free thyroxine were observed in male and female rats. However, no changes in total or free serum triiodothyronine were observed in aged rats of either sex.

Segal et al. (1982) performed a study in rats ranging in age from 10 days to 12 months. They found that serum thyroxine levels were consistently higher in the males for all age groups. However, serum triiodothyronine levels were consistently higher in the females for all age groups. Concentrations of thyroxine and triiodothyronine reached a peak between the first and second month of age for both males and females, but showed a progressive decline thereafter. Thyroxine levels decreased significantly by six months, while

triiodothyronine decreased significantly by two to three months. Azizi (1979) had found similar results in young adult male rats. As the age of the rats increased from one to five months, there was a 50% decrease in levels of thyroxine, but no change in levels of triiodothyronine were evident.

There is considerable speculation relating age to thyroid function in both rats and humans, but whether or not the data obtained from the rat studies can be extrapolated to humans is not certain since both species show confusing and contradictory results. Work done by Grad and Hoffman (1955) and Kumaresan and Turner (1967) have shown that older rats secrete less thyroxine than younger rats. Panda and Turner (1967) explain this decline in secretion rate by suggesting that the sensitivity of the thyroid gland to TSH decreases with age. Huang et al. (1980) found no change in levels of TSH, but a decrease in thyroxine and triiodothyronine with aging. They also attributed this reduced thyroid function to the thyroid gland, because no alterations in the hypothalamo-pituitary activity were detected. Significantly increased levels of TSH with aging were found by Choy et al. (1982). They feel the decrease in thyroxine and increase in TSH is a manifestation of an impaired pituitary-thyroid axis in aged rats.

Conflicting reasons have been suggested for changes in human thyroid levels with aging. Snyder and Utiger (1972a and 1972b) found a lower TSH response to TRH in aged men, but not in aged women. This suggests a tendency for the pituitary thyrotroph cells to become less sensitive with increasing age. Olsen et al. (1978) and Shigemasa et

al. (1981) reported basal serum TSH to be lower in healthy elderly subjects than younger subjects. Lower circulating levels of TSH may be an explanation for decreased thyroid hormones in the elderly. However, neither Olsen et al. (1978) or Shigemasa et al. (1981) found any significant changes in levels of thyroid hormones of elderly subjects. A possible mechanism for lowered basal TSH was proposed by Shenkman et al. (1973). They found that small doses of triiodothyronine suppressed the TSH response to TRH. In addition, Szabolcs et al. (1981) were able to show that the lower concentrations of triiodothyronine present in older persons compared to younger persons were able to suppress TSH more effectively.

In contrast to these reports, Ohara et al. (1974) found a higher TSH responsiveness to small doses of TRH, as well as slightly elevated basal serum TSH concentrations in the elderly. They explained this finding to be due to a latent hypothyroid-condition. Sawin et al. (1979) were also able to detect slightly elevated TSH levels in elderly humans. They suggest that the elevation in the serum TSH levels in the elderly may compensate for some thyroid dysfunction, although no changes in thyroxine were detected.

Other possible reasons for changes in human thyroid hormone levels, due to aging, have been suggested. Hansen et al. (1975) found the absolute iodine uptake by the thyroid to be decreased with age in both sexes. This finding is in accordance with evidence suggesting thyroxine production, and hence secretion into the blood, is decreased. Oddie and Fisher (1967) demonstrated an age-related

decrease in TBG and an age-related increase in TBPA concentrations from childhood to adulthood. They believe that these changes in the thyroid hormone carrier proteins could be responsible for the observed lowering of PBI concentrations with aging. These data are supported by Braverman et al. (1966) who suggests that these changes in the blood carrier proteins may occur during adolescence because of the changing status of gonadal hormones. Rubenstein et al. (1973) found a significant progressive decrease in both sexes in triiodothyronine concentrations which they attribute to a possible decrease in the peripheral monodeiodination of thyroxine to triiodothyronine.

As can be seen from the above review, the effects of aging on thyroxine secretion are not at all well understood and the published data is confusing and contradictory.

Effects of Aging on Thyroxine Response to Low Ambient Temperatures

With the stress of low ambient temperatures, the need for an increased BMR is evident. The rate of metabolism must increase in order to produce enough heat so that animals can cope with this condition. The thyroid gland is an important organ in that it helps in regulation of the BMR. When the thyroid gland is hyperfunctioning, i.e. secreting large quantities of thyroid hormones, there is an increase in the BMR that has been associated with hyperthyroidism for years. Sellers et al. (1974) performed a study in which they found

hypothyroid rats to be less able to cope with an environmental temperature of 4°C than were euthyroid rats. This suggests that thyroid hormones are necessary in order to cope with cold environmental temperatures. However, the question remains as to whether or not there is an increase in thyroid hormones during exposure to low ambient temperatures. Furthermore, are these changes in thyroid hormones during exposure to cold affected by aging?

In a study by Storm and Associates (1981), rats were exposed to 4° C for three, seven and 14 days. The results indicated that plasma thyroxine concentrations were significantly decreased after three, seven and 14 days of exposure. Plasma triiodothyronine concentrations were significantly increased after seven and 14 days. Hence, the triiodothyronine/thyroxine ratio was significantly elevated. They explain these results by suggesting that the rate of thyroxine conversion to triiodothyronine is increased during exposure to cold. These results have been confirmed by Bernal and Escobar del Rey (1975). LaBlanc et al. (1982) also found a significant increase in levels of plasma triiodothyronine one hr after exposure to -15°C in rats. However, they did not measure levels of thyroxine. An interesting paper by Van Hardeveld et al. (1979) showed the effects of 4°C exposure in rats after one and four wks. They found that plasma thyroxine concentrations declined after one wk, but had returned to normal values after four wks. Triiodothyronine concentrations were elevated significantly after both one and four wks. They suggest that the initial decrease in thyroxine is due to quicker utilization

and deiodination thereby increasing the conversion of thyroxine to triiodothyronine. Restoration of thyroxine levels after four wks of 4°C exposure were probably due to the decrease in the renal clearance of iodine as the rats were exposed to cold for a longer period of time. This proposal was based on the work done by Galton and Nisula (1969).

Tuomisto et al. (1976) did a study on the effect of cold temperatures in humans. The volunteers were made to sit quietly in a swimming pool for 30 min in which the water was kept at 25 to 28°C. No significant changes in levels of triiodothyronine and the effective thyroxine ratio were reported, suggesting that the rate of thyroxine to triiodothyronine conversion is not a factor during exposure to cold in men. In contrast, Gambert and Barboriak (1982) reported an increase in rat serum triiodothyronine and a decrease in thyroxine to triiodothyronine conversion during exposure to cold. They reason that the elevation in serum triiodothyronine is probably due to the thyroid gland itself since in the rat, 50% of the circulating triiodothyronine comes directly from the thyroid gland as opposed to 20% in man. The difference in circulating levels of triiodothyronine content is made up by the peripheral conversion of thyroxine to triiodothyronine. For this reason, an increase in the thyroxine to triiodothyronine conversion would not be expected. However, they could not give a concrete reason as to why thyroxine to triiodothyronine conversion would decrease during exposure to cold in the rat.

Jobin and his group (1975) reported a significant increase in levels of plasma thyroxine during exposure to 5°C for a total of four

hrs in the rat. However, prolonged exposure (32 days at 5°C) did not show an increase in thyroxine concentrations. An increase in levels of thyroxine during exposure to cold was supported by Huang et al. (1980). They showed levels of serum thyroxine to increase by two hrs during exposure to 4°C, but thyroxine levels fell back to basal by the sixth hr.

In an experiment by Leppaluoto et al. (1974), levels of serum TSH were shown to be rapidly increased (by 15 min) during exposure to 5°C in rats. They feel that this fast increase in levels of TSH is due to some neural mechanisms involved in the response to short-term exposure to low ambient temperature, since it had been reported that hypothalamic TRH content (Sakoda and Nakabayashi, 1970) and that TRH-synthetase activity increase (Reichlin et al., 1972) during exposure to cold. From these results, the suggestion was made that exposure to cold in the rat represents a good model in which the interactions of the hypothalamo-pituitary-thyroid axis could be studied. In contrast to Leppaluoto's group (1974), Jobin et al. (1975) found no significant changes in levels of hypothalamic TRH during exposure to cold in the rat, yet they do report a significant increase in levels of both plasma and pituitary TSH. They explain that it is possible, during exposure to low ambient temperature, for an equal rise in release and synthesis of TRH so that any changes in hypothalamic TRH content go undetected. Other papers supporting an increase in levels of TSH during exposure to cold have been reported by Meuller et al. (1974), Tuomisto et al. (1976) and Huang et al.

(1980).

The effects of aging on the thyroxine response to low ambient temperatures have not been studied by many investigators and the literature available on this topic is scant. Gregerman (1963) determined the effects of age and exposure to cold in thyroxine metabolism. He found that exposure to 5°C for two wks increased the fractional turnover rate of thyroxine in both male and female rats, although the plasma thyroxine concentrations did not appear to be altered. In addition, he found a significant 7% decrease in thyroxine concentrations of 24 month-old rats as compared to 12 month-old rats. This was accompanied by an increase in the degradation of thyroxine with age. Huang et al. (1980) studied the effects of aging on serum thyroxine response to short-term cold exposure (4°C for six hrs) in the rat. Their results indicate that old rats ranging from 20 to 24 months of age, have reduced thyroxine response to the cold in comparison to six to eight month-old rats. Triiodothyronine and TSH responses to the cold were not significantly different between the two age groups. The effects of cold exposure on triiodothyronine in rats of increasing age were also studied by Gambert and Barboriak (1982). They agree with Huang's group (1980) that no changes in triiodothyronine response to cold occurs during the aging process. The effects of cold on thyroxine concentrations are a controversial subject and the effects of aging on thyroxine concentrations are even more so. The lack of a sufficient number of investigations as well as a concensus in this area indicate that more research needs to be done.

Effects of Aging on Cortisol Secretion

The aging adrenal cortex undergoes degenerative changes. These include an insignificant reduction in adrenal weight, an increase in nodule formation, an increase in the amount of connective tissue, an accumulation of pigment cells in the cortex and a loss of steroid-containing lipid in the zona fasiculata (Blichert-Toft, 1978). These morphological changes that occur during the aging process may have an effect on the functional capacity of the adrenal gland.

Early studies indicated that the metabolism of adrenocortical hormones changes with increasing age. Romanoff et al. (1961) reported that the 24-hr secretion rate of corticosteroids in elderly persons was 75% of that found in young adults. Samuels (1956) found that the biological half-life of cortisol increases with age so that only 50% of the cortisol secretion rate is required to maintain equal cortisol levels in both young and old humans. West et al. (1961) were able to find an age-dependant decline in the rate of cortisol removal from the circulation. However, none of these studies were able to show a difference in the circulating concentrations of cortisol between young and aged individuals.

Most of the earlier studies were unable to determine any changes in the levels of cortisol with increasing age. In contrast, a few recent reports have detected an age-related increase in the levels of circulating cortisol. These opposing results are presently being

disputed, but the evidence for an age-related increase in cortisol concentration is becoming rather convincing, especially since more sophisticated means for collecting and measuring blood for steroid hormones are now available.

Riegle and Nellor (1965 and 1967) reported no age differences in levels of plasma cortisol between young and old cattle. Similar results were obtained in goats (Riegle et al., 1968) and in rats (Hess and Riegle, 1970 and 1972). In man, Saruta et al. (1980) reported concentrations of plasma cortisol to be slightly lower in subjects over 61 years of age as compared to subjects under 41 years of age. However, this difference did not reach significance. Vermeulen et al. (1982) were also unable to detect any significant changes in plasma cortisol concentrations in man with aging.

In a study by Britton et al. (1975), rats of two, 12 and 24 months of age were analyzed for circulating levels of corticosterone. Corticosterone concentrations in the 12 and 24 month-old rats were slightly higher than the two month-old rats. This difference, however, was not significant and the experimenters explain that since blood was collected three min after ether anesthesia, a significant change in levels of corticosterone with aging may have been masked by the stresses of ether treatment and animal handling in the more stress susceptible two month-old rats. Riegle and Hess (1972), performed an experiment in rats on the effects of aging on stress responsiveness and dexamethasone treatment. The levels of corticosterone before treatment were higher in the old rats than in the young rats by 35%.

However, they made no mention of whether this difference was significant. Tang and Phillips (1978) report similar results, where old rats showed levels of corticosterone to be 100% higher than corticosterone concentrations in the young rats, but they reported that there were no age-related differences in the basal levels of corticosterone.

One of the earliest studies to indicate that levels of adrenocortical hormones increase with aging was performed by Friedman et al. (1969) in healthy elderly men. They reported elevated plasma cortisol levels at midnight in most of the elderly patients, but levels of cortisol concentrations at nine AM were not different from those found in the normal range. Interestingly, however, plasma cortisol concentrations in the elderly were not compared to those in the young adult subjects.

Lewis and Wexler (1974) looked at levels of serum corticosterone in rats of three age groups: three to four month-old, six to eight month-old and 15 to 18 month-old. They reported that levels of corticosterone were considerably elevated in the 15 to 18 month-old virgin males and in the six to eight month-old breeder males as compared to the three to four month-old virgin and breeder males. They associate this hyperadrenocorticoid activity to pathological changes including hemorrhages in the adrenal glands. Landfield et al. (1978) also used male rats of three age groups: young, mid-aged and old. They found the mid-aged rats to have significantly increased corticosterone concentrations as compared to young rats. However,

levels of corticosterone in the old rats tended to decrease. They suggest that these results could explain the reason for the conflicting data in the literature, since different studies used animals of different ages. Sapolsky and associates (1982) found that corticosterone concentrations in the rat showed an age-related elevation in basal titers under both light and dark conditions. They advise the use of more than two age groups per study in order to detect differences in corticosterone levels with aging. Dekosky et al. (1984) confirmed the above results on an age-related increase in levels of corticosterone. In summary, in the past, most investigators had the view that circulating levels of glucocorticoids, cortisol and corticosterone, do not change with age. However, in the past few years, evidence for an age related increase in levels of adrenocorticoids has accumulated.

Although some discrepencies still exist, there is a general agreement that older subjects have a reduced ability to respond to stress. It still remains to be seen where in the hypothalamo-pituitary-adrenal axis this age-related problem may exist. In the early studies of Riegle and Nellor (1965 and 1967), it was noticed that old cattle had a decreased responsiveness to ACTH infusion when compared to young cattle. They suggest that the reason for this decreased responsiveness in old cattle is due to a decrease in the functional capacity of the adrenal cortex. This means that the adrenal cortex in old cattle is secreting more cortisol in order to maintain normal circulating levels. Under stressful situations, the

cortisol reserves in the old cattle have thus already been depleted, hence the lowered response to stress in the aged cattle. Riegle et al. (1968) propose the same explanation in the goat and Hess and Riegle (1970) confirm this in the rat. In another experiment in the rat, Hess and Riegle (1972) measured corticosterone levels in young and aged rats after both acute and chronic ACTH-treatment. Acute ACTH-treatment showed a lowered corticosterone response in the aged rats as compared to the young rats. In contrast, chronic ACTH-treatment showed a lowered corticosterone response in the young rats as compared to the acute ACTH-treated young rats. The chronic ACTH-treated old rats revealed levels of corticosterone that were not significantly different from acute ACTH-treated old rats. From these results, the hypothesis of adreno-cortical exhaustion could not be supported. Instead, they explained the results by suggesting that the lowered adrenocortical responsiveness might be due to differently responding hypothalamic-pituitary control mechanisms in the two age groups. Later, Riegle and Hess (1972) and Riegle (1973) indicated that there is a decrease in the sensitivity of the adrenocortical control mechanism to the feedback inhibition in the old rats as compared to the young rats.

Tang and Phillips (1978) did not find a decreased adrenal responsiveness in old rats to stress. Instead, they noticed an age-dependant increase in levels of ACTH. They suggest that this increase is an adaptive process in response to the degenerative histopathological changes that occur in the aging adrenal to ensure

sufficient circulating corticosterone. In addition, Tang and Phillips (1978) found that as the stress became longer and more severe, the younger rats showed a greater ACTH response, but no change in levels of ACTH were detected in the older rats. They suggested a decrease in the pituitary ACTH reserve or a decline in CRF secretion in response to stress. In contrast, Sapolsky et al. (1982) feel that an age-related increase in basal corticosterone concentrations of male rats is due to an increase in the secretion rate of corticosterone by the adrenal, since there were no differences detected in the metabolic clearance rate between young and old rats.

Effects of Aging on Cortisol Response to Low Ambient Temperature

The adrenocortical steroids, cortisol and corticosterone, are very important hormones involved with the stress of low ambient temperatures. Like the thyroid hormones, the corticosteroids are concerned with the metabolic processes that help the body maintain homeothermy. Generally, it is believed that cold exposure elevates the corticosteroids in the circulation. However, care must be used in interpreting the data, since experimental variables, such as the temperature of the exposure, the duration of exposure and the animal species used, can produce variable results.

Lenox et al. (1980) conducted an experiment in rats, in which they wetted the fur of each animal with cold water for 15 sec. This was followed by placing the rats in a chamber at 4^{o}C for five min.

The results of this acute exposure indicate that corticosterone concentrations are significantly increased by more than three-fold. Takeuchi et al. (1977) studied the effects of acute cold exposure in rats. They found that exposure to 2^{0}C provoked a significant increase in levels of corticosterone within 15 min and remained elevated for the next three hrs. Jobin et al. (1976) exposed rats to 5^{0}C for a period of 45 min. Their results indicated that corticosterone concentrations were significantly increased from 5 ug/100 ml to 30 ug/100 ml within 15 min and to 45 ug/100 ml within 45 min. The experiments of Maickel and associates (1961) are in agreement with the above data. They found concentrations of corticosterone, in the rat, to be increased by 250% after two hrs of exposure to 4^{0}C. Levels of corticosterone remained elevated for up to eight hrs of exposure, but the concentrations of corticosterone were back to their normal level by 20 hrs.

Similar results have been reported in the dog by Egdahl and Richards (1956). They exposed mongrel dogs to -46 to -50^{0}C and -75 to -79^{0}C for a total of five hrs. In both temperature ranges, there was a significant increase in levels of 17-hydroxycorticosteroids which persisted from one to three hours. The work of Chowers et al. (1964) studied the effects of acute environmental cooling in the dog. The exposure time was 45 min at 0^{0}C. They found that levels of cortisol were significantly increased at 15, 30 and 45 minutes during the exposure. Egdahl and Richards (1956) reported that the environmental temperature should be decreased to -10^{0}C in order to elicit adrenal

activation in the dog, where as Chowers et al. (1964) demonstrated that a response in the dog could be elicited at 0^{o}C.

Humans exposed to low ambient temperatures have yielded comparable results. Suzuki et al. (1967) exposed volunteers to mild cold of 10 to 15^{o}C for one hr. They found plasma cortisol to increase slightly, but significantly towards the end of exposure. Wilson et al. (1970) found significantly higher cortisol levels throughout the exposure period of three hrs at -5^{o}C. In contrast to these results, Golstein-Golaire et al. (1970) reported a slight but significant decrease in levels of plasma cortisol at 30 min during exposure to 4^{o} C. However, they associate this decrease to the normal circadian rhythm. No other changes in levels of plasma cortisol were observed during the two-hr exposure to cold.

The cold-induced increase in levels of adrenal steroids is most likely associated with an increase in levels of CRF and, in turn, of ACTH. Usategui et al. (1977) reported an increase in levels of ACTH in the rat during a two-hr exposure to 2^{o}C. The ACTH concentrations were elevated within 15 min of exposure, but then returned to basal levels. This decrease is due to the feedback inhibition produced by increased levels of corticosteroids. This was supported by the experiments of Jobin et al. (1976) who found that dexamethasone, a synthetic corticosteroid, completely abolished the ACTH response to cold in the rat. In addition, plasma corticosterone concentrations were unaltered, a reflection of the blocked ACTH response to cold.

There is very little literature concerning the effects of aging on corticosteroid response to low ambient temperatures. Sapolsky et al. (1982) has performed one of the only two studies available in which the effect of aging on adrenocortical response to cold was studied. Corticosterone concentrations were measured in young and old rats that had been exposed to $4^{o}C$ for four hrs. The results indicate that old rats have a significantly greater and longer response to cold. This is in contrast to most other studies which suggest that older rats have a reduced ability to respond to stresses of other kinds. However, they explain their results by saying that old rats have an impaired capacity to adapt to and recover from stress. They suggest that aged rats and younger rats both have the same ability to perceive the stress of cold, the only difference being that the old rats have an impaired negative feedback mechanism which fails to inhibit the secretion of ACTH. Finch et al. (1969) found a slight decrease in the corticosterone response to cold in old mice as compared to young mice. However, this difference did not reach significance. They concluded that the adrenal activity in aged mice was not significantly altered due to the similar corticosterone concentrations in both age groups. To my knowledge, the effects of aging on cortisol responses to cold in humans have not been investigated and the paucity of data in this area should encourage more research.

Interrelationship Between the Thyroid and Adrenal Cortex

There is evidence, although not a great deal, of a relationship between the functions of the thyroid and adrenal glands. The role of the glucocorticoids in the regulation of thyroid function was studied by Nicoloff et al. (1970). They found that the corticosteroids are capable of severely reducing thyroid function by inhibiting TRH. The work of Otsuki et al. (1973) confirms these results. They also found that corticosteroids can also inhibit TSH at the pituitary level. This reasoning stems from the fact that patients with Cushing's disease receiving glucocorticoid treatment and given exogenous TRH showed little or no rise in TSH concentrations. Kemppainen et al. (1983) and Woltz et al. (1984) both showed a reduction in levels of thyroxine and triiodothyronine after multiple injections of prednisone, a synthetic corticosteroid, in the dog. Pathological examination of the thyroid gland revealed an accumulation of cytoplasmic colloidal droplets within the follicles. They suggest that lysosomal hydrolysis of this colloid is inhibited by the prednisone and in turn reduces the concentrations of the thyroid hormones.

The effects of reduced thyroid hormones on adrenal function has been investigated by Fortier et al. (1970). They found that thyroidectomized rats showed a depression in the release and synthesis of ACTH. A reduction in the circulating levels of ACTH in turn leads to a decrease in corticosterone concentrations and in adrenal weight. These results have also been confirmed by Murakami et al. (1984),

Ottenweller and Hedge (1981), Meier (1976) and Martin et al. (1963). Shapiro and Leathem (1971) suggest that the high incidence of hypothyroidism in aged rats is associated with adrenal changes.

Cortisol Circadian Rhythm

Biological rhythms exist in all forms of life, from the simplest unicellular organism to the largest multicellular animals. These rhythms have been linked to the four geophysical cycles: the tides, the day-night cycle, the lunar cycle and the seasons. Development of these biological rhythms means an adaptive process in which organisms have an inborn ability to incorporate these geophysical cycles into a self-oscillating endogenous rhythm. The duration of these rhythms closely match the duration of the particular geophysical cycle to which it corresponds. Franz Halberg, of the University of Minnesota, coined the term "circadian" (from the Latin circa, about and dies, day) to describe the 24-hr day-night cycle. Similarly, there are circatidal, circalunar and circannual rhythms (Aschoff, 1980).

The presence of endogenous rhythms has become very evident among the secretion of hormones, especially the circadian periodicity of the adrenal corticosteroids. The circadian rhythm of cortisol is a well documented phenomenon in man. It typically shows a zenith during the day and a nadir during the night. Faiman and Winter (1971) found cortisol concentrations in man to be highest at 0800 hours and lowest at 0400 hours during a 24-hr period. Similar 24-hr profiles in humans have been shown by Orth et al. (1967) and Arendt et al. (1982).

Circadian rhythms have also been investigated in other animals. Monkeys show a 24-hr cortisol rhythm that is much the same as the human cortisol rhythm (Spies et al., 1979). Circadian variation of cortisol has also been observed in bulls, cats, and ewes (Thun et al., 1981; Krieger and Rizzo, 1969; Fulkerson and Tang, 1979). In contrast, to our knowledge, no one has ever been able to show a cortisol circadian rhythm in the dog. All the papers that have investigated circadian periodicity in the dog have turned up negative results (Breznock and McQueen, 1969; Johnston and Mather, 1978; Takahashi et al., 1981; Kemppainen and Sartin, 1984). In nocternal animals, such as the rat, the circadian rhythm of corticosterone is as well defined as it is in humans. However, the circadian periodicity of corticosterone in the rat is opposite that of man. D'Agostino et al. (1982), reported a zenith in levels of corticosterone between 1700 and 0100 hours and a nadir at 0500 hours. For this reason, care should be exercised when extrapolating rat data to humans.

The secretion of cortisol during a 24-hr period is not a nice smooth circadian profile, as most studies would have you believe, but rather, the secretion of cortisol is an episodic event. Wietzman et al. (1971) showed this episodic secretion in man. Furthermore, they stipulate that the frequency and duration of cortisol secretory episodes, at different times during the 24-hr period, are related to the actual rhythmicity of cortisol. High levels of cortisol are characterized by an increase in frequency and duration of episodes, while the opposite is true for low levels of cortisol. These results

are supported by Gallagher et al. (1973) and Jacoby et al. (1974). Other investigators have also found the secretion of cortisol to be episodic while also displaying a typical circadian rhythm. However, they did not report a relationship between episodic secretion and circadian rhythmicity (Quabbe et al., 1982; Thun et al., 1981; Fulkerson and Tang, 1979). In a study by Kemppainen and Sartin (1984), evidence for an episodic, but not circadian activity in plasma cortisol was reported in the dog.

Gallagher and Associates (1973) have shown that ACTH fluctuates with the same rhythmicity as cortisol. Since the secretion of ACTH is also episodic, they feel that there is central nervous system control of ACTH (and therefore cortisol) in a reasonably constant pattern under steady state conditions. The work of Graber et al. (1965) has shown a persistence of plasma ACTH circadian rhythm even in patients with Addison's disease. Plasma levels of ACTH were increased as expected, suggesting that there is a negative feedback mechanism at work. But levels of ACTH at six AM were consistently greater than levels of ACTH at six PM. From this data, Graber et al. (1965) believe that the persisting influence of an ACTH circadian rhythm is superimposed on the low ACTH concentrations. Work done by Glass et al. (1984) has shown that cortisol circadian rhythms can exist in patients with Cushing's disease. Their results have some interesting implications. First of all, the ACTH circadian rhythm seems to be generated by the hypothalamus, even in Cushing's disorder. Secondly, the persistence of cortisol circadian periodicity, in patients with

Cushing's disease, suggests that there may be two physiologically, and perhaps anatomically separate regulatory sites for the release of ACTH. This assumption was made since the levels, but not the circadian activity of cortisol were altered. Nicholson et al. (1985) performed a study in which the hypothalamo-pituitary-adrenocortical axis of the rat was studied and found that the circadian variations of CRF, ACTH and corticosterone exhibited similar fluctuatons during a 24-hr period. Therefore, it seems that the circadian cyclicity is initiated in the hypothalamus. In turn, the responsiveness of the pituitary gland to CRF and the adrenal cortex to ACTH are what determine the amplitudes of the peaks and troughs observed in the corticosterone rhythm.

Krieger and Rizzo (1969) have found evidence in the cat that serotonin is the neurotransmitter involved in the mediation of 17-hydroxycorticosteroid circadian cyclicity. Injections of reserpine, which deplete the brain catecholamines and serotonin, failed to obliterate the circadian rhythm of cortisol. In contrast, injections of cinanserin, a competitive inhibitor of serotonin, was able to block the circadian rhythm of cortisol. These results have been confirmed in the rat by Scapagnini et al. (1971). Moore and Eichler (1972) report the loss of circadian corticosterone rhythm in the rat following suprachiasmatic lesioning. The suprachiasmatic nuclei are very concentrated in serotonergic terminals and are believed to be the biological time clock regulating most of the circadian rhythms in the rat (Moore, 1978). In contrast to

serotonergic stimulation, Van Loon et al. (1971) have found evidence for adrenergic neural inhibition of ACTH in the rat. This suggests that injections of norepinephrine or dopamine would inhibit the circadian cyclicity of corticosterone. Similar results have also been reported by Scapagnini et al. (1972).

Probably the two most potent external factors that effect corticosteroid circadian rhythms are sleep and light. The effects of sleep on normal corticosteroid circadian rhythms have been shown by many investigators. Weitzman et al. (1983) demonstrated that the behavioral complex of sleep inhibits cortisol secretion in man. They found that if sleep had occurred during the time of day when subjects were normally awake, cortisol concentrations would be significantly decreased. Similar results have been found by Scheving and Pauly (1966) on the effects of light on plasma corticosterone rhythm in the rat. During the light hours, corticosterone concentrations are usually high and, during the dark hours, corticosterone concentrations are low (keep in mind that the rat is a nocturnal animal). When lighting conditions were reversed from light-dark to dark-light, the relative concentrations of corticosterone were also reversed.

The thyroid hormones have also been shown to affect steroid circadian rhythms in man. Martin et al. (1963) provides evidence that spontaneous hyperthyroidism or injections of triiodothyronine decrease the levels of free plasma 17-hydroxycorticosteroids and exaggerate the circadian rhythm of conjugated 17-hydroxycorticosteroids. The opposite was true when low levels of thyroid hormones were present.

Murakami et al. (1984) have found that the thyroid hormones are essential in order to maintain circadian rhythmicity in the rat. They suggest that the thyroid hormones have the ability to synthesize and release ACTH. Other papers confirming the need of thyroid hormones for normal corticosteroid circadian periodicity have been reported by Ottenweller and Hedge (1981) and Meier (1976).

Effects of Aging on Cortisol Circadian Rhythm

The effects of aging on cortisol circadian rhythm have not been investigated in any detail. Touito et al. (1982 and 1983) have recently performed studies in young men, elderly men and women and demented elderly men and women in order to determine the effects of aging on circadian rhythms of total and free cortisol. The results of these experiments failed to show any differences in either total or free cortisol circadian rhythms with aging. Serio et al. (1970) found a circadian rhythm in both young and old humans. According to the cosinor analysis method, which they used to analyze the 24-hr cortisol profile, the only difference they were able to detect was a slight shift in the rhythm, of older persons. The timing of the rhythm in older persons seems to occur later than in young adults. Blichert-Toft (1978), found no major disturbances in the circadian rhythm of aged people as compared to young adults. He also warns that abnormal circadian rhythms in the elderly may be more frequent due to disorders often present in the aged, such as insomnia, psychic distress and cardiac insufficiency. Grad et al. (1971) have also

found a higher frequency of abnormal cortisol circadian rhythm in elderly subjects with cardiac insufficiency.

MATERIALS AND METHODS

Animals

Female beagle dogs of three age groups of five each were used: puppies, young adults and old. The average (±Std. Err.) ages of the three age groups are 7.6±0.1 wks., 3.0±0.2 yrs. and 11.4±0.1 yrs. respectively. The mean (±Std. Err.) body weights are as follows: 2.4± 0.2 kg for the puppies, 10.7±0.6 kg for the young adults and 9.4±0.4 kg for the old. All dogs were housed individually in aluminum cages in a controlled environment (lights on 0700-1900 hrs; temperature 22±2^{0} C). Purina Puppy Chow was fed to the puppies and Kansas State University Dog Chow to the other dogs. All dogs were fed once daily at 1100 hrs and tap water was given ad libitum. At the time of experimentation, all young adult and old dogs were in anestrus.

Blood Collecting

Blood samples (3 ml) were collected from the cephalic vein in young adult and old dogs and from the jugular vein in the puppies. For blood collection, a three cc tuberculin syringe and a 22 gauge hypodermic needle were used. For serum extraction in experiment one, the blood was dispensed into a 12 x 75 mm test tube and allowed to clot in the refrigerator for 24 hrs. After this, the blood was centrifuged and the serum removed with a Pasteur pippette and transferred to another 12 x 75 mm test tube. The serum was stored at -20^{0}C until the time of assay. For plasma extraction in experiment

two, the blood was dispensed into a 12 x 75 mm test tube containing heparinized saline (5 IU/50 ul saline/1 ml blood). The blood was immediately centrifuged in a small clinical centrifuge for five minutes. The plasma was removed with a Pasteur pippette and transferred to another 12 x 75 mm test tube and stored at -20°C until the time of assay.

Experiment One: Effects of Aging on 24-Hr Profiles of Serum Cortisol in the Dog

The aims of this experiment were two fold: 1) to determine if there is a 24-hr rhythm in serum cortisol in the dog and 2) to determine how this periodicity is affected by aging. For this purpose, blood samples were collected during three 24-hr periods. On day one, blood collection began at 1200 hours and continued at three-hr intervals for the next 24 hrs; on day two, blood collection began at 1300 hours and continued at three-hr intervals for the next 24 hrs; and on day three, blood collection began at 1400 hrs and continued at three-hr intervals for the next 24 hrs. With this design we were able to obtain a complete 24-hr profile of serum cortisol without subjecting the animals to the stress of excessive blood collection. The dogs were allowed to rest for one day between any two 24-hr bleeding periods. Lights were turned on for a brief time (10 min) for blood collection during the dark hrs. As an additional precaution, hematocrit values were checked periodically.

Experiment Two: Effects of Aging on Plasma Thyroxine and Cortisol Responses to Low Ambient Temperatures in the Dog

The purpose of this experiment was to determine the effects of aging on plasma thyroxine and cortisol responses to low ambient temperatures in the dog. Before using the animals in this experiment, they were made familiar with the bleeding procedures and the environmental chamber for several days. Two dogs at a time were used during each exposure to various temperatures in the chamber. The animals were provided with food and water ad libitum while in the chamber. The dogs were exposed to four temperatures in the following order: $22^{o}C$, $10^{o}C$, $4^{o}C$ and $-5^{o}C$. They were allowed to rest for several days between any two exposures. Blood samples were collected at 0.0, 0.5, 1.0, 2.0, 3.5 and 5.0 hrs during exposures to various ambient temperatures. At the end of the five-hr exposure period. The temperature in the environmental chamber was elevated to $22^{o}C$ and blood samples were collected at 1.0 and 3.0 hours after the termination of exposure. Hematocrit values were checked frequently.

Radioimmunoassay

Total plasma thyroxine concentrations were determined with the use of solid phase radioimmunoassay reagents obtained from Abbott Laboratories. This assay has a sensitivity of 0.36 ug/dl and average ($\pm$SD) interassay and intraassay variabilities of $6.9\pm2.1\%$ and $4.9\pm1.0\%$ respectively. For cortisol radioimmunoassay, label was obtained from New England Nuclear (Boston, MA) and antisera from Radioassay Systems

Laboratories Inc. (Carson, CA). This assay has a sensitivity of <10 pg and average (±SD) interassay and intraassay variabilities of 4.3± 1.9% and 3.1±0.9% respectively. Methodological details of this assay are similar to those of other steroid assays (Quadri et al., 1979).

Calculations and Statistical Analysis

Cortisol concentrations (ng/ml) were determined using a computer program developed in our laboratory for the PDP 11/34. Thyroxine concentrations (ug/dl) were determined using a standard curve after logit-log transformation.

In the first experiment, to determine the existence of circadian periodicity in serum cortisol, a non-linear regression, utilizing the cosine curve as a model, was fit to all the individual dogs using the data obtained from the three 24-hr profiles. Similarly, cosine curves were fit to the young adult and old dogs as a group. F-tests were performed to determine the relative fit for the two age groups. Student's t-tests were employed to analyze differences in serum cortisol concentrations during the three 24-hr periods. Significance is indicated by $P < 0.05$.

In the second experiment, differences in basal levels of plasma thyroxine and cortisol in the three age groups were analyzed using Student's t-test. Plasma thyroxine and cortisol responses to the four ambient temperatures within each age group were also analyzed using Student's t-test. In order to analyze differences between age groups at the various ambient temperatures, percent changes in plasma

concentrations of thyroxine and cortisol were used. Comparisons among the three age groups were made between corresponding percent change values utilizing Student's t-test. Significance is indicated by $P < 0.05$.

RESULTS

Experiment One: Effects of Aging on 24-Hr Profiles of Serum Cortisol in the Dog

24-Hr Serum Cortisol Profiles in the Young Adult Dogs

The absolute values of 24-hr serum cortisol concentrations in the young adult dogs can be found in appendix A (Table 1). In the young adult dogs, 24-hr serum cortisol profiles with superimposed 24-hr period cosine curves are shown in Figure 1. From the cosine curves, the highest levels of serum cortisol ranged from 0600 to 1300 hrs and the lowest levels of serum cortisol ranged from 1800 to 2400 hrs. The hypothesis that a single cosine curve would fit all the young adult dogs as a group just missed being true ($P < 0.06$). However, when t-tests were performed, the levels of at the nadir were significantly lower ($P < 0.05$) than levels at the zenith. From the average cosine curve (Figure 3), the zenith serum cortisol concentration was 8.4 ng/ml and occurred at 1000 hrs and the nadir serum cortisol concentration was 5.6 ng/ml and occurred at 2200 hrs. The mean ($\pm$Std. Err.) 24-hr period serum cortisol concentration was 7.1$\pm$0.25 ng/ml.

24-Hr Serum Cortisol Profiles in the Old Dogs

The absolute values of 24-hr serum cortisol concentrations in the old dogs can be found in appendix A (Table 2). In the old dogs, 24-hr serum cortisol profiles with superimposed 24-hr period cosine curves

are shown in Figure 2. From the cosine curves, the highest levels of serum cortisol ranged from 1200 to 1900 hrs and the lowest levels of serum cortisol ranged from 2400 to 0700 hrs. The hypothesis that a single cosine curve would fit all the old dogs as a group was rejected and t-tests did not reveal any differences in the fluctuations of serum cortisol during the 24-hr period. From the average cosine curve (Figure 3), the zenith serum cortisol concentration was 11.4 ng/ml and occurred at 1700 hrs and the nadir serum cortisol concentration was 8.8 ng/ml and occurred at 0500 hrs. The mean (±Std. Err.) 24-hr period serum cortisol concentration was 10.1±0.54 ng/ml.

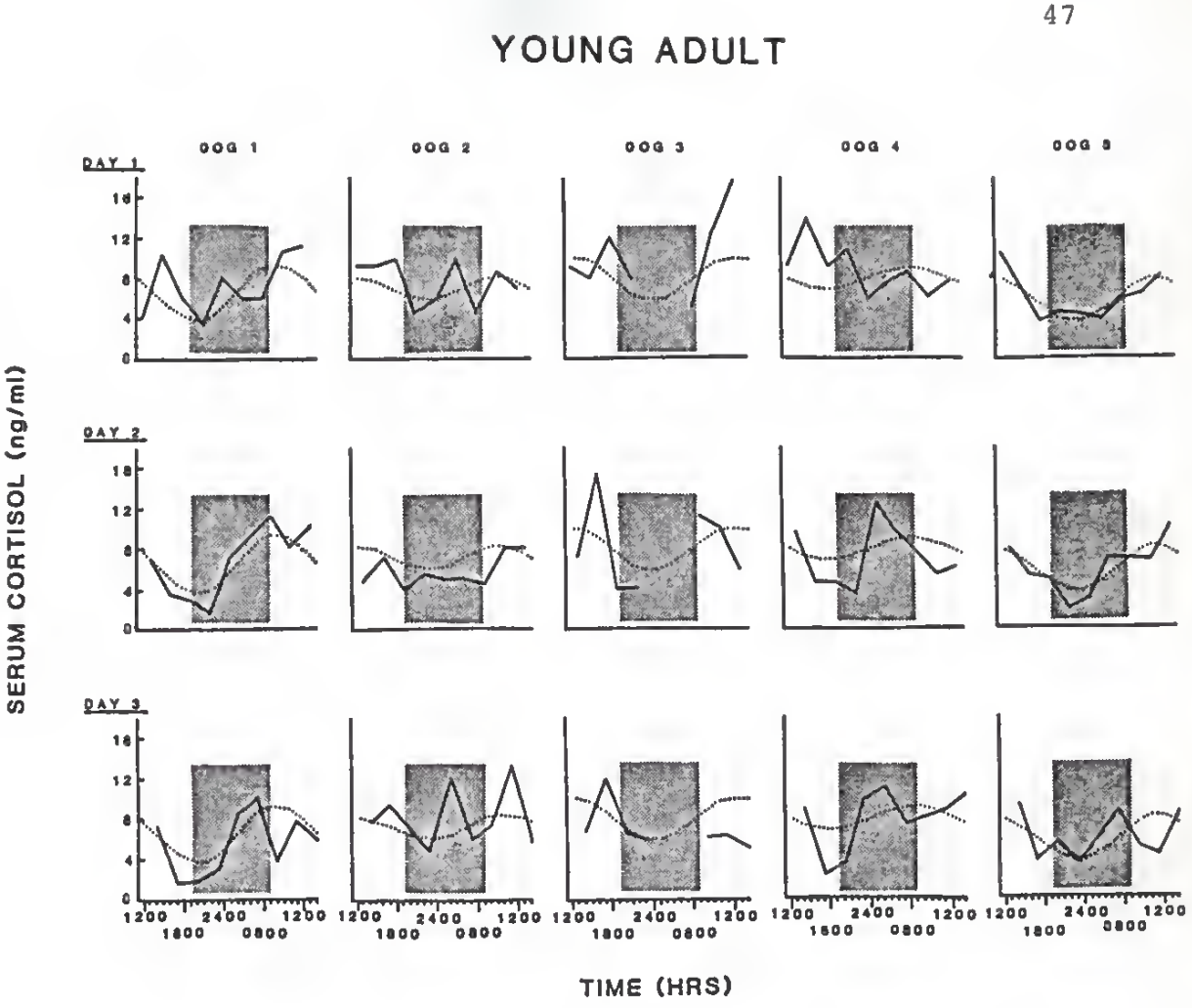

Fig. 1 Twenty-four-hour serum cortisol profiles in young adult dogs. Serum cortisol concentrations (solid line) were determined at three-hr intervals during three 24-hr periods (day 1, 2 and 3). The dotted line represents the 24-hr period cosine curve calculated for each dog using the cortisol concentrations for the three days. The shaded area indicates the time interval during which the lights were turned off.

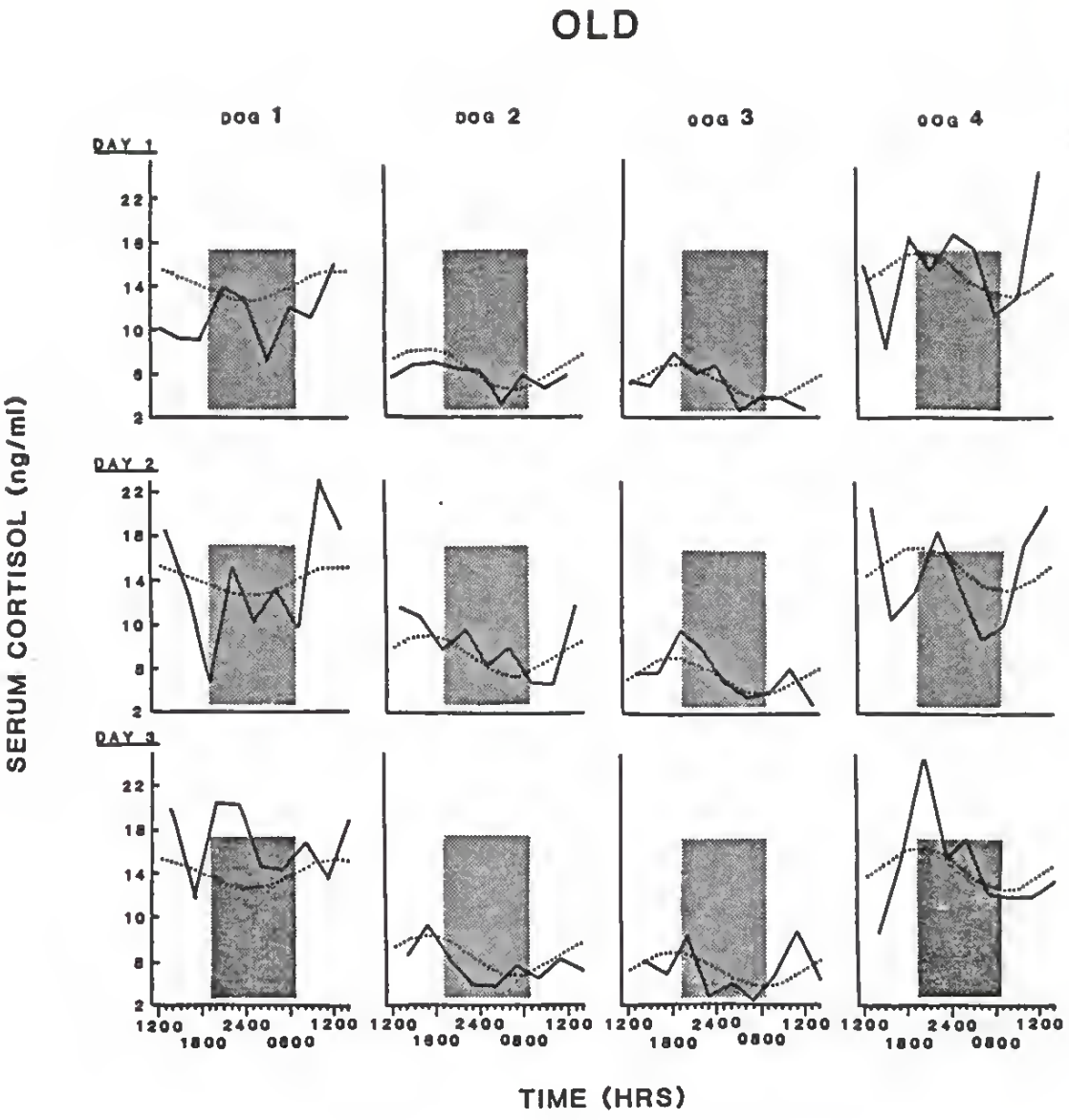

Fig. 2 Twenty-four-hour serum cortisol profiles in old dogs. Serum cortisol concentrations (solid line) were determined at three-hr intervals during three 24-hr periods (day 1, 2 and 3). The dotted line represents the 24-hr period cosine curve calculated for each dog using the cortisol concentrations for the three days. The shaded area indicates the time interval during which the lights were turned off.

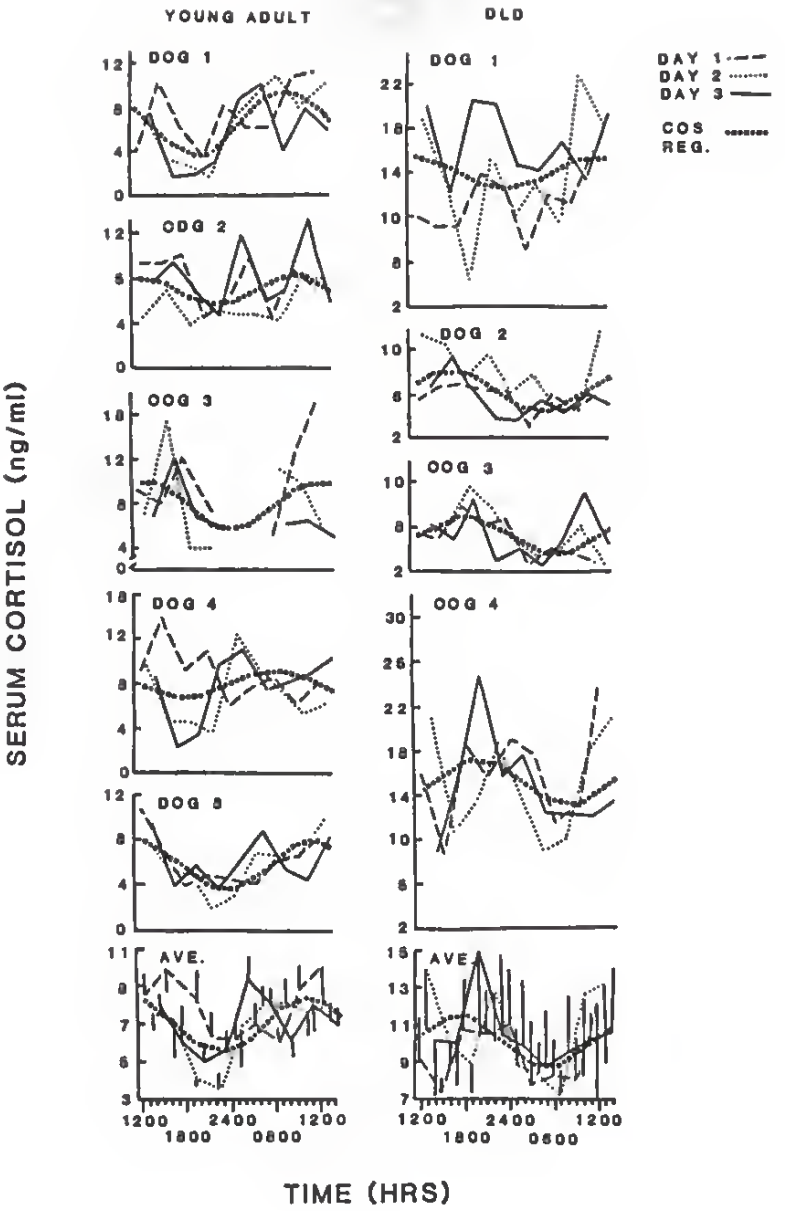

Fig. 3 Comparison of 24-hr serum cortisol profiles in young adult and old dogs. Each panel contains three 24-hr cortisol profiles and the calculated cosine curve (heavy dotted line) in one dog. The two panels at the bottom contain the average cortisol profiles for each of the three days and the cosine curve for the young adult (left) and old dogs (right). In the young adult dogs, the cosine curve fit well and the serum cortisol profile showed statistically significant fluctuations during the 24-hr period. By contrast, in the old dogs the cosine curve did not fit well and there were no significant differences in serum cortisol concentrations during the 24-hr period.

Experiment Two: Effects of Aging on Plasma Thyroxine and Cortisol Responses to Low Ambient Temperatures in the Dog

Thyroxine

Effects of Aging on Basal Plasma Thyroxine Concentrations

The basal plasma thyroxine concentrations at 0.0 hrs in the three age groups can be found in appendix B (Tables 3, 5, 7 and 9). The mean (±Std. Err.) plasma thyroxine concentrations in the three age groups are shown in Figure 4. Plasma thyroxine basal levels in the puppies, young adult and old dogs were 4.4±0.2, 4.2±0.2, and 2.8±0.1 ug/dl respectively. Thyroxine levels in the old dogs were significantly lower ($P < 0.001$) than those in the puppies and young adult dogs. There were no significant differences between thyroxine levels in the puppies and young adult dogs.

Effects of Low Ambient Temperatures on Plasma Thyroxine in the Three Age Groups

Plasma thyroxine concentrations in the three age groups during exposures to the various ambient temperatures can be found in appendix B (Tables 3, 5, 7 and 9). The percent changes in plasma thyroxine concentrations during these exposures are also found in appendix B (Tables 4, 6, 8 and 10) and are graphed in Figure 5. No changes in plasma thyroxine concentrations were observed in the three age groups

during exposure to 22^{0}C (control). Exposures to 10^{0}C and 4^{0}C also showed no significant differences in plasma thyroxine concentrations in the three age groups. In the young adult dogs, exposure to -5^{0}C produced significant elevations ($P < 0.005$) in plasma thyroxine concentrations of 116.4% at 3.5 hrs and 130.8% at 5.0 hrs. These levels remained high even after exposure to -5^{0}C was terminated. In the old dogs, exposure to -5^{0}C produced a 70.7% increase ($P < 0.05$) in plasma thyroxine concentrations at 3.5 hrs during exposure. However, levels of plasma thyroxine returned to control levels by five hrs exposure. In sharp contrast, the puppies produced no significant responses to the exposure of -5^{0}C.

Cortisol

Effects of Aging on Basal Plasma Cortisol Concentrations

The basal plasma cortisol concentrations at 0.0 hrs in the three age groups can be found in appendix B (Tables 11, 13, 15 and 17). The mean (±Std. Err.) plasma cortisol concentrations in the three age groups are shown in Figure 6. Plasma cortisol basal levels in the puppies, young adult and old dogs were 7.2±1.1, 14.4±2.4 and 21.1±3.1 ng/ml respectively. Cortisol levels in the puppies were significantly lower ($p < 0.005$) than those in the young adult and old dogs. Even though cortisol levels in the young adult dogs were lower than those in the old dogs, this difference did not reach significance.

Effects of Low Ambient Temperatures on Plasma Cortisol Responses in the Three Age Groups

Plasma cortisol concentrations in the three age groups during exposure to the various ambient temperatures can be found in appendix B (Tables 11, 13, 15 and 17). The percent changes in plasma cortisol concentrations during these exposures are also found in appendix B (Tables 12, 14, 16 and 18) and are graphed in Figure 7. No changes in plasma cortisol concentrations were observed in the three age groups during exposure to 22°C (control). Exposures to 10°C and 4°C also showed no significant differences in plasma cortisol concentrations in the three age groups. In the young adult dogs, exposure to -5°C produced significant elevations ($P < 0.05$) in plasma thyroxine concentrations of 186.5% at 0.5 hrs, 278.6% at 1.0 hours, 194.8% at 2.0 hrs, 158.3% at 3.5 hrs and 148.7% at 5.0 hrs. These levels returned to control levels within one hr after the termination of exposure to -5°C. In contrast, both the puppies and old dogs failed to show a significant response to -5°C exposure.

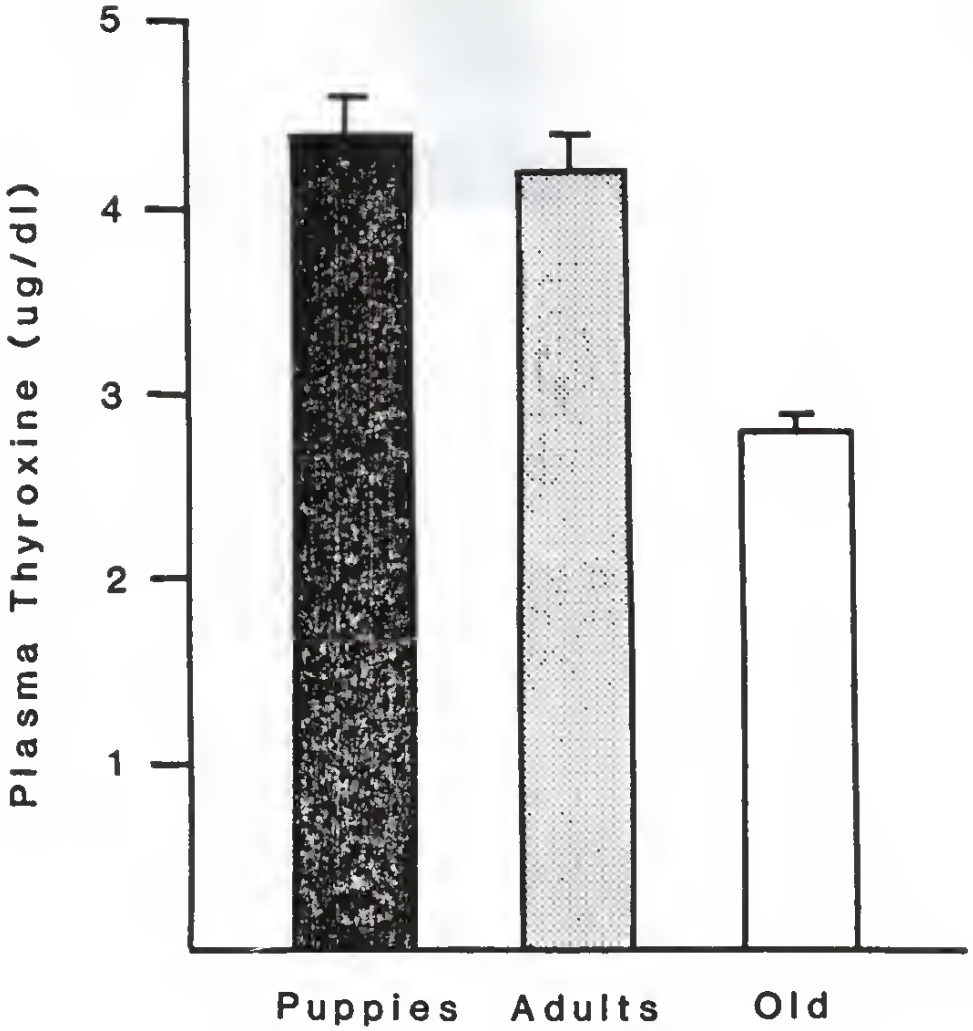

Fig. 4 Effects of aging on plasma thyroxine in the female beagle dog. Plasma thyroxine levels in the old dogs were significantly lower ($P < 0.001$) than those in the young adult and puppies. There were no significant differences in plasma thyroxine levels of the puppies and the young adults.

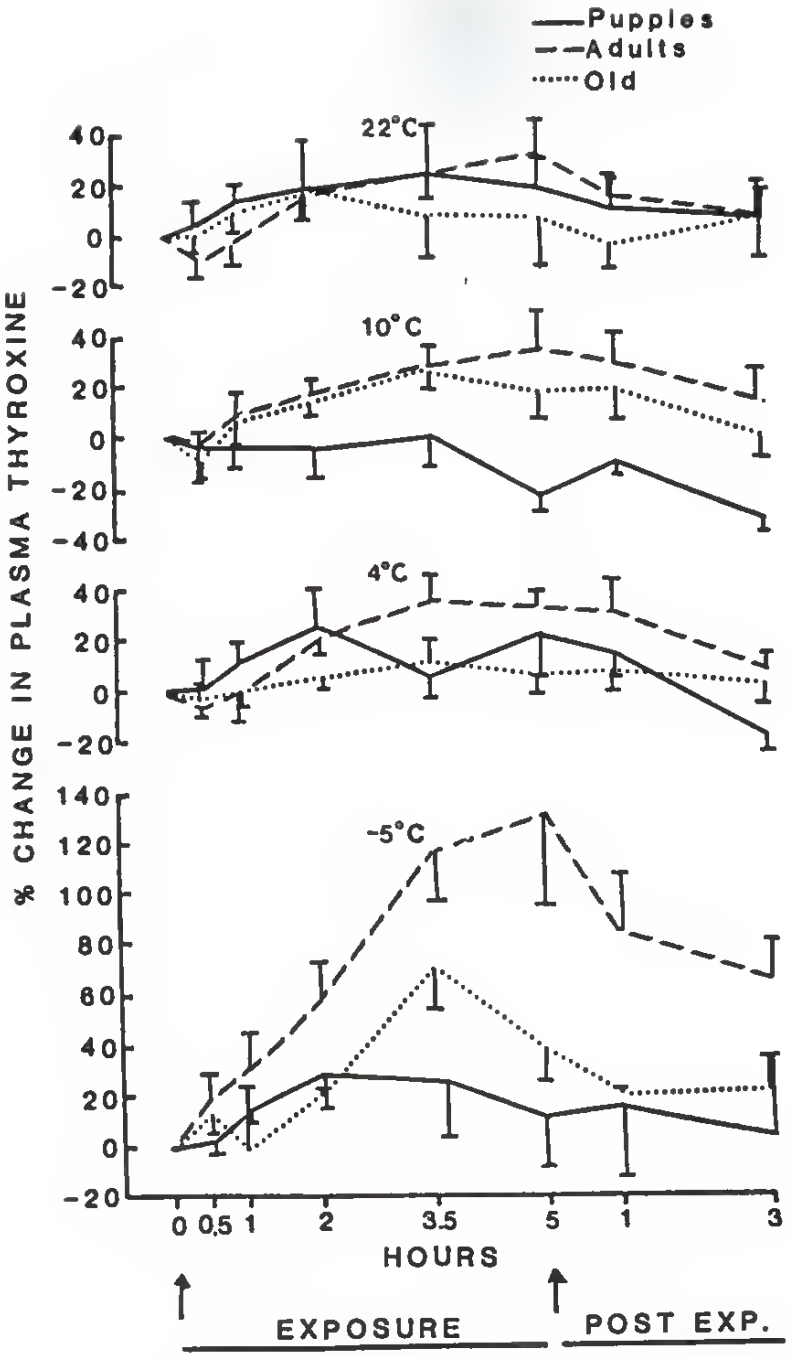

Fig. 5 Effects of exposures to 22° (control), 10°, 4° and -5°C on plasma thyroxine in the puppies, young adult and old dogs in terms of per cent change. The only significant changes in plasma thyroxine occurred after exposures to -5°C between 2 to 5 hrs in the young adult ($P < 0.05$) and at 3.5 hrs in the old dogs ($P < 0.05$).

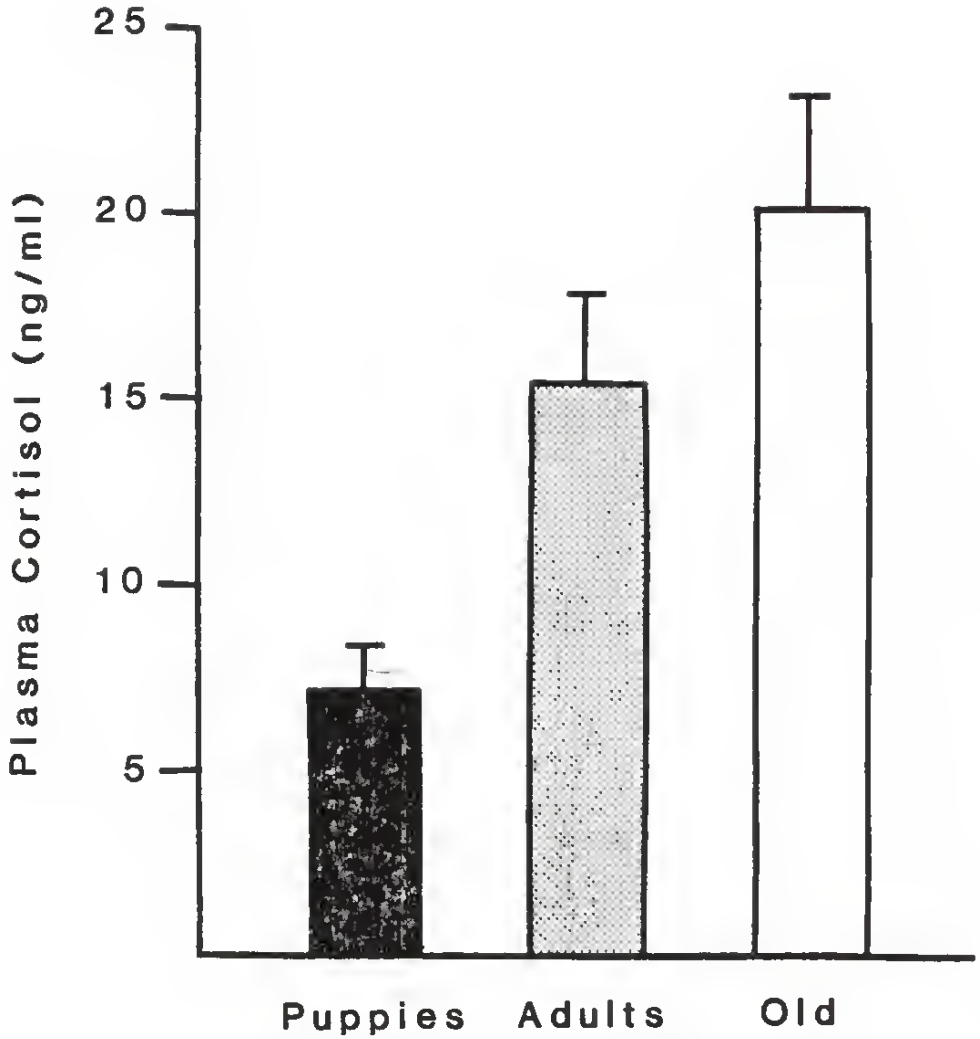

Fig. 6 Effects of aging on plasma cortisol in the female beagle dog. Plasma cortisol levels in the puppies were lower than those in the young adult ($P < 0.005$) and old dogs ($P < 0.005$). Cortisol levels in the old dogs were higher than the young adult dogs, but the difference did not reach significance.

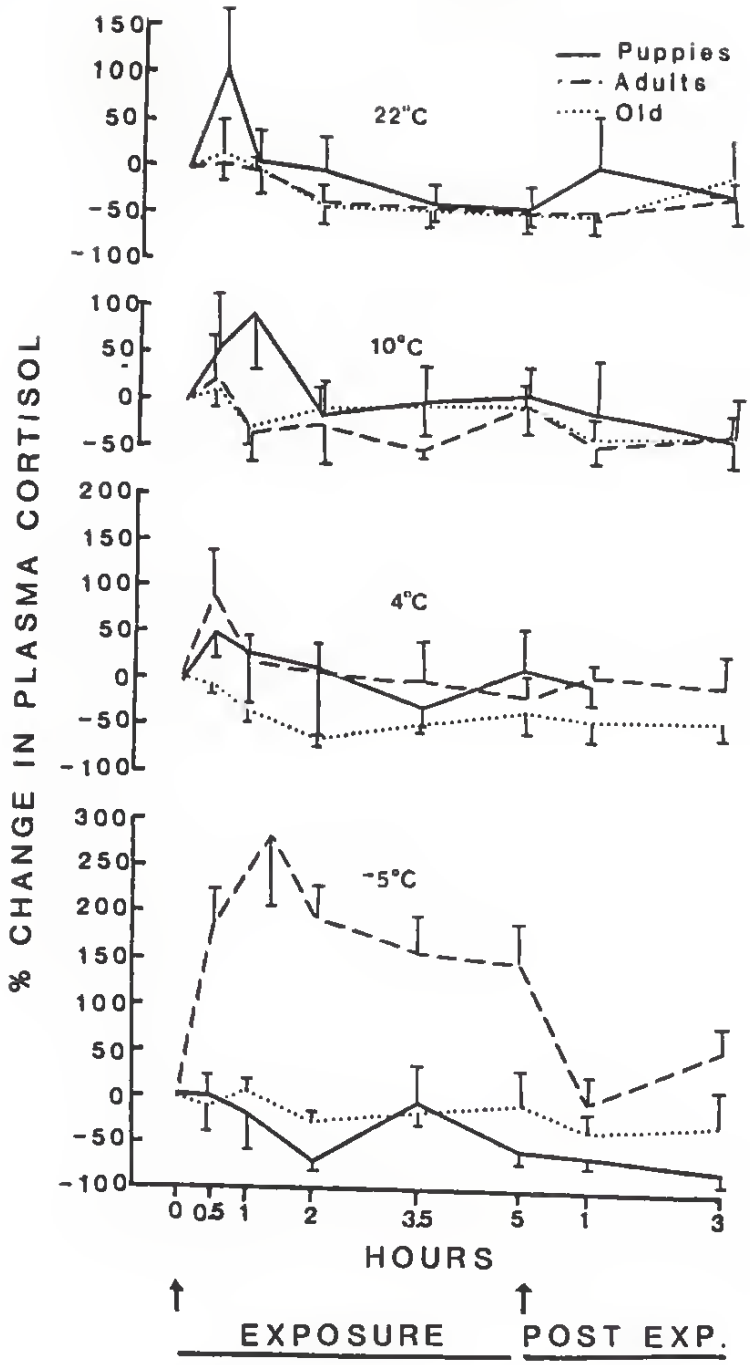

Fig. 7 Effects of exposures to 22° (control), 10°, 4° and -5°C on plasma cortisol in the puppies, young adult and old dogs in terms of per cent change. Exposure to none of these temperatures had any effect on cortisol levels in the puppies and old dogs. In the young adults, exposure to -5°C produced a significant increase ($P < 0.05$) which was sustained for the duration of the exposure.

DISCUSSION

Effects of Aging on Cortisol Circadian Rhythm

Our results show that there is a definite circadian periodicity in serum cortisol levels in the dog and that this rhythm is dampened in old age. Investigations of the 24-hr cortisol profiles in dogs (Breznock and McQueen, 1969; Johnston and Mather, 1978; Takahashi et al., 1981; Kemppainen and Sartin, 1984) have failed to show circadian periodicity. Such negative results are probably attributable to the fact that these previous studies used mongrel dogs of various ages and sex. Because of such differences in the experimental subjects, large variabilities in the data are created. Johnston and Mather (1978) show a 24-hr cortisol profile in dogs that resembles a circadian rhythm. However, they were unable to find statistical significance in fluctuations of 24-hr cortisol levels because of the large standard errors.

In contrast, the present results demonstrate the presence of a circadian rhythm in serum cortisol levels in the young adult dogs. The average cosine curve showed a peak serum cortisol concentration at 1000 hrs and a nadir serum cortisol concentration at 2200 hrs. The differences between the peak and the nadir cortisol levels were found to be statistically significant, confirming the results obtained in humans, bulls, cats and sheep (Faiman and Winter, 1971; Thun et al., 1981; Krieger and Rizzo, 1969; Fulkerson and Tang, 1979).

Two recent studies in man have failed to find any age-related

effects on the circadian rhythmicity of cortisol (Touito et al., 1982 and 1983). By contrast, in our studies we were not able to detect a circadian rhythm of cortisol levels in old dogs. A likely explanation for the absence of circadian periodicity in cortisol is the age-related decrease in plasma thyroxine levels (as is shown in the results of experiment 2). It appears that thyroid hormones are necessary for the synthesis and hence secretion of ACTH (Fortier et al. 1970). In addition, Murakami et al. (1984) have reported that thyroid hormones are essential in order to maintain circadian periodicity in the rat. Martin et al. (1963) reported the same results in humans. Thus, the decreased basal thyroxine levels found in the old dogs are probably responsible for the lack of a significant cortisol rhythm. To our knowledge, this is the first demonstration of a significant effect of aging on cortisol rhythm in any species.

Effects of Aging on Thyroxine and Cortisol Responses to Low Ambient Temperatures

Our results demonstrate that with aging there is a significant decrease in basal plasma thyroxine levels and a significant elevation in basal plasma cortisol levels in the dog. Our results also show that aging reduces responses of these hormones to the stress of cold. These results are in agreement with Herrmann et al. (1974) and Hesch et al. (1976) in man and Eleftheriou (1975) and Pekary et al. (1983) in rodents. Yet, other investigators have reported contrasting results. Some indicate an age-dependant increase (Britton et al.,

1975) in circulating levels of thyroxine. These inconsistencies could be attributed to inadequate control of variables which effect thyroxine secretion (Olsen et al., 1978). In our study, the dogs were carefully matched within an age group and screened for illnesses.

A tendency toward hypothyroidism has been reported to occur in aged humans (Sawin et al., 1979). Yet, a clear explanation as to why there should be a decrease in circulating levels of thyroxine is still in dispute. It has been speculated that degenerative processes in aging thyroid glands (Ingbar, 1978) could cause a decrease in the secretion rate of thyroxine (Chen and Walfish, 1978). Another study found that aging decreases absolute iodine uptake by the thyroid (Hansen et al., 1975), thus, providing further support for an age-related decrease in levels of circulating thyroxine.

Our results demonstrate that in addition to age-related differences in thyroxine, differences in thyroxine responses to low ambient temperatures exist among the three age groups. Exposure to -5^{0} C produced greater and longer responses in the young adult than in the old dogs. These results agree with the results of Huang et al. (1980) in rats. This reduced thyroid function in old dogs may cause the decreased levels of circulating thyroxine. From these results, it is apparent that young adult dogs are better able to cope with the stress of cold than old dogs, especially since the old dogs were observed to be shivering throughout the cold exposure. In contrast, the puppies did not respond to cold exposure of -5^{0}C, even though thyroxine levels in the puppies are comparable to those found in young adult dogs. One

plausible explanation for this occurrence is the fact that newborn animals have higher BMRs than adult animals. In response to cold, an increase in BMR is dependant on the presence of thyroxine (Sellers et al., 1974) to ensure the survival of the animal. Since puppies have a higher BMR than adult dogs, a need for elevated thyroxine concentrations during exposures to $-5^{o}C$ may not be necessary. Another possibility is that the puppies are not capable of responding to cold stress as well as the young adult dogs.

In this experiment, TSH responses to cold were not determined. However, Huang et al. (1980) and Gambert and Barboriak (1982) found no indication of age-related changes in TSH response to cold in the rat, suggesting comparable capabilities of the pituitary to respond to cold in both young and old rats. Furthermore, Pekary et al. (1983) found the TSH response to TRH to be similar for both young adult and old rats. On the basis of these results, it appears that there is a decrease in the thyroid sensitivity to TSH with increasing age.

To our knowledge, an age-related increase in basal levels of circulating cortisol has never been shown before in the dog. Most investigators indicate that cortisol concentrations remain stable throughout the life of the animal. This has been shown by Breznock and McQueen (1969) in dogs, Riegle and Nellor (1967) in cattle, Riegle et al. (1968) in goats, Hess and Riegle (1972) in rats and Saruta et al. (1980) in man. On the other hand, Dekosky et al. (1984) and Sapolsky et al. (1983) found an age-related increase in corticosterone concentrations of the rat, which support our results in the dog. Tang

and Phillips (1978) reported no change in basal levels of corticosterone in the rat, yet a close examination of their data indicates a 100% increase in the old rats as compared to the young rats. Sapolsky et al. (1983) have emphasized the need to use more than two age groups for investigation of aging effects on hormone levels. We agree with their opinion. Lewis and Wexler (1974) looked at levels of serum corticosterone in rats of three age groups. They were also able to find an age-dependant increase in levels of corticosterone, which they associated with pathological changes in the aging adrenal glands.

In our study, exposure to -5 C produced significant elevations in plasma cortisol levels in the young adult dogs. Cortisol levels remained elevated throughout the exposure period. Similar results in the dog were obtained by Egdahl and Richards (1956) and Chowers et al. (1964). In sharp contrast to the young adult dogs, exposure to $-5^{o}C$ did not produce any response in either the puppies or old dogs. The lack of adrenocortical responsiveness in old dogs can be attributed to a diminished sensitivity of the hypothalamo-pituitary-adrenocortical axis to cold. A clear explanation as to why puppies show no response to $-5^{o}C$ cold exposure is not available. Possibly, the hypothalamo-pituitary-adrenocortical axis has not developed efficiently enough to show a response in the puppies. Another possibility may be that the puppies have an alternate mechanism to respond to cold. Therefore, elevated cortisol levels may not be necessary since the puppies did not appear to be stressed during any

of the exposures to low ambient temperatures.

SUMMARY AND CONCLUSIONS

Young adult dogs show a definite circadian rhythm in serum cortisol concentrations. In the old dogs, a clear and statistically significant circadian rhythm in serum cortisol does not exist.

Our results also demonstrate that there is an age-related decrease in basal plasma levels of thyroxine and an increase in basal plasma levels of cortisol. In the puppies, exposures to 22^{0} (control), 10^{0}, 4^{0} and -5^{0}C produced no change in plasma concentrations of thyroxine and cortisol. In the adult dogs, exposures to 22^{0}, 10^{0} and 4^{0}C had no effect, but exposure to -5^{0}C produced significant elevations in both plasma thyroxine and cortisol levels. In the old dogs, a small but significant elevation in thyroxine, but not cortisol was observed during exposure to -5^{0}C. Exposure to other temperatures above -5^{0}C had no effect on plasma levels of these hormones.

From these results, it can be concluded that aging changes basal levels of thyroxine and cortisol in the dog. The age-related decrease in levels of thyroxine may be partially responsible for the deterioration of the cortisol circadian rhythm seen in old dogs. In addition, aging alters thyroxine and cortisol responses to low ambient temperatures in the dog. The thyroxine response to cold is reduced with aging. The primary cause for this lack of response may be a decrease in thyroid sensitivity to TSH. The cortisol response to cold is also reduced with aging. The reason for this is still unclear.

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APPENDIX A

Table 1

Serum Cortisol (ng/ml) Concentrations During a 24-Hr Period in Young Adult Beagle Dogs

Day 1

Time (hrs)	1200	1500	1800	2100	2400	0300	0600	0900	1200
Adult #									
1	4.2	10.5	6.1	3.7	8.2	5.9	6.2	10.7	11.2
2	9.4	9.4	9.9	4.7	5.9	9.7	4.6	8.7	7.2
3	9.3	8.3	12.2	8.0	---	---	5.0	12.5	17.5
4	9.4	14.1	9.3	10.8	6.1	7.6	8.6	6.3	7.3
5	10.6	6.9	3.8	4.7	4.4	4.0	6.0	6.4	8.3
MEAN	8.6	9.8a	8.3	6.4	6.2b	6.8	6.1b	8.9	10.3
STD. ERR.	1.1	1.2	1.5	1.3	0.8	1.2	0.7	1.2	1.9

Day 2

Time (hrs)	1300	1600	1900	2200	0100	0400	0700	1000	1300
Adult #									
1	7.1	3.5	2.9	1.8	6.9	9.3	10.9	8.3	10.3
2	4.8	7.2	3.8	5.6	5.2	5.2	4.6	8.2	8.3
3	7.3	15.4	3.9	4.1	---	---	11.3	10.2	6.1
4	9.6	4.6	4.6	3.4	12.8	9.6	7.5	5.5	6.2
5	8.0	5.4	5.0	2.1	3.0	6.9	6.0	6.0	9.7
MEAN	7.4a	7.2	4.0bc	3.4be	7.0	7.8df	8.1df	7.6df	8.1df
STD. ERR.	0.8	2.1	0.4	0.7	2.1	1.0	1.3	0.9	0.9

Day 3

Time (hrs)	1400	1700	2000	2300	0200	0500	0800	1100	1400
Adult #									
1	7.3	1.7	1.8	3.0	8.6	10.2	4.2	7.8	5.9
2	7.6	9.4	6.7	5.1	12.2	6.2	7.3	13.3	5.8
3	6.7	11.9	6.5	6.0	---	---	6.1	6.3	5.0
4	8.9	2.6	3.5	9.9	11.0	7.6	8.1	8.8	10.2
5	9.3	3.6	5.6	3.5	5.7	8.4	5.0	4.2	8.2
MEAN	8.0b	5.8	4.8a	5.5	9.4b	8.1b	6.1	8.1	7.0
STD. ERR.	0.5	2.0	0.9	1.2	1.4	0.8	0.7	1.5	1.0

a = Significantly different from b ($P < 0.05$).
c = Significantly different from d ($P < 0.05$).
e = Significantly different from f ($P < 0.05$).

Table 2

Serum Cortisol (ng/ml) Concentrations During a 24-Hr Period in Old Beagle Dogs

Day 1

Time (hrs)	1200	1500	1800	2100	2400	0300	0600	0900	1200
Old #									
1	10.1	9.2	9.2	13.8	12.7	7.3	11.9	11.2	15.7
2	5.6	6.8	7.0	6.4	6.2	3.3	5.9	4.7	5.9
3	5.4	4.9	7.8	6.1	6.7	2.6	4.0	3.6	2.7
4	15.7	8.4	18.6	15.5	18.4	17.6	11.3	12.9	24.6
MEAN	9.2	7.3	10.7	10.5	11.0	7.7	8.3	8.1	12.2
STD. ERR.	2.4	0.9	2.7	2.5	2.9	3.5	2.0	2.3	5.0

Day 2

Time (hrs)	1300	1600	1900	2200	0100	0400	0700	1000	1300
Old #									
1	18.5	13.3	4.8	15.3	10.6	12.9	9.7	22.8	18.4
2	11.5	10.7	7.6	9.6	6.2	7.9	4.8	4.5	11.6
3	5.5	5.6	9.5	7.8	5.1	3.5	4.1	6.1	2.8
4	20.5	10.5	13.1	18.6	13.8	8.7	9.9	17.1	20.4
MEAN	14.0	10.0	8.8	12.8	8.9	8.3	7.1	12.6	13.3
STD. ERR.	3.4	1.6	1.7	2.5	2.0	1.9	1.6	4.4	4.0

Day 3

Time (hrs)	1400	1700	2000	2300	0200	0500	0800	1100	1400
Old #									
1	19.8	11.6	20.4	20.2	14.5	14.3	16.7	13.7	18.8
2	6.7	9.3	6.2	3.9	3.7	5.6	4.6	6.2	5.3
3	6.1	4.8	8.5	3.0	4.1	2.5	4.9	8.9	4.4
4	8.4	15.1	24.7	15.4	17.3	12.2	11.8	11.8	13.3
MEAN	10.3	10.2	15.0	10.6	9.9	8.7	9.5	10.2	10.5
STD. ERR.	3.2	2.2	4.5	4.3	3.5	2.8	2.9	1.6	3.4

APPENDIX B

Table 3

Effects of Aging on Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 22°C (Control)

	Plasma Thyroxine (ug/dl)							
Ambient Temperature	<---------- 22°C ---------->							
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	6.0	8.0
Puppy								
1	4.8	5.2	5.0	4.5	4.2	4.6	4.3	3.3
2	3.9	5.0	5.2	---	---	4.6	3.9	4.7
3	5.2	3.7	---	4.3	5.2	5.1	4.9	5.8
4	2.4	2.7	2.6	3.4	4.0	3.5	3.5	3.0
5	3.5	3.7	3.9	5.5	5.2	5.3	4.7	4.1
MEAN	4.0	4.1	4.2	4.4	4.7	4.6	4.3	4.2
STD. ERR.	0.5	0.5	0.6	0.4	0.3	0.3	0.3	0.5
Adult								
1	2.4	2.6	3.0	3.1	3.2	---	2.3	2.8
2	5.5	4.1	4.2	5.0	5.9	---	5.6	5.4
3	3.7	3.4	3.1	4.1	4.2	4.0	4.7	2.5
4	3.3	2.9	3.3	4.5	4.7	4.7	4.3	4.6
5	3.8	3.5	4.2	4.6	4.9	5.7	4.9	4.8
MEAN	3.7	3.3	3.6	4.3	4.6	4.8	4.4	4.0
STD. ERR.	0.5	0.3	0.3	0.3	0.4	0.5	0.6	0.6
Old								
1	2.8	2.9	3.1	3.2	3.0	3.0	2.8	3.0
2	2.3	2.7	2.8	3.3	3.5	3.7	2.7	3.5
3	2.2	2.4	2.6	2.7	2.9	3.0	2.6	2.6
4	3.1	2.9	2.8	2.3	2.5	2.2	3.0	3.0
5	3.0	2.4	---	2.5	2.3	2.1	1.9	2.1
MEAN	2.7	2.7	2.8	2.8	2.8	2.8	2.6	2.8
STD. ERR.	0.2	0.1	0.1	0.2	0.2	0.3	0.2	0.2

Table 4

Effects of Aging on Percent Change in Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 22°C (Control)

	Percent Change in Plasma Thyroxine							
Ambient Temperature	<---------- 22°C ---------->							
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	6.0	8.0
Puppy								
1	---	8.3	4.2	-6.2	-12.5	-4.2	-10.4	-31.3
2	---	28.2	33.3	---	---	17.9	0.0	20.5
3	---	-28.8	---	-17.3	0.0	-1.9	-5.8	11.5
4	---	12.5	8.3	41.7	66.7	45.8	45.8	25.0
5	---	5.7	11.4	57.1	48.6	51.4	34.3	17.1
MEAN	---	5.2	14.3	18.8	25.7	21.8	12.8	8.6
STD. ERR.	---	9.3	6.5	18.1	19.0	11.6	11.4	10.2
Adult								
1	---	8.3	25.0	29.2	33.3	---	-4.2	16.7
2	---	-25.5	-23.6	-9.1	7.3	---	1.8	-1.8
3	---	-8.1	-16.2	10.8	13.5	8.1	27.0	-32.4
4	---	-12.1	0.0	36.4	42.4	42.4	30.3	39.4
5	---	-7.9	10.5	21.1	28.9	50.0	28.9	26.3
MEAN	---	-9.1	-0.9	17.7	25.1	33.5	16.8	9.6
STD. ERR.	---	5.4	8.8	7.9	6.5	12.9	7.4	12.5
Old								
1	---	3.6	10.7	14.3	7.1	7.1	0.0	7.1
2	---	17.4	21.7	43.5	52.2	60.9	17.4	52.2
3	---	9.1	18.2	22.7	31.8	36.4	18.2	18.2
4	---	-6.5	-9.7	25.8	-19.4	-29.0	-3.2	-3.2
5	---	-20.0	---	-16.7	-23.3	-30.0	-36.7	-30.0
MEAN	---	0.7	10.2	17.9	9.7	9.1	-0.9	8.9
STD. ERR.	---	6.5	7.0	9.9	14.6	17.9	10.0	13.5

Table 5

Effects of Aging on Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 10°C

	Plasma Thyroxine (ug/dl)							
Ambient Temperature	<---------- 10°C ---------->						<---- 22°C ---->	
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	4.6	3.9	4.0	5.5	5.3	4.6	4.5	3.8
2	5.5	3.2	3.7	3.7	4.0	4.0	5.3	4.3
3	5.3	6.9	6.1	4.5	6.2	4.1	4.3	3.3
4	3.6	3.9	3.7	4.0	2.9	2.6	3.0	2.1
5	5.0	5.2	5.4	5.0	6.0	3.6	4.9	3.1
MEAN	4.8	4.6	4.6	4.5	4.9	3.8	4.4	3.3
STD. ERR.	0.3	0.7	0.5	0.3	0.6	0.3	0.4	0.4
Adult								
1	2.7	3.0	3.6	3.4	3.8	5.0	4.4	4.2
2	5.2	4.6	5.3	5.8	5.8	5.7	5.7	5.2
3	4.1	4.4	4.1	5.2	5.5	5.2	5.1	5.0
4	4.6	3.9	3.9	4.4	5.0	4.9	4.9	4.2
5	4.1	3.9	5.0	5.4	6.2	6.2	6.2	4.5
MEAN	4.1	4.0	4.4	4.8	5.3	5.4	5.3	4.6
STD. ERR.	0.4	0.3	0.3	0.4	0.4	0.2	0.3	0.2
Old								
1	2.5	2.7	2.4	2.8	3.1	2.3	2.6	2.6
2	2.6	2.1	2.8	3.4	3.5	3.4	3.2	2.3
3	2.1	1.9	2.9	2.3	2.9	3.0	2.8	2.6
4	2.6	2.4	3.0	3.0	3.4	3.3	4.0	---
5	2.6	2.2	2.2	2.8	2.9	2.7	2.4	2.4
MEAN	2.5	2.3	2.7	2.9	3.2	2.9	3.0	2.5
STD. ERR.	0.1	0.1	0.2	0.2	0.1	0.2	0.3	0.1

Table 6

Effects of Aging on Percent Change in Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 10°C

	Percent Change in Plasma Thyroxine							
Ambient Temperature	<---------- 10°C ---------->					<---- 22°C ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	-15.2	-13.0	19.6	15.2	0.0	-2.2	-17.4
2	---	-41.8	-32.7	-32.7	-27.3	-27.3	-3.6	-21.8
3	---	30.2	15.1	-15.1	17.0	-22.6	-18.9	-37.7
4	---	8.3	2.8	11.1	-19.4	-27.8	-16.7	-41.7
5	---	4.0	8.0	0.0	20.0	-28.0	-2.0	-38.0
MEAN	---	-2.9	-4.0	-3.4	1.1	-21.1	-8.6	-31.3
STD. ERR.	---	12.1	8.5	9.3	10.1	5.4	3.7	4.9
Adult								
1	---	11.1	33.3	25.9	40.7	85.2	63.0	55.6
2	---	-11.5	1.9	11.5	11.5	9.6	9.6	0.0
3	---	7.3	0.0	26.8	34.1	26.8	24.4	22.0
4	---	-15.2	-15.2	-4.3	8.7	6.5	6.5	-8.7
5	---	-4.9	22.0	31.7	51.2	51.2	51.2	9.7
MEAN	---	-2.6	8.4	18.3	29.2	35.9	30.9	15.7
STD. ERR.	---	5.1	8.6	6.6	8.3	14.7	11.3	11.2
Old								
1	---	8.0	-4.0	12.0	24.0	-8.0	4.0	4.0
2	---	-19.2	7.7	30.8	34.6	30.8	23.1	-11.5
3	---	-9.5	38.1	9.5	38.1	42.9	33.3	23.8
4	---	-7.7	15.4	15.4	30.8	26.9	53.8	---
5	---	-15.4	-15.4	7.7	11.5	3.8	-7.7	-7.7
MEAN	---	-8.8	8.4	15.1	27.8	19.3	21.3	2.2
STD. ERR.	---	4.7	9.1	4.1	4.7	9.3	10.8	7.9

Table 7

Effects of Aging on Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of $4^{o}C$

	Plasma Thyroxine (ug/dl)							
Ambient Temperature	<---------- $4^{o}C$ ---------->					<---- $22^{o}C$ ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	5.0	3.9	---	6.0	5.9	6.1	6.6	3.9
2	3.9	5.3	5.0	5.6	4.7	6.8	5.0	---
3	4.6	5.0	4.5	5.1	5.4	6.1	4.2	4.7
4	3.4	3.5	4.3	5.8	3.2	2.9	3.4	2.7
5	4.8	3.9	4.6	3.8	3.9	4.5	6.1	3.4
MEAN	4.3	4.3	4.6	5.3	4.6	5.3	5.1	3.7
STD. ERR.	0.3	0.3	0.1	0.3	0.5	0.7	0.6	0.4
Adult								
1	2.9	2.8	2.9	3.9	4.3	4.3	5.0	3.6
2	5.4	5.3	5.9	6.5	5.7	6.0	5.4	5.0
3	4.1	4.1	4.1	5.0	5.3	5.3	5.5	4.0
4	4.4	3.9	3.9	4.7	5.7	5.9	5.3	4.6
5	4.1	3.7	4.1	4.8	6.9	6.0	5.5	5.2
MEAN	4.2	4.0	4.2	5.0	5.6	5.5	5.3	4.5
STD. ERR.	0.4	0.4	0.5	0.4	0.4	0.3	0.1	0.3
Old								
1	2.7	2.8	3.1	2.9	2.5	2.2	2.5	2.6
2	2.9	2.3	3.2	3.0	3.4	3.2	3.0	3.2
3	2.4	2.8	2.8	---	3.2	2.9	3.0	2.3
4	2.6	3.0	1.6	---	3.0	2.7	3.0	3.3
5	2.5	2.5	2.5	---	2.4	3.0	---	2.2
MEAN	2.6	2.7	2.6	3.0	2.9	2.8	2.9	2.7
STD. ERR.	0.1	0.1	0.3	0.1	0.2	0.2	0.1	0.2

Table 8

Effects of Aging on Percent Change in Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of $4^{o}C$

	Percent Change in Plasma Thyroxine							
Ambient Temperature	<----------		$4^{o}C$	----------		---------->	<---- $22^{o}C$	---->
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	-22.0	---	20.0	18.0	22.0	32.0	-22.0
2	---	35.9	28.2	43.6	20.5	74.4	28.2	---
3	---	8.7	-2.2	10.9	17.4	32.6	-8.7	2.2
4	---	2.9	26.5	70.6	-5.9	-14.7	0.0	-20.6
5	---	-18.8	-4.2	-20.8	-18.8	-6.2	27.1	-29.2
MEAN	---	1.3	12.1	24.9	6.2	21.6	15.7	-17.4
STD. ERR.	---	10.5	8.8	15.4	7.9	15.8	8.3	6.8
Adult								
1	---	-3.4	0.0	34.5	48.3	48.3	72.4	24.1
2	---	-1.9	9.3	20.4	5.6	11.1	0.0	-7.4
3	---	0.0	0.0	22.0	29.3	29.3	34.1	-2.4
4	---	-11.4	-11.4	6.8	29.5	34.1	20.5	4.5
5	---	-9.8	0.0	17.1	68.3	46.3	34.1	26.8
MEAN	---	-5.3	-0.4	20.2	36.2	33.8	32.2	9.1
STD. ERR.	---	2.2	3.3	4.5	10.5	6.7	11.8	6.9
Old								
1	---	3.7	14.8	7.4	-7.4	-18.5	-7.4	-3.7
2	---	-20.7	10.3	3.4	17.2	10.3	3.4	10.3
3	---	16.7	16.7	---	33.3	20.8	25.0	-4.2
4	---	15.4	-38.5	---	15.4	3.8	15.4	26.9
5	---	0.0	0.0	---	-4.0	20.0	---	-12.0
MEAN	---	3.0	0.7	5.4	10.9	7.3	9.1	3.5
STD. ERR.	---	6.8	10.2	2.0	7.5	7.2	7.1	6.9

Table 9

Effects of Aging on Plasma Thyroxine Concentrations

in Beagle Dogs During Exposure to Ambient Temperature of $-5^{0}C$

	Plasma Thyroxine (ug/dl)							
Ambient Temperature	<----------		$-5^{0}C$		---------->	<----	$22^{0}C$	---->
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	5.6	5.6	6.1	4.9	5.6	5.4	5.4	4.1
2	4.9	5.5	5.9	5.8	4.0	3.9	3.6	3.6
3	3.5	3.2	4.1	5.6	6.7	5.3	6.9	7.5
4	3.6	3.4	4.4	4.4	5.3	4.7	4.3	3.2
5	4.6	5.2	4.6	7.0	5.1	4.5	4.2	3.5
MEAN	4.4	4.6	5.0	5.5	5.3	4.8	4.9	4.4
STD. ERR.	0.4	0.5	0.4	0.4	0.4	0.3	0.6	0.8
Adult								
1	3.8	4.2	4.6	5.4	7.7	7.3	6.1	6.3
2	4.6	6.4	5.1	7.7	9.3	13.8	---	8.4
3	6.2	8.4	10.0	12.0	16.0	18.5	13.0	13.0
4	4.0	3.5	5.1	5.1	8.1	5.3	---	4.4
MEAN	4.7	5.6	6.2	7.5	10.3a	11.2a	9.6	8.0
STD. ERR.	0.5	1.1	1.3	1.6	1.9	3.0	2.4	1.8
Old								
1	4.4	4.4	4.0	5.2	6.3	5.7	5.3	5.9
2	3.6	4.2	5.0	4.5	6.8	4.5	4.4	---
4	3.0	3.7	2.1	3.6	5.4	4.8	3.5	3.3
MEAN	3.7	4.1	3.7	4.4	6.2a	5.0	4.4	4.6
STD. ERR.	0.4	0.2	0.9	0.5	0.4	0.4	0.5	1.3

a = Significantly different from values at 0.0 hrs ($P < 0.05$).

Table 10

Effects of Aging on Percent Change in Plasma Thyroxine Concentrations in Beagle Dogs During Exposure to Ambient Temperature of -5°C

	Percent Change in Plasma Thyroxine							
Ambient Temperature	<---------- -5°C ---------->					<---- 22°C ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	0.0	8.9	-12.5	0.0	-3.6	-3.6	-26.8
2	---	12.2	20.4	18.4	-18.4	-20.4	-26.5	-26.5
3	---	-8.6	17.1	60.0	91.4	51.4	97.1	114.3
4	---	-5.6	22.2	22.2	47.2	30.6	19.4	-11.1
5	---	13.0	0.0	52.2	10.9	-2.2	-8.7	-23.9
MEAN	---	2.2	13.7	28.1	26.2	11.2	15.5	5.2
STD. ERR.	---	4.5	4.1	13.0	19.5	13.0	21.7	27.4
Adult								
1	---	10.5	21.1	42.1	102.6	92.1	60.5	65.8
2	---	39.1	10.9	67.4	102.2	200.0	---	82.6
3	---	34.5	61.3	93.5	158.1	198.4	109.7	109.7
4	---	-12.5	27.5	27.5	102.5	32.5	---	10.0
MEAN	---	17.9	30.2	57.6	116.4a	130.8a	85.1b	67.0
STD. ERR.	---	11.9	10.9	14.5	13.9	41.4	24.6	21.0
Old								
1	---	0.0	-9.1	18.2	43.2	29.5	20.5	34.1
2	---	16.7	38.9	25.0	88.9	25.0	22.2	---
4	---	23.3	-30.0	20.0	80.0	60.0	16.7	10.0
MEAN	---	13.3	-0.1	21.1	70.7	38.2	19.8	22.1
STD. ERR.	---	6.9	20.4	2.0	14.0	11.0	1.6	12.1

a = Significantly different from corresponding values in the puppies ($P < 0.05$).
b = Significantly different from corresponding values in the old ($P < 0.05$).

Table 11

Effects of Aging on Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 22°C (Control)

	Plasma Cortisol (ng/ml)							
Ambient Temperature	<---------- 22°C ---------->							
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	6.0	8.0
Puppy								
1	4.4	9.3	7.1	2.2	6.6	0.3	13.9	9.1
2	6.9	21.3	10.9	9.2	1.6	5.1	4.8	4.4
3	0.9	3.5	---	1.6	0.4	1.1	0.2	0.9
4	3.6	3.4	3.4	1.1	1.6	3.3	4.6	1.2
5	13.1	4.1	1.1	---	7.0	2.3	1.2	2.5
MEAN	5.8	8.3	5.6	3.5	3.4	2.4	4.9	3.6
STD. ERR.	2.1	3.4	2.1	1.9	1.4	0.8	2.4	1.5
Adult								
1	21.6	30.2	15.9	26.2	19.7	---	10.5	22.7
2	18.6	20.1	13.2	2.3	6.5	---	9.0	17.8
3	28.4	14.4	6.4	8.5	10.6	5.9	9.8	10.2
4	22.0	37.0	38.6	14.4	11.8	14.1	21.8	22.8
5	11.1	6.0	6.8	9.9	11.1	9.3	7.8	6.4
MEAN	20.3	21.5	16.2	12.3	11.9	9.8	11.8	16.0
STD. ERR.	2.8	5.5	5.9	4.0	2.1	2.4	2.5	3.3
Old								
1	27.3	25.4	18.5	12.6	11.2	7.4	8.7	8.4
2	32.0	31.4	34.3	18.2	19.1	18.0	15.6	18.3
3	12.2	29.5	12.3	5.7	8.7	5.8	5.3	5.1
4	12.1	8.9	14.7	15.5	14.5	16.7	18.1	30.3
5	13.9	9.3	---	2.1	3.3	2.4	4.5	17.4
MEAN	19.5	20.9	20.0	10.8	11.4	10.1	10.4	15.9
STD. ERR.	4.2	4.9	5.0	3.0	2.7	3.1	2.7	4.4

Table 12

Effects of Aging on Percent Change in Plasma Cortisol Concentrations

Dogs During Exposure to Ambient Temperature of 22°C (Control)

	Percent Change in Plasma Cortisol							
Ambient Temperature	<---------- 22°C ---------->							
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	6.0	8.0
Puppy								
1	---	111.4	61.4	-50.0	50.0	-93.2	215.9	106.8
2	---	208.7	58.0	33.3	-76.8	-26.1	-30.4	-36.2
3	---	288.9	---	77.8	-55.6	22.2	-77.8	0.0
4	---	-5.6	-5.6	-69.4	-55.6	-8.3	27.8	-66.7
5	---	-68.7	-91.6	---	-46.6	-82.4	-90.8	-80.9
MEAN	---	106.9	5.6	-2.1	-36.9	-37.6	8.9	-15.4
STD. ERR.	---	66.0	35.9	34.7	22.3	22.0	55.8	33.6
Adult								
1	---	39.8	-26.4	21.3	-8.8	---	-51.4	5.1
2	---	8.1	-29.0	-87.6	-65.1	---	-51.6	-4.3
3	---	-49.3	-77.5	-70.1	-62.7	-79.2	-65.5	-64.1
4	---	68.2	75.5	-34.5	-46.4	-35.9	-0.9	3.6
5	---	-45.9	-38.7	-10.8	0.0	-16.2	-29.7	-42.3
MEAN	---	4.2	-3.7	-36.3	-36.6	-43.8	-39.8	-20.4
STD. ERR.	---	23.2	27.1	19.7	13.6	18.6	11.3	13.9
Old								
1	---	-7.0	-32.2	-53.8	-59.0	-72.9	-68.1	-69.2
2	---	-1.9	7.2	-43.1	-40.3	-43.8	-51.3	-42.8
3	---	141.8	0.8	-53.3	-28.7	-52.5	-56.6	-58.2
4	---	-26.4	21.5	28.1	19.8	38.0	49.6	150.4
5	---	-33.1	---	-84.9	-76.3	-82.7	-67.6	25.2
MEAN	---	14.7	-0.7	-41.4	-36.9	-42.8	-33.8	1.1
STD. ERR.	---	32.3	11.4	18.7	16.3	21.4	22.3	40.8

Table 13

Effects of Aging on Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 10°C

	Plasma Cortisol (ng/ml)							
Ambient Temperature	<---------- 10°C ---------->						<---- 22°C ---->	
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	16.4	3.5	20.6	3.7	7.8	12.1	5.6	6.1
2	7.2	7.2	20.1	6.9	12.1	4.0	0.0	2.4
3	1.5	3.5	2.6	0.8	3.2	2.1	2.0	0.6
4	1.0	3.6	3.7	1.8	0.4	1.7	3.0	1.8
5	6.5	4.3	0.7	---	1.4	---	0.5	3.3
MEAN	6.5	4.4	9.5	3.3	5.0	5.0	2.2	2.8
STD. ERR.	2.8	0.7	4.4	1.3	2.2	2.4	1.0	0.9
Adult								
1	21.1	17.6	6.9	4.1	8.4	17.0	2.4	2.7
2	17.7	17.2	6.3	---	8.7	24.9	6.7	12.6
3	19.4	6.8	4.0	11.2	7.0	6.7	10.0	15.2
4	14.9	40.0	24.2	27.8	13.2	30.3	21.0	22.3
5	46.4	---	---	14.7	18.2	23.4	24.0	25.4
MEAN	23.9	20.4	10.4	14.5	11.1	20.5	12.8	15.6
STD. ERR.	5.7	7.0	4.7	5.0	2.1	4.0	4.2	4.0
Old								
1	31.9	19.8	10.9	7.8	5.6	19.3	5.5	11.2
2	52.4	42.8	25.2	33.4	26.4	18.0	18.0	18.8
3	11.3	14.8	5.2	9.0	8.2	14.9	5.8	---
4	14.1	25.6	21.9	25.7	31.4	19.8	21.0	---
5	11.6	12.2	8.0	12.5	14.9	15.0	8.7	23.0
MEAN	24.3	23.0	14.2	17.7	17.3	17.4	11.8	14.0
STD. ERR.	8.0	5.5	3.9	5.1	5.0	1.0	3.2	4.4

Table 14

Effects of Aging on Percent Change in Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 10°C

	Percent Change in Plasma Cortisol							
Ambient Temperature	<---------- 10°C ---------->					<---- 22°C ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	-78.7	25.6	-77.4	-52.4	-26.2	-65.9	-62.8
2	---	0.0	179.2	-4.2	68.1	-44.4	-100.0	-66.7
3	---	133.3	73.3	-46.7	113.3	40.0	33.3	-60.0
4	---	260.0	270.0	80.0	-60.0	70.0	200.0	80.0
5	---	-33.8	-89.2	---	-78.5	---	-92.3	-49.2
MEAN	---	56.2	91.8	-12.1	-1.9	9.9	-5.0	-31.7
STD. ERR.	---	62.0	62.0	34.2	38.7	27.0	56.5	28.1
Adult								
1	---	-16.6	-67.3	-80.6	-60.2	-19.4	-88.6	-87.2
2	---	-2.8	-64.4	---	-50.8	40.7	-62.1	-28.8
3	---	-64.9	-79.4	-42.3	-63.9	-65.5	-48.5	-21.6
4	---	168.5	62.4	86.6	-11.4	103.4	40.9	49.7
5	---	---	---	-68.3	-60.8	-49.6	-48.3	-45.3
MEAN	---	21.1	-37.2	-26.2	-49.4	1.9	-41.3	-26.6
STD. ERR.	---	50.9	33.4	38.4	9.8	31.2	21.8	22.2
Old								
1	---	-37.9	-65.8	-75.5	-82.4	-39.5	-82.8	-64.9
2	---	-18.3	-51.9	-36.3	-49.6	-65.6	-65.6	-64.1
3	---	27.4	-54.0	-20.4	-27.4	31.9	-48.7	-73.5
4	---	81.6	55.3	82.3	122.7	40.4	48.9	---
5	---	5.2	-31.0	7.8	28.4	29.3	-25.0	98.3
MEAN	---	11.6	-29.5	-8.4	-1.7	-0.7	-34.6	-26.1
STD. ERR.	---	20.7	21.9	26.4	36.0	21.6	23.0	41.5

Table 15

Effects of Aging on Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of $4^{o}C$

	Plasma Cortisol (ng/ml)							
Ambient Temperature	<---------- $4^{o}C$ ---------->					<---- $22^{o}C$ ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	6.7	12.6	10.8	10.1	2.5	14.6	8.6	---
2	8.9	13.6	---	---	9.3	7.5	9.8	---
3	0.7	1.5	1.9	1.6	1.0	1.3	1.1	---
4	9.1	5.3	3.3	4.2	5.7	3.5	7.6	---
5	9.6	11.9	3.8	2.3	1.9	0.8	1.8	---
MEAN	7.0	9.0	5.0	4.6	4.1	5.5	5.6	---
STD. ERR.	1.7	2.4	2.0	1.9	1.5	2.6	1.8	---
Adult								
1	8.3	19.2	5.3	3.0	2.9	4.0	7.2	0.5
3	8.4	11.0	12.7	13.9	4.7	5.2	9.7	11.4
4	26.4	22.6	33.3	21.9	27.2	20.9	28.2	38.0
5	12.8	41.1	18.7	18.6	27.7	18.9	17.4	16.7
MEAN	14.0	23.5	17.5	14.4	15.6	12.3	15.6	16.7
STD. ERR.	4.3	6.4	5.9	4.1	6.8	4.4	4.7	7.9
Old								
1	26.8	18.0	15.2	7.4	9.8	4.3	6.9	7.4
2	54.0	49.1	27.5	26.3	22.6	31.6	18.7	22.0
3	8.6	10.0	2.8	---	5.5	4.6	4.1	1.9
4	22.6	15.1	24.0	---	18.2	13.7	28.7	21.8
5	15.1	16.7	10.8	---	6.8	24.6	---	12.4
MEAN	25.4	21.8	16.1	16.9	12.6	15.8	14.6	13.1
STD. ERR.	7.8	7.0	4.5	9.5	3.3	5.4	5.7	4.0

Table 16

Effects of Aging on Percent Change in Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of 4°C

	Percent Change in Plasma Cortisol							
Ambient Temperature	<---------- 4°C ---------->					<---- 22°C ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	88.0	61.2	50.7	-62.7	117.9	28.4	---
2	---	52.8	---	---	4.5	15.7	10.1	---
3	---	114.3	171.4	128.6	42.9	85.7	57.1	---
4	---	-41.8	-63.7	-53.8	-37.4	-61.5	-16.5	---
5	---	24.0	-60.4	-76.0	-80.2	-91.7	-81.3	---
MEAN	---	47.5	27.1	12.4	-26.6	13.2	-0.4	---
STD. ERR.	---	27.1	56.2	47.6	22.5	40.5	23.5	---
Adult								
1	---	131.3	-36.1	-63.9	-65.1	-51.8	-13.3	-94.0
3	---	31.0	51.2	65.5	-44.0	-38.1	15.5	35.7
4	---	-14.4	26.1	-17.0	3.0	-20.8	6.8	43.9
5	---	221.1	46.1	45.3	116.4	47.7	35.9	30.5
MEAN	---	92.3	21.8	7.5	2.6	-15.8	11.2	4.0
STD. ERR.	---	52.6	20.1	29.6	40.5	22.1	10.2	32.8
Old								
1	---	-32.8	-43.3	-72.3	-63.4	-84.0	-74.3	-72.4
2	---	-9.1	-49.1	-51.3	-58.1	-41.5	-65.4	-59.3
3	---	16.3	-67.4	---	-36.0	-46.5	-52.3	-77.9
4	---	-33.2	6.2	---	-19.5	-39.4	27.0	-3.5
5	---	10.6	-28.5	---	-55.0	62.9	---	17.9
MEAN	---	-9.6	-36.4	-61.8	-46.4	-29.7	-41.3	-39.0
STD. ERR.	---	10.4	12.3	10.5	8.2	24.5	23.2	19.4

Table 17

Effects of Aging on Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of -5°C

	Plasma Cortisol (ng/ml)							
Ambient Temperature	<---------- -5°C ---------->						<---- 22°C ---->	
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	17.6	20.0	4.4	1.6	10.9	4.8	13.7	---
2	8.3	12.4	19.9	3.1	19.7	8.0	0.7	4.6
3	3.1	1.2	0.5	1.7	1.5	0.5	---	---
4	9.8	7.4	1.4	1.6	1.0	3.2	5.1	0.9
5	7.7	8.8	9.8	3.0	9.6	4.3	1.2	0.8
MEAN	9.3	10.0	7.2	2.2	8.4	4.2	5.2	2.1
STD. ERR.	2.4	3.1	3.6	0.3	3.5	1.2	3.0	1.3
Adult								
1	5.2	16.9	21.0	20.7	18.7	17.1	7.3	9.9
2	5.0	17.8	28.3	14.5	16.3	16.1	---	12.3
3	6.1	8.0	12.4	13.3	15.5	9.7	7.1	7.5
4	5.6	17.5	20.8	13.9	12.0	17.1	---	7.6
5	5.1	15.7	---	16.3	7.1	6.6	2.0	5.0
MEAN	5.4	15.2a	20.6a	15.7a	13.9a	13.3a	5.5	8.5
STD. ERR.	0.2	1.8	4.6	1.3	2.0	2.2	1.7	1.2
Old								
1	8.6	12.8	11.7	5.5	11.5	16.5	8.5	15.4
2	22.0	28.7	24.2	14.0	16.2	15.9	---	11.7
3	13.9	8.8	13.0	10.7	11.1	9.3	5.9	2.1
4	10.5	2.7	9.8	9.8	7.3	6.2	6.6	6.3
MEAN	13.8	13.3	14.7	10.0	11.5	12.0	7.0	8.9
STD. ERR.	3.0	5.6	3.3	1.8	1.8	2.5	0.8	2.9

a = Significantly different from values at 0.0 hrs ($P < 0.05$).

Table 18

Effects of Aging on Percent Change in Plasma Cortisol Concentrations in Beagle Dogs During Exposure to Ambient Temperature of $-5^{o}C$

	Percent Change in Plasma Cortisol							
Ambient Temperature	<---------- $-5^{o}C$ ---------->					<---- $22^{o}C$ ---->		
Time (hrs)	0.0	0.5	1.0	2.0	3.5	5.0	1.0	3.0
Puppy								
1	---	13.6	-75.0	-90.9	-38.1	-72.7	-22.2	---
2	---	49.4	139.8	-62.7	137.3	-3.6	-91.6	-44.6
3	---	-61.3	-83.9	-45.2	-51.6	-83.9	---	---
4	---	-24.5	-85.7	-83.7	-89.8	-67.3	-48.0	-90.8
5	---	14.3	27.3	-61.0	24.7	-44.2	-84.4	-89.6
MEAN	---	-1.7	-15.5	-68.7	-3.5	-54.3	-61.6	-75.0
STD. ERR.	---	18.9	44.2	8.3	39.7	14.2	16.2	15.2
Adult								
1	---	225.0	303.8	298.1	259.6	228.8	40.4	90.4
2	---	256.0	466.0	190.0	226.0	222.0	---	146.0
3	---	31.1	103.3	118.0	152.5	59.0	16.4	23.0
4	---	212.5	271.4	148.2	114.3	205.4	---	35.7
5	---	207.8	---	219.6	39.2	29.4	-60.8	-2.0
MEAN	---	186.5ab	278.6ab	194.8ab	158.3ab	148.7ab	-1.3	58.6
STD. ERR.	---	39.7	75.0	31.1	39.4	43.1	30.5	26.6
Old								
1	---	48.8	36.0	-36.0	33.7	91.9	-1.2	79.1
2	---	30.5	10.9	-36.4	-26.4	-27.7	---	-46.8
3	---	-36.7	-6.5	-23.0	-20.1	-33.1	-57.6	-84.9
4	---	-74.3	-6.7	-6.7	-30.5	-41.0	-37.1	-40.0
MEAN	---	-7.9	8.4	-25.5	-10.8	-2.5	-32.0	-23.2
STD. ERR.	---	28.8	10.1	7.0	15.0	31.6	16.5	35.5

a = Significantly different from corresponding values in the puppies.
b = Significantly different from corresponding values in the old.

THE EFFECTS OF AGING ON THYROXINE AND CORTISOL RESPONSES TO LOW AMBIENT TEMPERATURES AND ON CIRCADIAN RHYTHM OF CORTISOL IN THE DOG.

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AN ABSTRACT OF A MASTER'S THESIS

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Puppies (average age 7.6±0.1 wks), young adult (average age 3.0± 0.4 yrs) and old (average 11.4±0.2 yrs) female beagle dogs were used in two sets of experiments. In the first experiment, we wanted to determine if there was a circadian rhythm in serum cortisol and if this circadian rhythm is affected by aging. In the second experiment, the effects of aging on basal levels of plasma thyroxine and cortisol and their responses to cold were determined.

1. In the first experiment, the dogs were kept in a 12-hr light, 12-hr dark cycle. Young adult and old dogs were bled at three-hr intervals during three 24-hr periods. Blood sampling began at 1200 hrs during the first 24-hr period, 1300 hrs during the second 24-hr period and 1400 hrs during the third 24-hr period. The dogs were given a one-day rest between any 24-hr bleeding periods. Blood samples (3 ml) were collected by venipuncture from the cephalic vein and serum cortisol concentrations were determined by radio-immunoassay.

In adult dogs, a definite cortisol circadian rhythm was found, with a zenith at 1000 hr (8.4 ng/ml) and a nadir at 2200 hrs (5.6 ng/ml). The mean (±Std. Err.) 24-hr serum cortisol concentration was 7.1±0.25 ng/ml. In old dogs, no significant circadian rhythmicity was detected and there was no significant difference in cortisol values at the zenith at 1700 hrs (11.4 ng/ml) and at the nadir at 0500 hrs (8.8 ng/ml). The mean (±Std. Err.) 24-hr period serum cortisol concentration was 10.1±0.54 ng/ml.

2. In the second experiment, puppies, young adult, and old dogs

were exposed to 22^{0}(control), 10^{0}, 4^{0} and -5^{0}C. Plasma thyroxine and cortisol concentrations were determined at 0.0, 0.5, 1.0, 2.0, 3.5 and 5.0 hrs during and 1.0 and 3.0 hrs after end of exposure. Blood samples (3 ml) were collected from the jugular vein from the puppies and the cephalic vein from the young adult and old dogs. Basal thyroxine levels in the puppies, young adult and old dogs were 4.4 $\pm$ 0.2, 4.4$\pm$0.2 and 2.8$\pm$0.1 ug/dl respectively. Thyroxine levels in the old dogs were significantly lower ($P < 0.001$) than those in the puppies and young adult dogs. There were no significant differences between puppies and young adult dogs. None of the dogs showed any significant changes in thyroxine levels during exposures to 22^{0}, 10^{0} and 4^{0}C. Exposure to -5^{0}C significantly elevated ($P < 0.05$) thyroxine levels by 116.4% at 3.5 hrs and 130.8% at 5.0 hrs in the young adult dogs and by 70.7% at 3.5 hrs in the old dogs. No changes in thyroxine response to -5^{0}C were observed in the puppies.

Basal cortisol levels in the puppies, young adult and old dogs were 7.2$\pm$1.1, 14.4$\pm$2.4 and 21.1$\pm$3.1 ng/ml respectively. Cortisol levels in the puppies were significantly lower ($p < 0.005$) than those in the young adult and old dogs. Even though cortisol levels in the young adult dogs were lower than those in the old dogs, this difference did not reach significance. Exposures of adult dogs to -5^{0} C significantly elevated ($P < 0.05$) cortisol levels by 186.5% at 0.5 hrs, 278.6% at 1.0 hr, 194.8% at 2.0 hrs, 158.3% at 3.5 hrs and 148.7% at 5.0 hrs during exposure. No changes in cortisol responses to low ambient temperatures were observed in these dogs during exposures to

22^{0}, 10^{0} and 4^{0}C. None of the four temperatures produced a significant change in plasma cortisol in the puppies or old dogs. It is concluded that aging decreases plasma thyroxine and cortisol responses to cold.