THE INFLUENCE OF THE THYROID HORMONE ON THE ASCORBIC ACID CONTENT OF THE ADRENAL CORTEX

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THE INFLUENCE OF THE THYROID HORMONE ON THE ASCORBIC ACID CONTENT OF THE ADRENAL CORTEX

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INTRODUCTION

With the current interest in the physiology of the thyroid gland, and the possible use, in certain phases of animal production, of thyroidally active iodinated proteins, and the goitrogenic thioureas, it becomes of interest to know what the effects of the thyroid hormone, or a lack of it, are on the animal body with respect to productive processes, and specific organs affected.

One of the most important endocrine glands in the animal body, indeed one essential to life, is the adrenal cortex. This study is concerned with the effects of varying levels of thyroidal stimulation on the adrenal cortex.

There is extensive evidence in the literature suggesting an interrelationship between the thyroid gland and the adrenal cortex. The nature of this interrelationship is, however, rather imperfectly understood. It has recently been demonstrated that the cholesterol and ascorbic acid concentrations in the adrenal cortex are reliable indicators of the functional activity of this organ. Using the determination of ascorbic acid in the adrenal cortex as a criterion of activity, the following facets of the thyroid-adrenocortical relationship have been investigated.

I. The effect of varying dosage levels of thyroidally active iodinated casein (Protamone) administered in the diet at levels of physiological stimulation.

II. The effects of varying dosage levels of thyroxine administered parenterally above physiological levels of stimulation.

III. The concentration of ascorbic acid in the adrenals at various time intervals during a six week regimen of thyroxine injections at a constant dose.

IV. The effect of concurrent dosage of varying levels of adrenocortical extracts with administration of a constant dosage level of thyroxine.

V. The influence of thyroidectomy.

VI. The effects of a constant dosage level of thyroxine on the adrenal cortices of pregnant rats, and on the adrenal cortices of the new born young of these mothers.

VII. The influence of sex on normal concentrations of ascorbic acid found in the adrenals.

REVIEW OF THE LITERATURE

Evidence of an interrelationship between the thyroid gland and the adrenal cortex exists in abundance. For the sake of convenience this has been divided into four principal categories, the first of which includes the effects of thyroxine administration on the adrenals.

Effect of Thyroxine on the Adrenals

The first work reported concerning the effects of thyroid administration on the adrenals was that of Hoskins (1). In this memorable but often overlooked paper, Hoskins reported that feeding desiccated thyroid to guinea pigs produced adrenal hypertrophy. Subsequently this work was confirmed for the adrenals of the rat by Herring (2), Cameron and Carmichael (3), Korenchevsky, et. al. (4), Cohen (5), Schmidt and Schmidt (6), DeWesselow and Griffiths (7), Ingle and Kendall (8), Uotila (9), Korenchevsky and Hall (10), and Lowenstein and Zwemmer (11).

Ingle and Higgins (12), in a somewhat different approach, enucleated one adrenal in a rat, and found that thyroxine administration caused extensive hypertrophy in the intact gland. In the enucleated gland, there was an increase in size of the zona glomerulosa, but there was no significant increase between the weight of this gland and an enucleated adrenal in a control animal not receiving thyroxine injections. The enucleated gland of a thyroxine-treated rat will regenerate faster, and to a greater extent, than will an untreated control.

Hypertrophy not due to Raised Metabolism.

The increase in metabolism following thyroxine administration is not the cause of adrenal hypertrophy. This was demonstrated by DeWesselow and Griffith (7), when they administered dinitrophenol to rats. This compound raised the metabolic rate of the rats, but did not cause adrenal hypertrophy. Further evidence that this compound acts differently from thyroxine was demonstrated by Cutting and Tainter (13). They failed to note growth acceleration in tadpoles following dinitrophenol adminstration. The metamorphosis accelerating effect of thyroxine in this species is well known.

If a hyperthyroid condition produces adrenal hypertrophy, it seems logical to expect that the opposite condition of hypothyroidism should produce adrenal atrophy. The second category of evidence concerning the thyroid-adrenal relationship is thus concerned with the effects of hypothyroid states on the adrenal.

Effect of Hypothyroidism on the Adrenals

The literature concerning the effects of hypothyroid conditions on the adrenal seems somewhat confusing. Gley (14) as cited by Koelsche and Kendall (15), found that there was an enlargement of the suprarenals after atrophy of the thyroid gland or after thyroidectomy, but upon histological examination, this increase in size was attributed to an increase in the lipoid content of the cortical cells. He concluded, that the enlargement could not be due to hyperfunction of the adrenals. Kennedy and Purves (16), noted adrenal hypertrophy in rats fed goitrogenic seeds of the Brassicae family. Their histological studies confirmed Gley's work, as they found an increase in the fat content of the cortical cells.

In recent years, a number of the sulfa drugs have been found to possess goitrogenic activity as well as the thioureas, and thiouracil compounds. Leblond and Hoff (17), administered sulfadiazine, sulfathiazole, and thiouracil to three groups of rats and thyroidectomized a fourth group. The animals were kept on these respective treatments for three weeks, and all animals except those receiving sulfadiazine, showed a decrease in the size of the adrenals. The most marked decrease occurred in the thiouracil group, followed by the thyroidectomized, and sulfathiazole groups. Sulfadiazine had no effect.

The influence of thiouracil on the adrenals was confirmed by Baumann and Marine (18). They administered thiouracil to rats for four months, and noted an involution of the adrenals to half their former size. Histological examination of the adrenals of these animals, showed the adrenals were still capable of hypertrophy, however, because if these hypothyroid animals were subjected to stresses, the atrophied adrenals would enlarge.

Glock (19), fed rats 0.5% thiourea in the diet, and noted that the animals remained at constant weight for four weeks. After this period death often ensued, but if adrenocortical extracts were administered, the rats sometimes recovered. Histologically, the adrenals of the rats in moribund condition, showed marked congestion, particularly of the zona reticularis, depletion of the lipoid content of the cortex, hemorrhages, and sometimes areas of necrosis. This work possibly indicates a toxic action of the particular thiourea used, as these results have not been reported for other goitrogens.

The third division of this discussion deals with the effects of adrenalectomy and sublethal adrenocortical injury on the thyroids.

Effect of Total Adrenalectomy

Most of the evidence concerned with the effects of adrenal extirpation on the thyroids, is related to metabolism studies and is therefore indirect. Gradinescu (20), as cited by Aub et. al. (21), noted a drop in the metabolism of three adrenalectomized cats and one dog. He attributed this drop to "a lack of regulatory mechanism for the peripheral vessels whose movements are normally controlled by adrenin from the suprarenal capsules." This drop in metabolism has been confirmed in cats by Webster et. al. (22) and Aub et. al. (21), in dogs by Harrop et. al. (23), and in rats by Carr and Beck (24). It has been noted by Lerman (25), and Aub et. al. (39), that basal metabolism often is low in Addison's syndrome.

Metabolic Depression and Adrenalin.

Aub et. al. (21), noted that when the thyroid is removed from animals, about three days elapse before a lowering in the metabolic rate is seen. When the hypophysis or the gonads are removed, metabolic effects are not seen for even longer periods of time. When the adrenals are removed, however, the decline in metabolic rate is abrupt, and prompt, and the authors suggest from these facts, that the metabolic depression following adrenalectomy is not related to other organs of internal secretion. In support of this idea, the following facts are cited:

1. Under anaesthesia, the lowered basal metabolic rate due to adrenalectomy may be returned to normal by intravenous injection of adrenalin, and they suggest that the metabolic fall is due to a lack of epinephrine.

2. Adrenalectomy causes a drop in metabolism in a thyroidectomized animal, and thus the thyroid is not involved.

3. Adrenaline injections increase metabolism in thyroidectomized animals.

4. In thyrotoxic animals, adrenalectomy does not cause a return to normal of the elevated metabolism.

5. Metabolism of resting frog muscle is greatly increased by bathing in adrenaline solutions. The same holds true for heart muscle.

Aub et. al. (39), in a subsequent paper, reported that under urethane anaesthesia there is a greater flow of adrenaline than normal. If the adrenals are removed under this anaesthetic, there is a prompt and progressive fall in metabolism averaging 12%, and this drop occurs after removal of the thyroid. Thus the thyroid is not essential to this reaction. Adrenaline administered intravenously causes a distince rise in metabolism under urethane anaesthesia, and the authors suggest as before, that this fall in metabolism is due to a lack of adrenaline.

From this work Aub et. al. (39) conclude that there are two physiological mechanisms for the elevation of basal metabolic rate. The adrenaline mechanism is a quick reaction while that of thyroxine is for sustained periods of time. Aub et. al. (39) failed to note any metabolic effects following the injection of adrenocortical extracts into adrenalectomized cats.

Effects of Adrenocortical Extracts in Adrenalectomized Animals.

The work of Webster et. al. (22), contradicts some of Aub's work. Webster et. al. (22) noted a 50% drop in metabolism in bilaterally adrenalectomized cats, and administration of adrenocortical extracts could bring this back to normal in 24 to 48 hours. They also noted a 15% to 30% increase in metabolism in thyroidectomized cats following adminstration of adrenocortical extracts, and this effect occurred in a short period of time. They further observed that adrenocortical extracts exerted no metabolic effects in normal animals.

Harrop et. al. (23), confirmed some of Webster's work in dogs. He observed that large amounts of adrenaline-free adrenocortical extracts had no metabolic effects on normal dogs. This has been confirmed in human subjects by Hitchcock et. al. (26). The adrenocortical extracts could, however, prevent a drop of 20%-25% in metabolism following adrenalectomy. Harrop noted in contradiction to Aub et. al. (21), that the metabolic decline following adrenalectomy does not occur in a dog maintained with cortin for a "considerable interval" after the withdrawal of adrenocortical extracts, and is more or less coincident with a fall in body temperature. This paper also reported the histological condition of the thyroids of adrenalectomized dogs maintained with adrenocortical extracts. An absence of hyperplasia was observed, as well as frequent calcification of the colloid.

It has thus been adequately demonstrated that following total extirpation of the adrenals, there is a fall in metabolism. This fall has not been observed following sublethal adrenocortical injury, and in many cases a rise in metabolism has been noted.

Effects of Sublethal Adrenocortical Injury

The first work reported on sublethal adrenocortical injury and its effect on metabolism was that of Golyakowski (27), as cited by Webster et. al. (22). Golyakowski (27) almost completely ligated the blood vessels supplying the adrenals in dogs, and noted a 30% increase in heat production in those dogs surviving six weeks or longer.

Similar work on other species has been reported by Marine and Baumann (28), (29), Scott (30), Davis and Hastings (31), and Carr and Beck (24). Marine and Baumann (28), noted in rabbits when the adrenals were damaged by freezing with ethylene chloride, or were removed, a disturbance in metabolism characterized by increased heat production and carbon dioxide output was seen. (As a good many rabbits have accessory adrenocortical tissue, adrenalectomy in those rabbits which survive the operation, is really equivalent to sublethal injury of the adrenal cortex.) This heat rise continued for periods up to three weeks, after which there was a gradual decline until the death of the animal. The symptom complex suggested in these rabbits was that of exophthalmic goiter. From this work Marine and Baumann (28), suggested that there is an antagonism between the adrenal cortex and the thyroid. They believe that the adrenal cortex exerts an inhibiting effect over the thyroid secretion, and when its influence is absent, the thyroid brings about the observed metabolic changes. In addition to these changes in metabolism, Marine and Baumann (28) also noted sleeker fur in those rabbits with injured adrenocortical tissue, as well as a vicious disposition which is characteristic of a hyperthyroid rabbit.

In a subsequent paper, Marine and Baumann (29), noted that thyroidectomy preceding destruction of adrenocortical tissue in the rabbit, prevented the previously observed heat rise.

Scott (30) repeated this work in cats, and noted that adrenal injury by partial ligation of the blood supply, or freezing with ethylene chloride, caused a significant and prolonged increase in heat production followed by a gradual decline to the death. The injury to the adrenals must be such that there is enough functional adrenocortical tissue present to just maintain the animal. If there is too much functional tissue present, the rise in heat production is not observed. Scott (30) observed in cats with the adrenals injured to the optimum degree, that there was an unusual sleekness of the fur, and an increase in appetite which suggested increased thyroid activity.

The decline in heat production in the terminal stages of life could be prevented by increasing the iodine content of the diet. Scott suggests that the gradual decline in heat production following the initial heat rise, is due to exhaustion of the thyroid, because 30 days after the addition of iodides to the diet, the thyroids were hypertrophied in those cats which showed an increase in heat production after suprarenal injury.

Barlow (32) noted in the cat after unilateral adrenalectomy, an initial drop in total metabolism, which gradually returned to normal or above. In bilaterally adrenalectomized cats, there was an initial depression in metabolism followed by a rise toward normal for the first six hours after the operation, and then a gradual decline in metabolism until the death of the animal at 40 hours.

Davis and Hastings (31), reported similar work in the mouse. This animal like the rabbit, possesses accessory adrenocortical tissue, and therefore adrenalectomy really amounts to sublethal adrenocortical injury in those animals surviving the operation. Davis and Hastings (31) noted that excised muscle from adrenalectomized mice showed an increase in "aerobic" metabolism 40-70 days following adrenalectomy. If adrenalectomy was preceded by thyroidectomy, this increase in heat production was not observed. Davis also noted that the activity of adrenalectomized mice was similar to intact mice receiving thyroxine, and further, that adrenalectomized mice show a much greater sensitivity to thyroxine. This observation has been made by Lerman (25), in human patients with Addisons disease. He reports in such patients a lowering of the basal metabolic rate, and if thyroid is administered to correct this condition, the patient may be thrown into a state of Addisonian crisis.

Davis and Hastings (31) from their work suggest that there is an interrelationship between the thyroid and the adrenal cortex such that when there is a deficiency of adrenocortical hormones, there is an increased production of the thyroid hormone, and this overproduction of the thyroid hormone continues until sufficient adrenocortical tissue has regenerated to make up the deficiency.

Inhibitory Effects of Adrenocortical Hormones on the Thyroid.

Further evidence of a regulatory effect of the adrenocortical hormones on the thyroid was presented in a review by Marine (33). He points out that in Graves disease, the adrenals are usually small, and he attributes the etiological factor in Graves disease to adrenocortical insufficiency. He believes that the gonads may be involved, but the thyroid changes in this disease are due to a compensatory mechanism arising from the lack of adrenocortical hormones. In addition to his rabbit work (previously cited) he points out the fact that in the new born human infant, the adrenal cortex begins to involute about the 8th day after birth, premature or otherwise. With this involution there is a rapid rise in heat production. Marine believes that in intrauterine life, the adrenal cortex controls tissue oxidations, and that the adrenal cortex involutes after birth to increase heat production.

From the evidence presented, it would appear that the adrenocortical hormones have an inhibitory effect on the thyroid secretion, as a lack of these hormones will cause an increase in heat production which is not observed when the thyroid is absent.

Effect of Adrenocortical Hormones on Metabolism

If the adrenocortical hormones have this inhibitory effect on the thyroid, their administration should logically depress metabolism in some way. The fourth category of evidence concerning the adrenocortical-thyroid relationship is thus concerned with the effects of adrenocortical extracts on metabolism and on the thyroid gland itself.

Evidence concerning the effects of adrenocortical hormones on metabolism is contradictory. Marine et. al. (34), fed rabbits glycerol emulsions of ox adrenal glands and noted a depression in metabolism of 6%-27%. The fall in metabolism began 5-7 days after beginning the treatments which is about the length of time required for thyroidectomy to show its effects. He suggested that the metabolism depressing substance exerts its effects either directly or indirectly through the visceral nervous system on the thyroid cells, either to inhibit the formation of thyroxine, or to prevent its secretion. In a subsequent paper Marine et. al. (35), reported that this same glycerol emulsion caused beneficial effects in Graves disease, causing an increase in muscle strength and body weight. Marine (33), also found that feeding ovarian corpora lutea had some beneficial effects in Graves disease.

These results have not been confirmed by Aub et. al. (39), Webster (22), or Hitchcock (26). Aub et. al. (39), found no metabolic effects following injection of adrenocortical extracts in the cat. Webster et. al. (22), confirmed these findings in the cat and rabbit. Hitchcock et. al. (26), injected adrenocortical extracts into humans and noted no effects on Oxygen consumption.

The author had occasion to check oxygen consumption of hyperthyroid rats receiving 20 gamma of thyroxine per 100 grams of body weight coincident with the administration of 1 unit of the commercial adrenocortical extract "Eschatin" (Parke-Davis) per 100 grams of body weight, and noted no difference in oxygen consumption between these rats and some receiving thyroxine at the aforementioned dosage. Thus the majority of evidence would seem to point to the fact that adrenocortical extracts exert no direct effects on energy metabolism.

Effects of Adrenocortical Hormones on the Thyroid Gland.

The evidence concerning the effects of adrenocortical extracts on the thyroid itself is meagre and contradictory. Black et. al. (36), fed an adrenal residue consisting of the "final filtrate from an aqueous extract of the gland after the removal of the nucleoproteins with acetic acid, and then the coaguable acid and alkali albumens. The resultant filtrate having been boiled at least twice, represents a slightly hydrolyzed preparation". This was administered per-os to dogs in experimental periods ranging from 1 to 6 months. Half of the dog's thyroid was analyzed for iodine content at the start of the experiment, and the other half analyzed at the conclusion of the experiment. With the extract used, an average increase of 70.4% in the iodine content of the gland was noted at the end of 45 days. Another adrenal extract consisting of the adrenal nucleoproteins was also fed to dogs, and this produced in the same length of time, an average increase in the iodine content of the dog thyroid, of 50.7%. Epinephrine had no effect.

Elmer et. al. (37), did not confirm these findings in guinea pigs. Using three groups of animals he injected the first group with thyrotrophin, the second group received cortin, and the third group received cortin and thyrotrophin. Cortin had no effect on the normal thyroid, and was unable to prevent the hypertrophy induced by thyrotrophin. The authors point out that these results are opposed to Marine's theory that Graves disease is the result of adrenocortical insufficiency.

Summary of Evidence on the antagonistic Interrelationship of the Thyroid and Adrenal Cortex

To summarize the arguments in favor of an interrelationship between the thyroid and the adrenal:

1. Thyroxine administration produces adrenal hypertrophy.

2. Administration of goitrogens or thyroidectomy, for the most part produces adrenocortical atrophy. Where there is hypertrophy, it is due to fatty infiltration of the cortex, and is not considered to be due to hyperfunction.

3. Adrenalectomy in those animals without accessory adrenocortical tissue like the dog and cat, will result in a decrease in metabolism until the death of the animal. This decline is sometimes preceded by a slight rise. The decline in metabolism can be prevented with adrenocortical extracts, and epinephrine.

4. In those animals with accessory adrenocortical tissue such as the rat and the mouse, adrenalectomy is tantamount to sublethal adrenocortical injury in those animals that survive the operation. In animals without accessory adrenals such as the dog and cat, if the adrenals are crippled by freezing, or by partial ligation, these animals are then equivalent to adrenalectomized animals with accessory adrenocortical tissue. In this condition, a rise in metabolism is noted immediately following adrenocortical injury, but is not seen where the thyroid is absent.

From these facts it is postulated that cortical hormones in some way exert an inhibiting effect on the thyroid, and when this secretion is lacking, the thyroid is free to exert its well known metabolic effects.

Evidence of a Synergism Between the Thyroid and Adrenal Cortex.

The evidence presented so far is conducive to the idea that there is an antagonism between the thyroid and the adrenal cortex. There is some evidence, however, that the adrenocortical hormones have a synergistic effect with thyroxine. In an abstract of work by Bock (38), it was observed that the addition of 5 cc of cortin to a bath of 500 cc of water containing 0.2 cc of thyroxine, accelerated the metamorphosis of tadpoles and axolotls beyond the rate attained by this concentration of thyroxine alone. This concentration of cortin alone, did not influence metamorphosis.

The adrenal Medulla and the Thyroid

No discussion of an interrelationship between the thyroid and the adrenals could be considered complete without some reference to the relationship of the adrenal medulla to the thyroid.

Levy (40), noted that the thyroid is induced to secrete by stimulation of the cervical sympathetic ganglia, and the thyroid secretion renders more excitable, the sympathetic structures acted on by adrenalin in raising arterial pressure. Cannon and Cattell (41), demonstrated that adrenalin causes an action current in the thyroid, and in a subsequent paper Cannon and Cattell (42), reported that there are non medullated fibers distributed to the thyroid. These fibers were found to be true secretory fibers, as they did not exert their effects through alterations in the blood supply to the thyroid. Cannon found that an action current is evoked in these fibers by adrenalin, and he suggests that this may indicate secretory activity by the thyroid.

Metabolism studies by Marine and Lenhart (43), show that there is an increased oxygen consumption following an injection of adrenalin in normal and thyroidectomized rabbits, but the onset of increased oxygen consumption in a thyroidectomized rabbit is delayed and does not last as long, as in a normal control animal.

The observation of Aub et. al. (39) that there are two mechanisms regulating metabolism in the body, one being adrenaline which serves to raise metabolism quickly and for short periods of time, while the thyroid exerts its effects more slowly, and for longer periods of time, may be worthy of repetition here.

Vitamin C and the Adrenal Cortex

Since the discovery of ascorbic acid in the adrenals by Szent-Gyorgyi (44), there has been a lot of evidence brought forth indicating that vitamin C is in some way concerned with adrenocortical activity. The highest concentrations of ascorbic acid found in the body are in the adrenals, Yavorsky et. al. (45). Harris and Ray (46), noted in guinea pigs, that even though the adrenals are very rich in vitamin C, there is only enough vitamin C present to supply the physiological needs of the guinea pig for 24 hours. With vitamin C administration, the adrenal ascorbic acid concentration does not increase. In scorbutic guinea pigs even when teeth lesions are evident, there are still considerable amounts of ascorbic acid present in the adrenals. During the progress of avitaminosis C, the decline in adrenal ascorbic acid is very slow, and at the death of the animal, there is still present in the adrenal, small amounts of the vitamin. Harris and Ray (46), concluded that vitamin C was necessary for the normal functioning of the adrenals. Quick (47), confirmed this work in guinea pigs. He observed a marked adrenal hypertrophy in guinea pigs dead of scurvey, and using the silver nitrate reduction method of Szent-Gyorgyi (44), for the determination of vitamin C, he noted that the adrenals were depleted of vitamin C. He also noted that there is a marked capillary permeability in vitamin C deficiency which results in diffuse hemorrhages. This condition is also seen in acute adrenocortical insufficiency. It thus appeared that vitamin C was not stored in the adrenals for the physiological needs of the body.

Vitamin C and Adrenocortical Activity.

The first direct evidence that Vitamin C is in some way concerned with adrenocortical activity was advanced by Kuchel and Mitchell (48), in an important, but often overlooked, piece of work. Kuchel and Mitchell (48) noted that when rats were stimulated by fear, injections of acetyl choline, light ether anaesthesia for 45 minutes, curare administration in conjunction with light ether anaesthesia for 45 minutes, electrical stimulation following ether anaesthesia, injections of morphine, injections of eserine, and any situation causing adrenal stimulation, there was a decline in the ascorbic acid content of the glands. Kuchel and Mitchell (48) further noted that adrenaline content bore no constant relationship to ascorbic acid and the ratio of glutathione to ascorbic acid was not altered by adrenal stimulation. Bowman and Muntwyler (49) confirmed some of Kuchel and Mitchell's work in rats and guinea pigs. Euler and Klussman (50), as cited by King (51), noted a 50% depletion in adrenal ascorbic acid in guinea pigs injected with methylene blue. Flexner and Grollman (52), in a series of experiments designed to augment or inhibit adrenocortical activity, observed that when adrenocortical tissue was stimulated by unilateral adrenalectomy, administration of thyrotrophic hormone, inanition due to starvation, and injection of tetrahydro-beta-napthylamine, a sympathetic stimulant, there was an increase in the osmic acid reducing ability of the adrenocortical tissue. Conversely, when the adrenocortical tissue was inhibited by administration of cortical hormones as a purified concentrate, or as a charcoal absorbate, there was a diminished reducing ability of the adrenocortical zones of the adrenal. The reducing substances possibly acting on the osmic acid, were thought by Flexner and Grollman (52), to be the "unsaturated constituents of visible droplets of lipoid," as well as ascorbic acid, glutathione, and cortical hormones.

Torrance (53), noted that a dose of diptheria toxin which will prove fatal to some of the test guinea pigs, will reduce the ascorbic acid content of the adrenals. Long (54), in a review article covering several years of work, noted that injection of adrenotrophic hormone, epinephrine injections, hemorrhage, burns, muscle trauma, cold, painful stimuli of peripheral nerves, and scalds caused depletion of ascorbic acid. Sayers and Sayers (55) in another review article add to this list of stimuli which cause adrenocortical depletion of ascorbic acid, benzene injections, estrogens, chloroform, insulin, tetanus toxin, anoxia, infectious disease, atropine, nicotine, histamine, heat killed typhoid organisms, dibenamine, and intraperitoneal injection of glucose. They further report that a relationship has been found between the intensity of cold, histamine, and epinephrine stimuli, and the absolute depletion of adrenocortical ascorbic acid.

Evidence that Vitamin C is Not Essential to Adrenocortical Activity.

Some evidence that vitamin C is not involved in the secretion of adrenocortical hormones may be gathered from the work of Vars and Pfiffner (56), Svirbely and Kendall (57), and Sure et. al. (58). Vars and Pfiffner (56), maintained dogs on scorbutic rations for periods of 1 to 4 years and did not note signs of scurvey, or adrenal deficiency of vitamin C. Guinea pigs on scorbutic rations do show a diminution of vitamin C in the adrenals and cortical hormones will not prevent this loss. Vars concludes that there is no evidence that the adrenals are concerned with synthesis or metabolism of vitamin C, and if the adrenals play a part, the action is conferred by the adrenocortical hormone.

Svirbely and Kendall (57) postulated that if ascorbic acid was essential to the physiological response of cortin, the absence of ascorbic acid in the diet might be reflected by the amount of cortin required to maintain a dog in normal condition. Failure in physiologic response to cortin would be demonstrated by a change in nitrogen metabolism if the animal was kept on a constant amount of cortin. In his experiment Svirbely adrenalectomized a male and a female dog and placed them on a scorbutic diet. He then injected them daily with ascorbic acid-free cortin and did not note any changes in daily nitrogen balance. The dogs did not show any signs of scurvey with these treatments, and it was suggested by the authors that the dog has the ability to synthesize vitamin C. In guinea pigs, cortin had no effect on the onset of scurvey.

One of the stresses reported by Flexner and Grollman (52) as causing an increase in the osmic acid reducing powers of the adrenal cortex is inanition due to starvation. This caused an increase in the reducing substances of the cortex, one of which was thought to be ascorbic acid. This result was not reported by Sure et. al. (58). They fasted rats for periods of 10-11 days and noted no changes in the concentration of vitamin C in the endocrine organs of the rat. As starvation is certainly a severe stress, it seems surprising that no changes were noted.

Chemical Evidence that Vitamin C is a Part of Adrenocortical Hormones.

The majority of evidence cited shows that vitamin C is somehow concerned with the secretion of adrenocortical hormones.

Some indication that Vitamin C is a side chain on some of the adrenocortical steroid hormones may be gained from the work of Zwemmer et. al. (59). They noted that after crystalline steroid compounds from the adrenal cortex had been crystallized from various solvents, the mother liquor was 10-100 times more potent with regard to adrenocortical activity than the best crystalline product. This proved that most of the hormone activity was tied up in compounds which were soluble not only in aqueous solutions, but also in other solvents as well, Purely steroid compounds are not water soluble, and as vitamin C is found in such large quantities in the adrenals, it led to speculation on a possible linkage between vitamin C and the steroid nuclei.

As Zwemmer et. al. (59), point out, "Evidence for such a combination would be, a) physiological activity of similar substances; b) its isolation from the adrenal glands, and c) enhancing the activity of cortically active steroids by linking them with ascorbic acid or a sugar".

Some of the cardiac glucosides such as Strophanthin, Quabain, Digitalis, and Digitora, are steroids and in addition possess a reducing sugar side chain. It was found that these substances, in common with some of the cortical hormones, will lower blood potassium, increase blood sugar, and decrease plasma protein. Strophanthin, would also diminish the lipoid content and the size of the adrenals as can be accomplished following administration of a wide variety of adrenocortical hormones, and could also protect rats and mice from an ordinarily lethal dose of potassium chloride and insulin.

By analogy then, it would appear that the more active water soluble adrenocortical steroids, have a reducing side chain which is probably vitamin C.

Vitamin C and Adrenocortical Activity in a Hyperthyroid State.

Little work has been done on the relationship of vitamin C to adrenocortical activity in the hyperthyroid state. Sure and Theis (60), reported that in rats fed dosages of thyroxine up to 0.5 mg per day, there was a striking decrease in the ascorbic acid content of the adrenals, the greatest decrease being observed on the 18th day. They further noted that administration of vitamin C and vitamin B_1 counteracted these losses. This work has been confirmed to some extent by Marine et. al. (61). They placed guinea pigs on a scorbutic diet and noted that when they were injected with thyrotrophin, they showed exophthalmous and thyroid hyperplasia more quickly than when adequate vitamin C is present in the diet. Thyroids of guinea pigs given thyrotrophin and vitamin C average 30% smaller than those given thyrotrophin alone. Also vitamin C prevented or lessened the adrenal hypertrophy seen in scurvey or following administration of thyrotrophin. From this work it was concluded that Vitamin C has an inhibiting effect on the thyrotropic hormone of the anterior pituitary.

Effects of Vitamin C Administration on Metabolism.

Belasco and Murlin (62), reported a direct effect on metabolism following administration of vitamin C to rats. They reported that vitamin C administration will reduce the metabolism from plus 59% to plus 35%, and from this they conclude that there is an increased requirement for vitamin C in a hyperthyroid animal. This work was confirmed by Lewis (63) in humans. He studied the vitamin C excretion of five hyperthyroid human patients and noted that before thyroidectomy, the vitamin C excretion was much lower, and following the operation, the vitamin C values in the urine rose to normal, in 4 out of the 5 patients. If there is a relation between the vitamin C content of the adrenals and their secretory activity, and if in a hyperthyroid state there is an increased demand for cortical hormones, then it appears logical to expect that the bodily requirements of vitamin C in a hyperthyroid animal are going to increase. In animals which must get their vitamin C from an exogenous source like the guinea pig, administration of vitamin C should prevent some of the strain on the adrenal caused by an insufficient supply of vitamin C, and thus to some extent prevent, hypertrophy as was shown by Marine et. al. (61).

Further proof that Vitamin C is essential to the secretion of adrenocortical hormones is intimately connected with the relationship of the anterior pituitary to the adrenal.

Relation of the Anterior Hypophysis to the Adrenal Cortex.

The first work reported on the relation of the hypophysis to the adrenal cortex was that of Smith (65). He reported a method of hypophysectomy, and noted as a result of this operation, growth stasis and a regression in size of the adrenal cortex. Intramuscular injections of rat pituitaries into hypophysectomized rats affected a "partial repair" of the adrenals.

This work was confirmed by McQueen-Williams (64). He implanted beef anterior pituitaries into intact rats and noted that this produced adrenal hypertrophy which was largely due to adrenocortical enlargement. He noted a very slight response of the adrenals in a thyroidectomized animal, and from this evidence he concluded that there are two adrenocorticotrophic hormones, one acting directly on the adrenals, and one acting by way of the thyroid. What probably happened here is that the thyrotrophic titer of the implants was higher than the titer of adrenocorticotrophin, which was the cause of the observed results.

The Anterior Hypophysis and Adrenocortical Vitamin C.

Tyslowitz (66), in further confirmation of Smith's work, (65) noted that following hypophysectomy, there was a loss in weight of the adrenals. This was accompanied by a coincident decline in the content of adrenal vitamin C, and also a decline in the vitamin C content of other organs. Tyslowitz (66) came to the rather odd conclusion that the observed decline in vitamin C content of the adrenals was not specific for that organ.

Bowman et. al. (67), confirmed Tyslowitz' work in that he also noted a decline of adrenocortical vitamin C following hypophysectomy to the extent of 45%.

The first anterior pituitary adrenocorticotrophic extracts were prepared simultaneously by Evans (68), and Collip et. al. (69). Evans (68) reported atrophy of the adrenals following hypophysectomy in the rat, and observed that the atrophy was largely restricted to the cortex. He further reported that an alkaline extract of the anterior pituitary will cause regrowth of this atrophied adrenocortical tissue. Collip et. al. (69), reported the first purified adrenotropic extracts. In completely hypophysectomized rats the left adrenal was removed 11 to 148 days following hypophysectomy. This was not followed by hypertrophy of the right adrenal normally seen in intact animals. In 6 control rats, the right adrenal which was removed 1 week after the left, was found to weigh the same or less than the previously removed left gland. A large number of different anterior pituitary extracts were injected into those hypophysectomized animals with the right gland intact, and adrenal repair was noted where the adrenal extract contained the adrenotropic factor. When it did not, no adrenal repair was seen. Collip et. al. (69) also noted that the adrenotropic principle is heat stable as boiling it for thirty minutes did not effect its potency.

In 1943 Li et. al. (70) and Sayers et. al. (71), presented methods for preparing the adrenotropic principle from the sheep and hog pituitary respectively, and also demonstrated the chemical properties of this hormone. Sayers et. al. (71), noted that 5 gamma of his hog pituitary preparation, could maintain the adrenals of a hypophysectomized rat in a normal condition.

Following the preparation of pure adrenocorticotrophic hormones, Sayers et. al. (72), observed that intraperitoneal injections of these extracts will produce in 20 minutes a diminution in the ascorbic acid of the rat adrenal gland to 2/3 of normal. A maximum decrease is seen in one hour and the vitamin remains at this low level for 2 to 3 hours and returns to the initial level nine hours later. Adrenal cholesterol shows a maximum decrease 3 hours after injection, and its restoration to normal levels is at a slower rate than that of vitamin C.

Sayers and Sayers (55) in a review article, sum up the evidence concerned with the regulation of adrenocortical vitamin C by the anterior pituitary, and conclude that measurement of adrenal vitamin C is an accurate measure of the activity of this gland. This conclusion is justified by the following facts, cited by Sayers and Sayers (55).

"a) Administration of purified A.C.T. and subjection to stress produce a depletion of ascorbic acid within a period of less than one hour.

b) Removal of the pituitary leaves the adrenal ascorbic acid nonresponsive to stress.

c) Purified A.C.T. free from other pituitary activities depletes ascorbic acid of the adrenal of the hypophysectomized rat. A quantitative relationship exists between the dose of A.C.T. injected and the amount of reduction of ascorbic acid."

Long (54), emphasizes that in response to stress, the first response of the organism is to secrete adrenotrophic hormone from the anterior pituitary, and this in turn, acting on the adrenals produces the observed changes in adrenal ascorbic acid. Presumably the effects of thyroxine would be exerted by way of the anterior pituitary, as it has been demonstrated that the adrenal cortex fails to respond in a hypophysectomized animal. It should be noted, however, that Miller and Riddle (73), reported maintenance of normal adrenal size in hypophysectomized pigeons injected with thyroxine.

Effects of Administration of Adrenocortical Extracts on the Adrenal Cortex

Mechanism of Atrophy.

There is extensive literature reporting that administration of adrenocortical extracts, and hormones structurally related to those secreted by the adrenal cortex, will produce adrenocortical atrophy. This has been observed by Korenchevsky et. al. (74), Ingle and Kendall (75), Ingle et. al. (76), Ingle (77), Selye (78), Selve and Dosne (79), Carnes et. al. (81) and Lowenstein (11). Ingle et. al. (75) (76) suggested a mechanism for this reaction. They observed that when adrenocorticotrophic hormone from the anterior pituitary, was injected coincident with large amounts of cortin, the otherwise observed atrophy was prevented. They suggested that the anterior hypophysis is sensitive to the amount of cortin, in the body fluids or to the physiological responses produced by cortin, and that changes in the adrenal cortex are influenced by the secretion of adrenocorticotrophic hormone from the anterior pituitary. When there is an amount of cortin present in the body above physiological requirements, the output of adrenocorticotrophic hormone is supressed. Ingle (77), further observed in support of his theory, that when the hypophysis is absent, and the size of the adrenals is maintained with adrenocorticotrophic hormone (henceforth referred to as A.C.T.) that cortin has no effect on the adrenals.

A Sex Difference in Response.

A sex difference in the response of the adrenals to adrenocortical and related hormones was reported by Ingle (82). He noted that in the female rat the adrenal is bigger, and does not show as marked an atrophy following the administration of a given amount of cortin, as in the male. The maximum extent of adrenocortical atrophy producable by Cortin administration was found to be comparable to the atrophy observed in the adrenals 1 week after hypophysectomy. Selye (80), in contradiction to Ingle's work, noted that the female adrenal was more sensitive to treatment with desocycorticosterone, and he suggested that the reason for this increased sensitivity was due to the presence of more adrenocortical tissue in the female. When this atrophied, it would produce a more striking decrease in weight. He also noted that the female adrenal was much more sensitive to the involuting action of progesterone, than was the male adrenal.

Negative Effects of Adrenocortical Hormones on the Adrenal.

No effects of adrenocortical extracts on the adrenal have been reported by Howard and Grollman (83), and King (84).

Howard and Grollman (83), injected the equivalent of 10 to 40 gms of fresh beef adrenocortical tissue into rats and did not note any changes in the adrenals. King (84), also failed to note changes in the adrenals of rats fed a charcoal absorbate of beef adrenals.

Adrenocortical Implants and the Adrenocortical Hormones.

Some rather interesting indirect evidence that adrenocortical hormones have an inhibiting effect on adrenocortical tissue, may be gathered from the work of Wyman and Tum-Suden (85). They found that autoplastic and homoplastic transplants of adrenocortical tissue will not grow in the presence of already functioning adrenocortical tissue. In a subsequent paper, Wyman and Tum-Suden (86), concluded that A.C.T. was necessary for growth of adrenocortical transplants. The fact that transplants will not grow in the presence of functioning adrenocortical tissue, is due to the inhibiting effects of the hormones secreted by this tissue, on the secretion of A.C.T. by the anterior pituitary.

Effect of Adrenocortical Hormones on the Adrenals in Conditions of Prolonged Stress.

In conditions of prolonged stress, adrenocortical hormones can prevent adrenocortical hypertrophy, as was demonstrated by Selye et. al. (87) (88). The stresses reported on were surgical shock and injection of 4% formaldehyde solution. It was noted that the adrenals of the female rat are more sensitive to such stresses, and that administration of desoxycorticosterone could prevent adrenal enlargement due to formaldehyde injections in this sex, but had no effect in the male.

A Contradiction to the Mechanism of Atrophy Suggested Previously.

The theory that adrenocortical hormones have an inhibiting effect on the secretion of A.C.T. would seem to explain these results very satisfactorily were it not for some interesting contradictory evidence reported by Selye (80). He noted that following administration of desoxycorticosterone to the rat, all three zones of the adrenal cortex atrophy. This is not true of hypophysectomy as the zona reticularis undergoes such rapid involution that numerous hemorrhages appear in this region. If adrenocortical hormones do inhibit A.C.T. secretion by the anterior hypophysis, then the histological appearance of the adrenals should be identical with that seen following hypophysectomy. No satisfactory explanation has yet been offered for the differences noted here.

Histological Changes in the Adrenals in Hyper- and Hypothyroid States

In the previously cited literature passing reference has been made to histological changes seen in the adrenals following different treatments. At this point it seems advisable to cite a reference concerning specific regions of the adrenal affected by hyper- and hypothyroid states. Deane (89), noted that in hypothyroidism induced either by thiouracil, or thyroidectomy, the zona fasciculata of the adrenal cortex atrophies and its ketosteroid content is reduced. The zona glomerulosa on the other hand, increases in activity, and contains an abnormal amount of steroid material. Following thiouracil administration, this zone becomes temporarily exhausted. In a hyperthyroid state, the zona glomerulosa is also active coincident with the zona fasciculata. This latter zone in prolonged stages of hyperthyroidism begins to show necrosis on its interior side. The zona glomerulosa becomes exhausted of ketosteroids. Deane (89) believes that A.C.T. has control only over the zona fasciculata, and in hypothyroidism this zone atrophies due to a lessened secretion of A.C.T. The zona glomerulosa, however, is not under pituitary control, but responds to changes in salt and water balance resulting from the different states.

MATERIALS AND METHODS

Male rats of the Michigan State College strain were used in all the phases of this investigation except as otherwise stated in the text.

Extraction Procedure

After a given experimental treatment, a uniform method was followed in killing the animals, and taking the desired observations. The animals were killed by decapitation and immediately afterwards the adrenals were removed and placed on a piece of moist filter paper. They were then carefully trimmed of adipose connective tissue, and weighed on a Roller Smith torsion balance to the nearest tenth of a milligram.

Following this they were placed in previously prepared 50 cc centrifuge tubes containing about 3.5 gms of course sand saturated with 1 cc of 3% metaphosphoric acid. The glands were macerated to as fine a degree as possible by gentle grinding with a glass rod. To the resulting mass, 10 cc of metaphosphoric acid was added and the whole stirred vigorously. The tubes were centrifuged at approximately 2500 revolutions per minute for five minutes; the resulting supernatant liquid was removed to a clean centrifuge tube, and the residue of sand and tissue was washed and centrifuged twice more with 10 and 5 cc portions of metaphosphoric acid.

To the resulting slightly turbid extract containing small amounts of suspended tissue, was added a small amount of Celite, a filtering aid, which material was stirred into the extract until it was milky white, and the resulting suspension was centrifuged for fifteen minutes. The supernatant liquid was completely freed of turbidity and floating pieces of tissue by this process.

A twenty cc aliquote of this clear extract was then buffered to a pH range of 3.0-3.5 with 10 cc of a sodium citrate buffer, after which it was ready for the estimation of ascorbic acid.

Method for Estimation of Ascorbic Acid

The method used for the determination of ascorbic acid was essentially that of Mindlin and Butler (90), as modified for plant and animal tissues by Bessey (91). The method is based on the ability of ascorbic acid to reduce a solution of the indicator dye, 2,6 dichlorophenol indophenol, the extent of reduction as measured by increased light transmission in a spectrophotometer, being an indication of the potency of the sample.

For all ascorbic acid determinations in this study, a Coleman Universal Spectrophotometer with a filter setting of 520 was used.

Five cc of the aforementioned adrenal ascorbic acid extract, was blown with an Ostwald pipette into a spectrophotometer cuvette containing 5 cc of the indicator solution 2,6 dichlorophenol indophenol and a reading was made 30 seconds afterward in comparison with a blank of distilled water. Occasionally gas bubbles would be noted in the cuvette immediately following transfer of the extract to the indicator solution, but it was always possible to dislodge these by gently jarring the cuvette against the side of a table. In the method described by Mindlin and Butler (90), after the final reading is made, a crystal of ascorbic acid is added to the cuvette in the spectrophotometer to further reduce any dye that remains, and the reading, if it is below 100, is compensated for in the calculations for ascorbic acid. In these determinations it was always possible to get readings of 98 to 100 when an ascorbic acid crystal was added and so this procedure was omitted.

Calculations.

The formula for the determination of ascorbic acid as derived by Mindlin and Butler (90), is: $C = K(\log Gs - \log Gb)$. C is the ascorbic acid content of the unknown sample. Log Gs, is the log of the galvanometer reading of the sample at 30 seconds. Log Gb is the log of a blank of citrate-buffered metaphosphoric acid and indicator dye solution read at 30 seconds. K is a constant and was calculated from ascorbic acid solutions of known concentration by the formula $\frac{C}{(\log Gs - \log Gb)} = K$. When the formula is applied, the resulting answer is the micrograms of ascorbic acid per cc of extract added to the indicator solution. This answer, corrected for the dilution to 36 cc of the total ascorbic acid present in the adrenals, results in the total amount of ascorbic acid present in the adrenals.

Preparation of Reagents

Musulin and King (92), found that metaphosphoric acid is a superior extractive agent for ascorbic acid, and exerts a positive protective action against oxidation of the ascorbic acid. Thus for all determinations, metaphosphoric acid in 3% solution was used.

The metaphosphoric acid was prepared by the method of Briggs (93). In this method sodium dihydrogen phosphate ($NaH_2PO_4 \cdot H_2O$) is heated in a muffle furnace at 700 degrees Centigrade for 1 hour. This drives off two molecules of water and in addition polymerizes the sodium metaphosphate ($NaPO_3$) to a glass-like compound of indefinite formula, $(NaPO_3)_x$. This molten glass-like material was poured onto an aluminum plate, and following cooling was made up into a 3% solution. This solution was stable indefinitely. Immediately before use 1 cc of glacial acetic acid was added per 100 cc of metaphosphate solution, to convert it to metaphosphoric acid.

The citrate buffer used in these determinations was made by dissolving 21 gms of citric acid in 200 cc of water and then adding enough sodium hydroxide pellets to raise the pH to a range of 3.0-3.5. The pH was determined with a beckman pH meter. The indicator solution of 2,6, dichlorobenzenone indophenol (Eastman), was made by dissolving approximately 10-12 Mg. of this dye in 500 cc of water.

EXPERIMENT I

The Effect of Time on the Response of the Adrenals to a Constant Level of Thyroidal Stimulation

It has been demonstrated by Long (54) and Sayers and Sayers (55), that time relationships are very important in the short time response of the adrenals to acute stress. This experiment was designed to investigate the response of the adrenals to the more prolonged stimulus of thyroxine injections.

Seven groups of 5 rats each, and 1 group of 8 rats were injected daily with a solution of d,l thyroxine at a dosage of 20 micrograms per 100 gm. of body weight. This dosage of thyroxine is about 5 times the normal secretion rate of the strain of rats used according to unpublished data of Dr. E. P. Reineke, who estimated this rate using the method of Dempsey and Astwood (94).

Four groups of controls were used. Group 1 was killed at the start of the experiment, and the second, third, and fourth groups were killed after 2, 4, and 6 weeks respectively. The latter three groups received daily injections of 0.1 cc of physiological saline solution per 100 gm of body weight.

Although there is some variation between the different control groups, no consistent trends in adrenal weight, or ascorbic acid content were noted. Consequently, the values for the adrenal weight and ascorbic acid content of the 4 control groups were pooled, and are shown graphically in Figure 1, as the base line or 100 percent. The mean control adrenal weights with standard deviation was 42.0 ± 5.98 mg. The value for ascorbic acid was 194.8 ± 52.7 micrograms. All the values for the experimental groups were calculated as percentages of

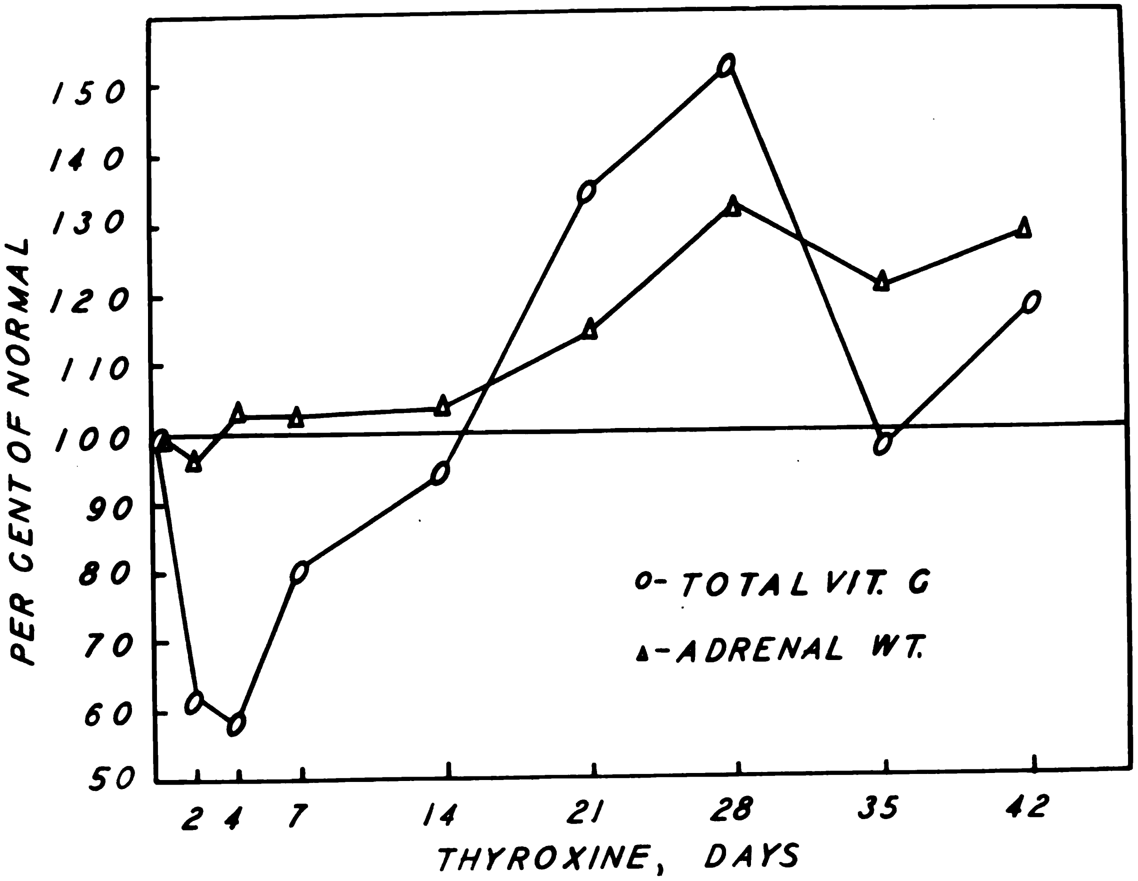

Fig. 1. Changes in the weight and ascorbic acid content of the adrenals brought about by a dosage of 20 micrograms of thyroxine per 100 gm. body weight during a six week experimental period.

TABLE 1, Experiment 1: The Effect of Time on the Ascorbic Acid Response of the Adrenals to a Constant Dosage of 20 Mcg. of Thyroxine per 100 gms body Weight.

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	TOTAL VIT. C Mcg.	Daily TREATMENT
1.	5	2	222 ± 27*	40.4 ± 5.61	118.7 ± 25.4	20 Mcg. of Thyroxine per 100 gms body weight
2.	5	4	209 ± 38	43.1 ± 4.51	111.2 ± 8.71	20 Mcg. of Thyroxine per 100 gms body weight
3.	5	7	213 ± 11.5	43.1 ± 4.8	134.5 ± 14.2	20 Mcg. of Thyroxine per 100 gms body weight
4.	4	14	231 ± 17	43.3 ± 3.8	183.3 ± 11.2	20 Mcg. of Thyroxine per 100 gms body weight
5.	5	21	238 ± 25	48.2 ± 5.3	260.9 ± 48.7	20 Mcg. of Thyroxine per 100 gms body weight
6	5	28	271 ± 19	55.5 ± 6.31	296.0 ± 15.4	20 Mcg. of Thyroxine per 100 gms body weight
7	4	35	270 ± 20	50.5 ± 2.91	188.6 ± 11.1	20 Mcg. of Thyroxine per 100 gms body weight

* Standard Deviation

TABLE 1 (Continued)

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	TOTAL VIT. C Mcg.	Daily TREATMENT
8	8	42	289 ± 46.0*	53.9 ± 8.4	228.4 ± 39.8	20 Mcg. of Thyroxine per 100 gms body weight
Controls 9	5	-	246 ± 6.62	44.8 ± 6.2	192.7 ± 46.8	No treatment
Controls 10	5	14	240 ± 15.7	36.9 ± 2.9	190.7 ± 29.2	0.1 cc. Physiological Saline per 100 gms. body weight
Controls 11	5	28	276 ± 23.3	48.9 ± 3.4	213.2 ± 21.8	0.1 cc. Physiological Saline per 100 gms. body weight
Controls 12	7	42	272 ± 14.0	38.7 ± 2.3	200.5 ± 17.4	0.1 cc. Physiological Saline per 100 gms. body weight
Pooled Controls	22	-	260 ± 20.0	42.0 ± 5.9	194.8 ± 52.7	0.1 cc. Physiological Saline per 100 gms. body weight

* Standard Deviation

this base.

It can be readily seen (Fig. 1) that the initial response of the adrenals to thyroidal stimulation was a sharp decline in vitamin C content. This decline was greatest at about 4 days; the values then began to rise. At 2 weeks, the total vitamin C in the adrenals was approximately normal, or a little below, and the adrenals had not as yet, begun to show signs of significant hypertrophy. At three weeks, the adrenals were significantly hypertrophied, and the vitamin C content was considerably increased. The zenith in response was reached at 4 weeks, after which there was a decline in ascorbic acid content at the fifth week to approximately normal values. The adrenals also showed a slight decrease in size. At the sixth week, there was again a slight increase in adrenal weight and a marked increase in vitamin C content.

The results of this experiment demonstrate that time relationships are very important in a study of the adrenal response to a prolonged stimulus. If we accept the fact that the vitamin C content of the adrenals is an index of secretion, it would further appear from these results, that the adrenals are discharging hormone into the blood stream in the early stages of induced hyperthyroidism. This may be deduced, from the immediate and abrupt decrease in adrenal vitamin C. Concurrently, with the development of more secreting units in the adrenal at the third week, as shown by increased weight, there is an increase in ascorbic acid content above normal values, which may mean that not only is there an increased rate of secretion, but also an increased rate of hormone synthesis. The supply of adrenocortical hormones probably balances the demand at the fourth week, as the vitamin C values and adrenal weights in response to this level of thyroidal stimulation do not increase beyond this time.

At 4 weeks there may even be an over supply of adrenocortical hormones, and when these are discharged, they inhibit further liberation of A.C.T. by the anterior pituitary. Thus at the fifth week, there was a significant decline in adrenal vitamin C, and a noticeable decrease in adrenal weight. At the sixth week, a balance may be presumed to be established, in which the size of the adrenals, and the rate of hormone synthesis and secretion, is adequate for the degree of thyroidal stimulation to which the adrenals are being subjected.

EXPERIMENT II

Effect of Varying Levels of Thyroidal Stimulation on Adrenal Weight and Ascorbic Acid Content

It has been demonstrated in Experiment I, that when a dosage of thyroxine, approximately five times the normal secretion rate of the strain of rats used, is injected daily, the greatest response of the adrenals is seen at 4 weeks. This experiment was designed to discover what the effects of varying levels of thyroidal stimulation, are on the response of the adrenals at 4 weeks.

In the first part of the experiment, 5 rats weighing about 150 gm, were thyroidectomized. Four other rats the same size were subjected to a sham operation in which the thyroid was exposed, and each lobe was grasped with a pair of forceps. As can be seen in Table 2, the thyroidectomized group ceased growth. The controls gained nearly 100 gm. in the 4 weeks following the sham operation. At the end of the experimental period the adrenals were dissected out, weighed, and analyzed for ascorbic acid. As may be observed in Table 2, there was a highly significant decrease in ascorbic acid content and adrenal weight of the group subjected to thyroidectomy. Thus it might be said that normal adrenal size, and presumably secretory activity are dependent to a great degree on the normal secretion rate of the thyroid.

Koger and Turner (95) showed that dosages of thyroprotein in the feed ranging from 0.01% to 0.16% produced noticeable and largely adverse effects in male rats with regard to growth,

TABLE 2, EXPERIMENT 2, PART 1. Effect of Thyroidectomy.

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL Wt. Mg.	TOTAL VIT. C Mcg.	TREATMENT
1	5	28	152 ± 17.0*	27.4 ± 2.5	125.6 ± 11.2	Thyroidectomy
2	4	28	241 ± 25.0	37.6 ± 5.0	235.0 ± 47.0	Sham Thyroidectomy
Part 2. Effect of Feeding Protamone at Physiological Levels of Stimulation.						
1	9	28	238 ± 24.0	38.5 ± 5.3	180.4 ± 22.6	0.01% Protamone in feed.
2	8	28	231 ± 22.0	35.0 ± 11.8	142.3 ± 27.8	0.02% Protamone in feed.
3	9	28	231 ± 36.0	41.3 ± 4.4	186.7 ± 26.4	0.04% Protamone in feed.
4	10	28	237 ± 7.0	43.7 ± 2.2	208.9 ± 40.7	0.08% Protamone in feed.
5	10	28	219 ± 23.0	40.3 ± 7.5	185.9 ± 46.2	0.16% Protamone in feed.
Controls	9	28	243 ± 26.0	39.9 ± 5.2	170.5 ± 27.8	No Treatment

* Standard Deviation

TABLE 2 (Continued), Part 3. Effect of Injections of Thyroxine at High Dosage Levels.

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	TOTAL VIT. C Mcg.	Daily TREATMENT
1	4	28	257 ± 12.0*	42.3 ± 3.3	204.6 ± 26.4	5 Mcg. of Thyroxine /100 gm. Rat.
2	4	28	251 ± 26.0	49.8 ± 3.3	246.5 ± 16.0	10 Mcg. of Thyroxine /100 gm. Rat.
3	5	28	271 ± 19.0	55.5 ± 6.3	296.0 ± 15.4	20 Mcg. of Thyroxine /100 gm. Rat.
4	5	28	236 ± 9.0	55.3 ± 5.5	282.4 ± 38.6	40 Mcg. of Thyroxine /100 gm. Rat.
5	5	28	223 ± 19.0	63.8 ± 5.9	401.7 ± 31.3	80 Mcg. of Thyroxine /100 gm. Rat.
Pooled Controls Exp. 1	22	-	260 ± 20.0	42.0 ± 5.9	194.8 ± 52.7	0.1 cc. Physiological Saline /100 gms. Rat.
Normal Females	8	-	220 ± 19.0	52.4 ± 6.8	229.9 ± 22.4	No Treatment

* Standard Deviation

the higher levels showing a definite toxic effect. The effects on the adrenals varied with 2 strains employed. One strain showed a hypertrophy of the adrenals which "paralleled roughly the level of thyroprotein fed." Females of the other strain showed this hypertrophy, but surprisingly, the males did not show any increase, and with some dosages used, the adrenal weights showed a statistically significant decrease.

In the second part of this experiment the dosages used, correspond to the first 5 dosage levels used by Koger and Turner (95) and range from 0.01% to 0.16% thyroprotein mixed in the feed. As can be seen in table 2, there is no significant trend in either adrenal weight or ascorbic acid content of any of the groups when compared with the controls. The animals remained on the treatment for 4 weeks, and no toxic symptoms were noted. It did appear, however, that as the dosage of thyroprotein was increased, the rate of feed consumption also increased. No figures are available for this however. It might be deduced from this work that the dosages of thyroprotein used were not sufficient to evoke any additional response from the adrenals which their normal size, and hormone producing ability could not take care of. Thus no significant hypertrophy or increase in Vitamin C content was noted.

The Protamone dosages used in the preceding work were comparatively low. To complete the picture of the effects of varying dosages of thyroidal stimulation on the adrenals, it was felt necessary to administer dosages of thyroxine at levels considerably above those heretherto employed.

Accordingly 5 groups of rats were placed on daily injections of thyroxine ranging from 5 micrograms to 80 micrograms per 100 gm. body weight. As can be seen from Part 3, Table 2, 5 micrograms per 100 gm. body weight had no effect either on adrenal weight or ascorbic acid content. One possible reason for this is that this daily dosage is approximately the same as the normal thyroid secretion rate of this strain of rats. A great increase is observed with 10 micrograms per 100 gm. body weight, both in adrenal size and ascorbic acid content. With 20 micrograms of thyroxine per 100 gm. body weight, an even greater increase is seen. At dosages of 10 and 20 micrograms of thyroxine per 100 gm. body weight, it appears that there is a progressive increase in size and ascorbic acid content of the adrenals. This progressive response is not seen when the dosage level is increased to 40 micrograms per 100 gm. body weight. It might be postulated that the adrenals in a rat receiving 20 micrograms of thyroxine per 100 gm. body weight are of a size and degree of secretory activity capable of coping with daily dosages of thyroxine ranging from 20 to 40 micrograms per 100 gms of body weight. When the dosage of thyroxine is again increased to 80 micrograms per 100 gm. rat per day, there is again a great increase in the size and ascorbic acid content of the adrenals.

It should be pointed out here that the influence of time was not investigated for all these dosage ranges, and whether on the higher dosages, the ascending or descending part of the curve, was intercepted is not known. Also, it is not known whether the effects of higher dosages would magnify the intensity of the decline and increase in the curve, or would lengthen or shorten the curve.

It has been reported by numerous authors that the adrenals of males are smaller than those of females. It was thought that if this fact were true, there might also be an appreciable difference in ascorbic acid content. Accordingly, in this experiment, a group of 8 females approximately the same age, were killed concurrently with the normal controls in Part 2. It was found that while the female adrenals were significantly heavier than the males, the ascorbic acid content was not significantly different in either sex, even though, as can be seen in table 2, the females did have a higher content of ascorbic acid.

EXPERIMENT III

A Determination of the Secretion Rate of the Adrenal Cortex in the Hyperthyroid Rat

A method for the determination of the secretion rate of the thyroid gland was proposed by Dempsey and Astwood (94), and has been successfully used by others for the determination of the secretion rates of the thyroids in various species of animals. The basis of this method is the inhibition of the secretion of the animal's own thyroid with thiouracil. This normally produces hypertrophy of the thyroid gland due to increased thyrotrophic stimulation from the anterior pituitary. If this increased thyrotrophin secretion is prevented by the simultaneous administration of thyroxine, such that the weight of the animal's own thyroid is maintained within normal limits, the dosage of thyroxine necessary to accomplish this is taken as the secretion rate of thyroxine for the species in question.

An analogous method for the determination of the secretion of adrenocortical hormones in hyperthyroid rats was tried in this experiment. As has been shown in the preceding experiment, the maximum response of the adrenals to a dose of 20 micrograms per 100 gm. of body weight is seen at the fourth week. It was thought that if this response could be wholly or in part prevented, by the simultaneous injection of adrenocortical hormones, the dose of cortical hormones necessary to accomplish this would be an indication of the increased secretion rate of the adrenals in animals subjected to thyroidal stimulation.

A preliminary trial in hyperthyroid rats, showed that the secretion rate expressed in dog units was going to be very high. (According to Cartland and Kuizenga (96), the normal secretion rate of adrenocortical hormones in the rat is about 300 times that of the dog.) Through the kindness of Dr. H. O. Von Rosenberg of Parke Davis Co., Detroit, Michigan, 3500 dog units of the suprarenal cortical extract "Eschatin" were supplied for use in these experiments. This extract was injected into 3 groups of 5 rats each which had been receiving thyroxine injections of 20 micrograms per 100 of body weight for 20 days. Group 1 received 10 units of Eschatin per day. Groups 2 and 3 received 20 and 40 units per day respectively. The animals were kept on the simultaneous injections of thyroxine and cortical extracts for 8 days, after which they were sacrificed, and the adrenals removed and analyzed for ascorbic acid.

Group 1, which received 10 units of Eschatin per day, showed no statistically significant decrease in either adrenal weight or ascorbic acid, when compared with the group receiving the same amount of thyroxine alone for 28 days in experiment 2. There is a noticeable decrease in adrenal weight, however, but not in vitamin C.

The next two groups receiving 20 and 40 units of eschatin per day respectively, in addition to thyroxine injections, showed no significant differences, when compared to each other, in either adrenal weight, or ascorbic acid. When these two groups were compared with the group receiving thyroxine alone for 28 days, a highly significant decrease in adrenal

TABLE 3, EXPERIMENT 3 - The Inhibition of Adrenocortical Hypertrophy Due to Thyroxine, With Adrenocortical Extract

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	TOTAL VIT. C Mcg.	DAILY TREATMENT
1	5	28	276 ± 12.7*	52.8 ± 5.9	289.6 ± 30.1	20 Mcg. of Thyroxine /100 gms rat + 10 Units Eschatin per day
2	5	28	262 ± 11.7	46.9 ± 4.5	249.2 ± 11.7	20 Mcg. of Thyroxine /100 gms rat + 20 Units Eschatin per day
3	5	28	261 ± 9.6	46.8 ± 5.7	261.2 ± 9.6	20 Mcg. of Thyroxine /100 gms rat and 40 Units Eschatin per day
4	5	28	271 ± 18.8	55.5 ± 6.3	296.0 ± 15.4	20 Mcg. of Thyroxine /100 gms rat

* Standard Deviation

size was seen as well as a significant decrease in ascorbic acid.

It appears from these data that the increase in secretion rate of adrenocortical hormones in rats receiving 20 micrograms of thyroxine per 100 gm of body weight, is somewhere between 10 and 20 dog units per day. Above the dosage of 20 units, which produces a maximum effect, additional adrenocortical hormone has no effect.

EXPERIMENT IV

The Effect of Thyroxine on the Adrenals of Pregnant Rats and on the Adrenals of Their Offspring

Evidence has been advanced in experiments 1 and 3 of this report, that the adrenals in a hyperthyroid rat are in a state of hypersecretion, and that the secretion rate is equivalent to 10 to 20 dog units of adrenocortical hormones.

Some question arose as to the effects of thyroxine on the adrenals of pregnant rats, and to its possible effects on the adrenals of the offspring. Also, if the adrenals of a hyperthyroid dam are in a state of hypersecretion, what is the effect of this increased secretion on the fetal adrenals?

Only one piece of work on this question has been found by the author. Hoskins (1), reported that when desiccated thyroid was fed to pregnant guinea pigs, the adrenals of the mother hypertrophied, and the adrenals of the offspring were atrophied. Hoskins (1) attributed this decrease in size of the adrenals of the offspring, which reached 53% in some cases, to "a reaction in the adrenals of the offspring to epinephrinemia caused by thyroidism in the mother". Hoskins also thyroidectomized some pregnant guinea pigs and noted that the adrenals of the offspring were hypertrophied, the average increase being 20%. He surmised that this hypertrophy was due to "a reaction to adrenal deficiency caused by hypothyroidism in the mother". The results of this paper seem to indicate that the adrenals of the fetus are sensitive to the secretory status of the dam's adrenals. When the dam is in a hyperthyroid condition the adrenals of the fetus atrophy due to adrenocorticotrophin suppression, brought about by the excessive adrenocortical secretion of the dam.

If these observations of Hoskins on guinea pigs were valid for rats, presumably the ascorbic acid content of the fetal adrenals should be appreciably altered if hyperthyroidism was induced in the mother.

Four pregnant rats were selected and injected daily with 20 micrograms of thyroxine per 100 gm. body weight, for periods ranging from 16 to 30 days, prior to parturition. Three rats served as controls. Approximately 12 hours after parturition in each case, the rats were killed and the adrenals weighed and analyzed for ascorbic acid. The adrenals of the offspring were treated in a somewhat different manner due to their small size. Instead of each pair of adrenals being analyzed separately, the adrenals from a litter of rats were pooled, after being individually weighed, and the total ascorbic acid content was divided by the number of baby rats which had contributed their adrenals to the sample in order to obtain the total adrenal vitamin C per rat.

It can be seen from Table 4 that there is no difference in either the weights or the vitamin C contents of the adrenals of the control litters, and the litters from hyperthyroid mothers, even though the adrenals of the hyperthyroid mothers reached tremendous weights, in one case a total of 106.1 mg.

Thus it may be deduced from this work that either the adrenals of the fetal rat are not responsive to thyroidal and adrenocorticotrophic stimulation, and adrenocortical

TABLE 4, EXPERIMENT 4, PART 1. The Effect of a Constant Dosage of Thyroxine on the Weight and Vitamin C Content of the Adrenals of Pregnant Rats.

FEMALE NO.	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	TOTAL VIT. C Mcg.	Daily TREATMENT
1	16	260	73.7	233.4	20 Mcg. of Thyroxine /100 gms. Rat.
2	30	272	94.4	369.7	20 Mcg. of Thyroxine /100 gms. Rat.
3	17	260	78.4	298.9	20 Mcg. of Thyroxine /100 gms. Rat.
4	17	300	106.1	427.2	20 Mcg. of Thyroxine /100 gms. Rat.
5	-	250	70.5	266.1	--
6	-	250	76.0	268.2	--
7	-	300	57.0	188.3	--

TABLE 4, EXPERIMENT 4, PART 2. The Response of the Fetal Adrenals to a Constant Dosage Level of Thyroidal Stimulation in the Mother.

GROUP	NO. OF ANIMALS	DAYS ON TRIAL	BODY WEIGHT Gm.	ADRENAL WT. Mg.	Mcg. VIT. C Per Rat
Thyroxine: Litter 1	8	16	5.8 ± 0.89	3.1 ± 0.8*	9.6
Thyroxine: Litter 2	10	30	5.6 ± 0.76	2.4 ± 0.51	7.9
Thyroxine: Litter 3	4	17	5.9 ± 0.547	2.6 ± 0.9	8.2
Thyroxine: Litter 4	11	17	6.0 ± .633	2.06 ± .565	7.1
Control: Litter 5	5	-	7.7 ± 0.55	2.7 ± 1.35	7.1
Control: Litter 6	8	-	7.3 ± 0.447	2.9 ± 0.947	8.3
Control: Litter 7	12	-	5.5 ± 1.2	2.2 ± 0.538	8.3

* Standard Deviation

suppression, from the mother, or that the placenta is not permeable to these hormones.

It should be pointed out however, that this work does not refute the work of Hoskins (1), because he used guinea pigs, and the reactions he observed may be a species peculiarity.

DISCUSSION

Claude Bernard has observed, as have others, that "Vital mechanisms have one object, preserving constant the internal environment". Numerous other workers have observed that biological organisms, like the societies of today, must achieve a state of balance, and that when, one part of the organism is subjected to a stress, the other parts of the organism attempt to compensate in one way or another for this stress. The degree of success attained, is measured by the ability of the individual to survive.

With these ideas in mind, one can proceed to the idea of balance between two individual organs, in particular the thyroid gland, and the adrenal cortex.

It has been shown in the experimental work of this report, that the level of thyroidal stimulation determines the size and secretory rate of the adrenal cortex to a large extent. Thus when the thyroid is removed, the adrenals atrophy and the ascorbic acid content decreases. There is a rather narrow range in which thyroidal stimulation has no appreciable effect on the adrenals, and this range is approximately the secretion rate of the normal animal. When the level of thyroidal stimulation is boosted above the "physiological" range, the adrenals respond with increased secretion, and this increase is for the most part progressive with increased dosage.

When measuring the "hormone content" of the adrenal, it might remain a question in the minds of some, whether an increased content of vitamin C meant increased synthesis and subsequent liberation of the adrenocortical hormones, or whether the increase in ascorbic acid is indicative of increased storage. In favor of the former interpretation is the fact that the initial response of the adrenals to adequate thyroidal stimulation, is a decrease in ascorbic acid. This decrease in ascorbic acid content has been shown to be due to liberation of adrenocortical hormones. Only when enough secretory units are laid down, so that the adrenals can balance the demand for their hormones, does the content of ascorbic acid show an increase.

Another piece of evidence that the adrenals in a hyperthyroid animal are in a state of hypersecretion, may be gathered from Experiment 3. Here it was demonstrated that the increase in adrenocortical secretion rate of a hyperthyroid rat on a dosage of 20 micrograms of thyroxine per 100 gm. body weight, is between 10 and 20 dog units of adrenocortical hormones, this dosage being necessary to cause atrophy of the adrenals. This is prima-facie evidence that the adrenals are in a state of active, if not hyper-secretion.

In experiment 4, some interesting questions are raised for further study. In this experiment it was demonstrated that thyroxine dosage will superimpose its effects on the adrenocortical hypertrophy produced by pregnancy. In addition to this, it was observed that thyroxine dosage has no appreciable effects on the adrenals of the offspring of hyperthyroid mothers. It is not clear at this time whether the reason for the negative response of the fetal adrenals is due to impermeability of the placenta to maternal adrenocorticotrophin, adrenocortical hormones, and exogenous thyroxine, or whether it is due to a lack of responsiveness of the fetal endocrine system.

The literature previously cited, has amply demonstrated that the mechanism of adrenocortical response to stimuli is mediated through the anterior pituitary. It has been demonstrated for at least one species, however, that thyroxine alone will maintain the normal size of the adrenals. (Miller and Riddle, (73), in pigeons.)

SUMMARY AND CONCLUSIONS

1. It has been demonstrated that time is a very important factor in the response of the adrenals to thyroxine stimulation. The initial adrenal response is a sharp decline in ascorbic acid followed by increases both in adrenal size and ascorbic acid content. The maximum response of the adrenals on a thyroxine dosage of 20 micrograms per 100 gm. body weight, is seen at the fourth week, followed by a slight decline in ascorbic acid and adrenal weight, at the fifth week and then a slight increase again at the sixth week.

2. In thyroidectomized rats, great decreases in adrenal weight and ascorbic acid are seen at 4 weeks. Thus the normal size and secretory activity of the adrenals are dependent to a great extent upon a normal rate of thyroid secretion.

3. Within "physiological" limits, thyroxine administration has no significant effect on the adrenals.

4. Above the "physiological" range, increased dosages of thyroxine will cause for the most part, progressive increases in adrenal size and ascorbic acid content at 4 weeks.

5. There is a significant difference in adrenal weight between male and female rats, the females having the heavier glands. There is an appreciable but not statistically greater content of ascorbic acid in the female adrenal, when compared with the male.

6. The adrenal hypertrophy induced by thyroxine administration at a dose of 20 micrograms per 100 gm. body weight, may be prevented by simultaneous administration of

adrenocortical extracts. The dose of adrenocortical extracts necessary to accomplish this is between 10 and 20 dog units. Thus the secretion rate of the adrenals of the male rat with this degree of hyperthyroidism is somewhere between 10 and 20 dog units.

7. Thyroxine administration to pregnant rats will superimpose its hypertrophic effects on the adrenal hypertrophy normally seen in pregnancy.

8. Thyroxine administration to pregnant rats will not cause any changes in size or ascorbic acid content of the fetal adrenals.

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