FACTORS AFFECTING THE MANIFESTATIONS OF TETRAETHYLLEAD IN PHOTOSENSITIZED RATS AND THE INFLUENCE OF OTHER MONOCHEMS ON THE ACTION OF TETRAETHYLLEAD

by

Heidi Helga Hartly

A Thesis

Submitted in partial fulfillment

of the requirements for the degree of

Master of Science

McGill University

Montreal, Quebec

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INTRODUCTION

Removal of the thyroid is not always followed by the impressive group of symptoms described in text-books of Endocrinology. In fact, thyroidectomized rats fed on the commercial diet, Purina Fox Chow, do not show the stunted growth and cretinoid syndrome associated with thyroidectomized animals. Paradoxically enough, thyroidectomized rats fed the diet supplied under the same name in 1943 had a full-blown thyroid deficiency.

The purpose of this work was to achieve a better understanding of what thyroid deficiency is, and especially to determine which factors are responsible for the appearance or suppression of the symptoms in thyroidectomized animals.

The presence of smaller or greater amounts of thyroxine in the diet was first suspected and an attempt was therefore made to prepare a virtually thyroxine-free diet. Further, in the hope of obtaining a syndrome of complete thyroid deficiency, the animals were exposed to a moderate cold, since the data in the literature showed that the requirements for thyroid hormone were greater at a low temperature. It was hoped that the nature of the symptoms obtained in the absence of thyroxine would throw some light on the fundamental mechanism of action of this hormone.

In the search for factors influencing the action of thyroxine, it was thought that the symptoms of thyroid deficiency and the action of thyroxine could be influenced by the hypo or hyper secretion of other glands. The influence of castration and testosterone, of adrenalectomy and cortisone, and of hypophysectomy on the action of thyroxine was therefore investigated. Of the end organs of thyroxine, the epidermis was made an object of special study of the interaction of these hormones.

HISTORICAL

A survey of the literature was undertaken to discover whether thyroidectomies without symptoms had previously been reported, and what, if any interpretations, had been advanced. Factors which were felt could influence the symptomology of thyroid deficiency such as diet, temperature, age, strain etc. were particularly investigated, with special emphasis on diet and cold.

Present theories on the peripheral mechanism of action of thyroxine have also been presented, as well as data on its interaction with other hormones. Finally a summary of the known hormonal influences on the epidermis was presented.

THE SYMPTOMATOLOGY OF THYROID DEFICIENCY

In descriptions of thyroid-deficient animals there was a certain set of symptoms on which there was general agreement

and which were therefore considered "typical". In the rat these symptoms included (1) a fall in the oxygen consumption and heart rates, (2) an inhibition of the growth of long bones, resulting in stunted animals with abnormal body proportions, (3) a dry scaly skin and loss of hair, (4) increased sensitivity to cold. All these symptoms also appeared in other species including the human and animals exhibiting such a syndrome were termed "cretinoid". The rat, unlike some other species however, did not show myxoedematous infiltrations of the epidermis.

On the other hand, there was disagreement as to the rate of survival of thyroidectomized animals. Thus, while Hammett, as early as 1926 claimed that he kept a rat which was thyroidectomized at thirty days alive for as long as two years and nine months in an active and healthy condition, though it never got heavier than 50 to 60 gr. Boldike reported in 1932 that none of his completely thyroidectomized rats survived the operation and even partially thryoidectomized animals showed a high death rate (66%). This mortality was presumably due to parathyroid deficiency tetany and may possibly be disregarded. Scow in 1944 found that animals thyroidectomized at birth showed a slow continuous increase in skeletal dimensions and body weight of 0.3 gm daily and survived very well for the duration of the experiment, while Salmon in 1936 reported that similar animals ceased gaining weight when they reached 20 to 25 grm and were very susceptible to infections, thus showing poor survival. None of these

results point to thyroid deficiency as being directly responsible for death.

Few reports were found in the literature of animals which although thyroidectomized, continued growing normally, and showed no signs of thyroid deficiency. If such animals were observed, perhaps their lack of symptoms was attributed to incomplete thyroidectomy. Most studies which were specifically concerned with the production of cretinoid symptoms were done in very young animals and these showed deficiency symptoms.

INFLUENCE OF DIET ON SYMPTOMS OF THYROID DEFICIENCY

DIET AS A SOURCE OF THYROXINE

Since thyroidectomized animals have been shown to be very sensitive to small amounts of thyroxine (Andik 1949 b) the presence of such small quantities of thyroxine in the diet could prevent the onset of a typical thyroid deficiency syndrome. The presence of thyroxine in meat was suggested by Kommerell in 1931, who found that when he fed meat to thyroidectomized dogs they failed to develop symptoms of thyroid deficiency unless the meat came from thyroidectomized dogs. He thus concluded that muscle protein contained thyroxine but only if it came from an animal with an intact thyroid. More recently, in 1949, Gross and Leblond confirmed this finding by a study of the peripheral of distributions of microgram amounts of radio-iodine labelled thyroxine. They found

an appreciable concentration of thyroxine in the muscle as well as in the liver and intestines. Since these latter two organs were known to be incorporated in most commercial feeds, they could severely limit the degree of thyroid deficiency of thyroidectomized animals fed such a diet.

DIET AS A SOURCE OF IODIDE

That an iodine-deficient diet could increase the symptoms of thyroid deficiency of thyroidectomized animals was reported by Chapman in 1944, who found a great decrease in the number of pituitary acidophils and also other thyroid deficiency symptoms in animals fed such a diet as compared to those fed a commercial diet. Grad (personal communication) also found that thyroidectomized rats fed a modification of the goitrogenic iodine-deficient diet, number 342, proposed by Remington in 1937, remained typical cretins while those on Purina Fox Chow continued growing normally. Whether the absence of iodine itself or the concomitant low thyroxine content of the diet was responsible for these changes was not shown.

FACTORS WHICH COULD COUNTER-ACT THE EFFECTS OF THYROXINE

The presence of substances in the diet which could counter-act the effects of thyroxine was suggested by Ershoff in 1949a, b and in 1950a, b, and by Betheil in 1949. These workers isolated an anti-pernicious anaemia factor in the liver which prevented the toxic effects of excess dosages of

thyroxine in hyperthyroid rats and mice. Similarly Emerson in 1949 found that when Vitamin B_{12}, also present in liver as well as in yeast, was given to animals receiving a diet devoid of animal protein in conjunction with thyroid powder, they grew at a rate double that of the control, although the food consumption over the fifteen-day test was the same. Whether these factors would have similar anti-thyroxine properties in normal or hypothyroid animals was not indicated.

NUTRITIONAL FACTORS WHICH COULD INFLUENCE THE EFFICIENCY OF UTILIZATION OF THYROXINE

Because of their lower metabolic rate, thyroidectomized animals were found to consume only about half as much food as normals (Persike 1948). In self-selection experiments performed by Warkentin in 1942, such animals were found to select the same proportions of foods as normal animals, with the exception of yeast. A curious finding that the symptoms of thyroidectomy could be qualitatively altered by a change in diet was reported by Schlotthauer in 1929, who found that thyroidectomized pigs fed a balanced diet showed cutaneous myxoedema and gained weight as controls, while, if they were fed a high carbohydrate diet they did not develop the oedema of myxoedema but lost weight. No specific mention was made of the influence of Vitamin deficiency on symptoms of thyroid deficiency, although a striking similarity existed between these.

INFLUENCE OF COLD ON SYMPTOMS OF THYROID DEFICIENCY

That cold could increase thyroid deficiency was generally accepted since there was need for an increased heat production in order to keep up the body temperature, and consequently increased requirements for thyroxine. Thus it was found that thyroidectomized animals soon succumbed at temperatures which were not lethal for normal animals. Bodansky in 1936 showed that thyroid deficient rats in individual cages had a low resistance to cold and became moribund or died after a one or two day exposure to a temperature of 3 - 5 °C. Ring in 1939 stated that one out of ten rats thyriodectomized for three weeks survived at 0 - 5 °C for more than nine days, the lone survivor being apparently incompletely thyriodectomized. A single injection of thyroxine (1 mg.) prolonged the life of the operated animals in the cold from 14 - 18 days. This work was confirmed by Leblond and Gross in 1942 and 1943 who remarked that if the animals were previously adapted to cold they survived longer.

In addition a number of publications showed that the mechanisms for maintaining normal body temperature was less efficient in thyroidectomized animals than in normals. Korenchvsky in 1926, working with the rabbit and Boldyreff in 1913 working the dog and cat, showed that the body temperature fell more rapidly in an animal exposed to the cold, after thyroidectomy. Similar results were reported in guinea pig by Pfeiffer in 1923 and in rats by Ring in 1939.

No specific mention of an actual increase in the severity

of the general symptoms of thyroid deficiency had, however, been reported. Most of the work dealt with the direct role of the thyroid in cold adaptation.

MECHANISM OF ACTION OF THYROXINE

Although there was a great deal of work done on the method of production of thyroxine, very little had been reported on the peripheral mode of action of this hormone. Cohen and Minz (1949) indicated that thyroxine played an important part as a catalyst for the transformation of pantothenic acid to Co-enzyme A, an enzyme necessary for the acetylation of oxal-acetic acid in the Krebs cycle of carbohydrate metabolism. Thibault in 1949 stated that thyroxine also acted by slowing down the oxidation of adrenalin and adrenochrome. Such a view was supported by Ring in 1942, who attributed the role of thyroxine in cold adaptation to an activation of adrenalin or rather to a sensitization of the tissues to adrenalin.

INFLUENCES OF OTHER HORMONES ON ACTIONS OF THYROXINE

As well as exerting its basic effects on general metabolism, thyroxine also played a part in maintaining the normal weight and histology of a number of peripheral organs. Here its action was often reinforced or inhibited by other hormones.

INTERACTION BETWEEN THYROID AND TESTES

It was well known that there was an interrelation between thyroxine and testosterone since thyroidectomy caused atrophy of the testes and seminal vesicles (Smelser, 1939) and thyroxine was found to increase the stimulation of the seminal vesicles caused by testosterone. (Caridroit, 1942). Castration was not reported to have had any effect on the thyroid.

Most reports on peripheral interactions of thyroxine and testosterone were highly contradictory, probably due to a variation from minimal effective to toxic dosage, which was not taken into consideration.

For instance Grad found in 1949 that contrary to reports by Eidelsberg (1940), Meyer (1942, and Kinsell (1944), physiological doses of testosterone did not stimulate energy metabolism, either alone or in conjunction with thryoxine. Grad also agreed with Noback (1949) and disagreed with Myda (1950) in that he did not observe any effects of testosterone on general body growth. He did, however, observe a synergistic action of thyroxine and testosterone on the weights of the heart, kidneys, submaxillary gland, and hypophysis of castrated-thyroidectomized rats. In histological sections he also noted a synergistic action on the serous tubules of the submaxillary gland, and an antagonistic action on the thickness of the epidermis.

Testosterone alone was found to stimulate the epidermis (Hooker, 1943), and the sebaceous glands (Hamilton, 1941).

INTERACTION BETWEEN THYROID AND ADRENAL

Adrenal involution has often been observed after thyroidectomy (Marine, 1935, Tonutti, 1943, Leblond and Hoff, 1944, b, and Zarrow, 1949) although there have been reports to the contrary (Kfouri, 1944, Deane, 1947). Baumann in 1945 implied a direct relationship between the thyroid and the adrenal and stated that the adrenal cortex exercised an inhibitory control over the thyroid, and interpreted the involution of the adrenal after administration of thiouracil as an attempt to compensate for loss of thyroid secretion. In support of this, Kfouri reported an increase in thyroid activity after adrenalectomy, while in contradiction Reiss (1949) found that in clinical cases of Addison's disease there was decreased thyroid function which could be remedied by the injection of Compound E acetate.

Relationships between action of these two glands

In addition to the influence the adrenal and thyroid seemed to exert on each other, there seemed to be a certain similarity of action and perhaps synergism between the hormones themselves. This suggested that functions commonly attributed to one of these glands might actually be effected indirectly through the other. For instance, cortisone and ACTH were found to cause an increase in basal metabolic rate, without changing the serum protein-bound iodine (representative of circulating thyroxine) (Hill, 1950, Beierwaltes,

1950, Wolfson, 1950, Kfouri, 1944). It has been suggested that this increase in basal metabolic rate represented an increased efficiency of action of thyroid hormone on peripheral tissue. Thus, hypothyroid patients with classical myxoedam showed marked improvement upon daily treatment with 1 gm. of cortisone. The metabolic rate went up, the patients were mentally more alert and less sensitive to cold. However, the appearance of the dry horny skin did not change and neither were myxoedematous infiltrations affected. (Beierwaltes, 1950.)

INTERACTION BETWEEN THYROID AND HYPOPHYSIS

That thyroidectomy caused a cessation or at least a marked retardation of growth was well established. Salmon (1936, 1938) found that thyroidectomized new-born rats ceased growing after they reached a weight of 20 to 25 gm. The administration of growth hormones did not restore growth. Evans (1939) however, found that if a sufficient dose of growth hormones was given to thyroidectomized rats, growth promotion in excess of normal (gigantism) could be produced, but that the effect was greater when the thyroid was present. Thyroxine, which promoted the growth of thyroidectomized animals to normal, did not have this effect when administered to thyroidectomized hypophysectomized animals. Evans concluded that thyroxine and growth hormone were synergistic in their action, and that whereas growth hormone could act in the absence ofthyroxine, thyroxine could not promote growth

in the absence of the hypophysis. Evans interpreted Salmon's lack of success in promoting the growth of thyroidectomized rats by the administration of growth hormone as showing merely that an amount of pituitary growth promoting substance inadequate for the promotion of growth, in the absence of the thyroid was adequate to promote growth when thyroid tissue was present. The synergistic action of these two hormones was confirmed by Scow (1944), Becks (1948a,b) Noback (1948). The latter attributed increase in general body proportions to the growth hormone, and maturation, that is, sexual development, epiphyseal closure, change from infant to adult hair type, to thyroxine

HORMONAL INFLUENCES ON EPIDERMIS AND APPENDAGES

As a section of the work to be presented will deal with the hormonal control of the thickness of the epidermis, a brief review on the known influences of the hormones of the thyroid, testis and adrenal on this organ will be presented.

Thyroid

The syndrome of dry, horny skin and hair as well as the loss of hair was typical of hypothyroidism, indeed, even diagnostic. Dieke (1947) reported that alpha-naphthyl thiourea inhibited hair growth and pigmentation in rats. Thyroidectomy was also found to decrease the growth of hair (in the rat (Emmens 1942, Dieke 1947), the rabbit (Asher 1924) and in the sheep (Simpson 1924c,) which condition was remedied by

thyroid therapy. The dryness of the skin seems to suggest an atrophy of the sebaceous glands, although this was not specifically stated. Thyroidectomy reduced growth of horns in the sheep, and the growth of feathers in fowl (Blivaiss 1946 and 1947).

A certain number of articles dealt with the effect of thyroxine on wound healing. Thus Barclay (1944) found that thyroid preparation reduced wound healing time by 11% or more; dinitrophenol however has a similar effect, thus this would seem to be due to general metabolic stimulation. Fleischmann (1947ab) and Smelser (1947) reported that superficial wounds of the cornea healed just as rapidly in thiouracil treated rats as in normals, but that, with colchicine, the number of mitoses was significantly reduced in the hypothyroid animals. Both the number of cells going into mitosis and the duration of each mitosis were reduced.

In studies on normal metabolism Bullough (1949a,1950) showed that the epidermal mitotic rate has a close correlation with the glycogen concentration. Thyroxine is known to deplete the glycogen stores of liver and heart (Defauw 1930, Fleischi 1933, Coggeshall 1933, Sternheimer 1939, McDonald 1938, Moses 1944) one would therefore expect thyroxine to have a similar action on the glycogen concentration of the epidermis, and also to cause a decrease in the mitotic activity of this end organ.

Testis

Graaf (1946) showed that testosterone proprionate caused an increase in all layers of the epidermis in the skin of rats taken from various sites of the body, as well as an increase in the size of the sebaceous glands. Hooker (1943) reported similar results.

Adrenal

Baker (1948) and Castor (1950) showed that adrenacortical steroids inhibited the growth of hair and caused a thinning of the epidermis when applied locally. Butcher (1939, 1941) and Ralli (1943) observed accelerated growth of hair after adrenalectomy, and conversely that administration of adrenal cortical extracts inhibited hair growth. Adrenocorticotrophin exerted the same effect, but the study by Baker (1948) indicated that the effect was directly on the skin and not associated with any other hormone. The question, however, was raised, as to whether perhaps thyroxine exerted its effect through the adrenal. No work has been reported on this phase of the problem, or on the interaction of gonadal hormones with either adrenal cortical hormones or thyroxine.

Combined action of Thyroxine, and testosterone on epidermis

The most recent work along these lines was carried out by Grad (1949) in this department. He observed that when thyroidectomized-castrated rats were given replacement doses

of either thyroxine or testosterone or both, thyroxine caused a decrease in the thickness of the epidermis, testosterone caused an increase, while both together combined their action to give a normal thickness.

ORIGINAL INVESTIGATIONS

METHODS AND TECHNIQUES

Techniques used in this work will herein be described with various degrees of detail. Routine techniques such as histological preparations, oxygen consumption and heart rate measurements, while employed extensively in this work, will only be outlined as to principle and mode of operation. Techniques developed or adapted in the course of this investigation will be dealt with more fully and the pilot experiments designed to assess the efficiency and accuracy of those techniques will be described.

The techniques to be reported are grouped under the following headings:

1. General animal techniques
2. Methods for inducing thyroid deficiency
3. Quantitative methods for detecting thyroid deficiency
4. Methods for studying the interaction of thyroxine and other hormones
5. Histological and histometric techniques

GENERAL ANIMAL TECHNIQUES

GENERAL SET-UP OF EXPERIMENTS

The animals used throughout this research were male albino rats of which some were bred in our own laboratory, while others were obtained from various commercial sources. Most of the animals were of the Wistar strain, the rest being of the Sherman strain. The same strain was used in any set of comparable experiments.

The animals were earmarked for identification and routinely weighed once or twice weekly. They were kept in cages of various sizes which were suspended in a rack. A tray filled with sawdust which was changed twice weekly, was placed about two inches below the cage for the collection of excreta. The drinking water was changed daily and the food supply replenished as required in order never to leave the animals without food.

Animals of approximately the same weight were used in any one experiment and were so distributed among the experimental groups that the average weight for each group was equal. In order to determine group size, whenever doubt existed as to what percentage of animals could be expected to survive a certain treatment, a pilot experiment was carried out. The function of such an experiment was to determine how many animals would be required to insure a statistically significant minimum survival at the proposed time of termination of the experiment. One or more control groups were also set up in almost every case.

ENVIRONMENTAL CONDITIONS

The room in which all the experiments (except those requiring a low environmental temperature), were conducted, was thermostatically maintained at 27- 30C. It was adequately ventilated and the daily amount of light was regulated by means of a fluorescent lamp which went on automatically at 8 am and out at 8 pm. The environmental conditions were thus kept as constant as possible and the

animals were not disturbed unnecessarily.

METHODS FOR INDUCING AND ACCENTUATING THYROID DEFICIENCY

OPERATIVE TECHNIQUE OF THYROID-PARATHYROIDECTOMY

Pre-Operative Treatment

One percent calcium lactate was given to animals as drinking solution one day before operation in order to prevent the death by tetany which otherwise would often occur within a few hours after the ablation of the parathyroids, which are removed jointly with the thyroid.

Operative Procedure

Clean but not sterile precautions were taken throughout all operations. The animal to be operated on was placed in an anaesthetizing chamber, containing cotton wool moistened with ether. When the animal's whiskers stopped moving (the animal could be watched through the glass walls of the anaesthetizing chamber), it was removed and if its head fell limply a sufficient depth of anaesthesia was considered to be present. Excessive anaesthesia was avoided by watching for blueness of feet and cessation of respiration. The anaesthetized animal was fastened to the operating board by catching the maxillary incisor teeth through the wire loop at the end of the board further from the operator and by slipping the four limbs under the rubber bands running lengthwise around the board.

The throat region was then washed with a minimum amount of 70% alcohol; a mid-ventral incision was made with

scissors from the level of the sternum to just above the larynx. The submaxillary glands and sternohyoid muscles were next retracted on either side. Finally, the small sterno-thyroid muscles were gently separated, thus leaving the thyroid exposed just below the larynx as two pink masses on either side of the trachea. These are joined by a thin bridge or isthmus of thyroid tissue.

The lower pole of the right thyroid lobe was then grasped with a pair of curved forceps held in the right hand and pulled upwards and sideways, while the muscles were retracted by a forceps held in the left hand, the left arm coming around the head of the animal. This thyroid lobe was then grasped with a second pair of forceps held in the left hand and was gently but firmly pulled in the direction of the space between the head and the left forearm of the animal until it snapped off. Care was taken to avoid damaging the recurrent laryngeal nerve which lies nearby, for its impairment causes laryngeal spasm with concomitant disturbance of respiration. Both pairs of forceps were then put back into 70% alcohol, where they were kept at all times in order to kill any adhering thyroid tissue which otherwise might be transplanted and then "take" rapidly. The other thyroid lobe was removed in a similar manner.

Hemorrhage from the thyroid arteries was stopped with cotton pads moistened with physiological saline (0.9% NaCl.).

When both lobes were out, the muscles were again

separated and the trachea examined to check on the completeness of the thyroidectomy. The muscles and overlying tissues were then pulled together but not sutured while the skin was sutured with three separate stitches each tied three times. These sutures did not have to be removed subsequently.

The animal could now be returned to its cage. It was found expedient to do all the operations of any one experiment on the same day in order to standardize that experiment.

Post-Operative Treatment.

Since the parathyroids were also removed in this operation, a 1% calcium lactate solution, provided instead of drinking water on the day before operation, was continued in order to prevent tetany. It was seldom necessary to continue this therapy longer than a week possibly due to a self-adaptative mechanism. The symptoms which were ascribed to thyroidectomy were those relieved upon the administration of replacement doses of thyroxine. Since, except for the transitory tetany, all noted symptoms were cured by thyroxine, the thyroid-parathyroidectomized animals used will, in future, be referred to simply as "thyroidectomized".

Verification of Completeness of Thyroidectomy by Examination of Hypophysis

In addition to a careful examination of the laryngeal region for thyroid remnants immediately following the extirpation of both lobes and again at autopsy, a further post-mortem

check on the completeness of the thyroidectomy was made possible by histological examination of the hypophysis. This organ was removed at autopsy, fixed in Susa and subsequently stained with haematoxylin and eosin. It has been shown by Leblond (1943) that if the thyroidectomy was complete there was complete absence of acidophils. All animals which showed the presence of acidophils in the hypophysis were thus omitted from the results.

PREPARATION OF VARIOUS THYROXINE DEFICIENT DIETS

In this department previous experience with thyroidectomized rats had shown that when such animals were fed commercial purina fox chow, they often continued growing normally and showed only mild symptoms of thyroid deficiency. One of the possible explanations of this phenomenon was that some thyroxine was being ingested in the diet. To reduce this possibility the animals were therefore put on a different diet whose ingredients, with the exception of 2% pig liver, were of vegetable origin. It was surmised that since this diet had a low iodine content, its thyroxine content should be similarly low. This diet, a modification of Remington's diet, number 342 (Remington 1937) had the following composition:

Table 1

COMPOSITION OF IODINE-DEFICIENT DIET

Ingredient	Percent Composition
Corn Meal	70.9
Wheat Gluten	16.4
Pig Liver	1.9
NaCl	0.9
$CaCO_3$	0.9
Yeast	9.0
Total	100.0

Since animals fed this diet still did not show very pronounced symptoms of thyroid deficiency, it was believed that the animals must still be ingesting small quantities of thyroxine, presumably found in the pig liver. An attempt was therefore made to produce a diet even more deficient in thyroxine-free proteins. On the assumption that the meat of a thyroidectomized animal would be devoid of thyroxine (Kommerell, 1931) a special diet was prepared in which the pig liver was replaced by various amounts of dried carcass of thyroidectomized rats. This latter ingredient was prepared in the following manner.

Preparation of Thyroidectomized Rat Meat Diet

Approximately 100 large rats (weighing over 100 gm) were thyroidectomized and put on the iodine deficient diet described above. After three weeks, i.e., when it was assumed that all the thyroxine secreted by the gland before its removal had been eliminated from the body, the animals were sacrificed with chloroform. If any of these animals had thyroid remnants, their tissues would obviously not be thyroxine-free. Therefore, an inspection of the thyroid region was done to check on the presence of such remnants. If they were found, the whole animal was discarded.

The skins were removed by making incisions up the hind legs to the midline, around the tail, and up the abdomen to the throat. Now the skins could be easily pulled over the head of the rat. The tail was cut off and abdominal viscera (with the exception of the liver, spleen, heart, lungs and kidneys) were removed. The carcasses were stored

in the refrigerator until the next step of the procedure.

The skinned carcasses were placed on a metal tray, covered by another such metal tray and cooked by placing them over a pan of boiling water. The carcasses themselves were fatty enough to prevent burning. The rats were thus steamed until cooked through which required approximately 2 hours.

The carcasses were again put in the ice-box in order to solidify the fat, thus making them easier to handle. When cold, the complete carcass, including the bones, was passed through a sturdy meat grinder. The ground meat was spread on metal trays and placed in a 60°C oven overnight or until thoroughly dried. The hard pellets thus obtained were put through a bone mill and came out as a fine powder. It was important that the meat was well dried before it was ground or it formed lumps after passing through the bone mill. (An electric grain pulverizing machine was tried in order to eliminate the mechanical labour of the hand-grinding but it was found that the heat generated caused lumping of the powder. Furthermore, dry ice was even ground with the meat, but this still did not help and therefore the use of this machine was discontinued.)

Such dried powder could be kept indefinitely in a closed container and in a dry place. It was used in the proportions indicated in the following table to make up diets of different protein levels.

TABLE 2

COMPOSITION OF RAT MEAT DIETS

Diet No.	Rat Meat % D	NaCl %	$CaCO_3$ %	Yeast %	Wheat Gluten %	Corn Meal %
1	5	1	1	10	18	65
2	10	1	1	10	18	55
3	15	1	1	10	18	50
4	30	1	1	10	18	35
5	45	1	1	10	18	20

Suitability of Diet as Food for Rats

A pilot experiment was carried out to ascertain whether the animals would eat this diet without deleterious effects and to determine what proportion of rat powder should be used.

Six groups of 15 male albino rats, weighing approximately 75 gm were thyroidectomized. They were then fed the special diet, containing graded amounts, namely 5, 15 and 45% of rat powder. All the animals were found to eat the diet readily. Furthermore, they did not suffer from diarrhea, as it was feared they might (young thyroidectomized rats on Remington's modified iodine deficient diet were often found to do so). Since the results were satisfactory, the experiment was discontinued after three weeks. There was not enough difference in the final body weights of the three groups to warrant the use of three diets (see Table 3) and therefore it was decided to use only 5% and 30% rat meat diets in future experiments.

TABLE 3

BODY WEIGHTS OF THYROIDECTOMIZED RATS FED GRADED AMOUNTS OF RAT MEAT DIET

Diet	Thyroxine per Day	Initial Body Weight In gm	Final Body Weight In gm
5% Rat Meat	0	75	98
15% Rat Meat	0	75	101
45% Rat Meat	0	76	103

ADJUSTMENT OF ROOM TEMPERATURE

In the hope of further intensifying the thyroxine deficiency of thyroidectomized rats, they were placed in a cool environment. A number of experiments were therefore conducted in which the animals were kept in a special room. The temperature of this room was thermostatically maintained at $16 \pm 2^{\circ}C$. The ventilation was adequate, and although the lighting was not automatically controlled, the light was generally turned on in the morning, left during the working day and turned off in the evening.

METHOD FOR DETECTION OF THYROID DEFICIENCY

The main criteria used to determine the degree of thyroid deficiency in rats were first, failure to grow and secondly, a fall in the basal metabolic and heart rates. The failure to grow was detected by weekly weighings of the animal. The apparatus used for the latter two measurements, as adapted for the rat, are described in detail by Grad (1949). These techniques will thus be only briefly reviewed here.

TECHNIQUES USED FOR THE MEASUREMENT OF THE OXYGEN CONSUMPTION IN THE RAT

The absorption by soda lime of the carbon dioxide expired by an individual rat during a given amount of time was the basic principle of the apparatus used for the measurement of the oxygen consumption.

Essentially, the apparatus consisted of a series of six 48-ounce glass jars, suspended up to their necks in a deep water bath, kept at 28°C, the "critical" temperature of the rat for measurement of the basal metabolic rate (Benedict 1928). Each bottle was partially filled with soda lime. This jar was connected in series with a Florence flask filled with oxygen and which in turn had an open tube dipping into the water bath - the only open part of the system. As the carbon dioxide was absorbed, a vacuum was created in the bottle and this was quickly replaced by oxygen from the Florence flask. The exit of oxygen in turn caused a negative pressure in the flask which in turn caused the water from the water bath to siphon in. This procedure was allowed to continue for twenty minutes, at the end of which time the amount of water in the Florence flask was taken as a measure of the oxygen which had followed out, which in turn was equal to the volume of CO_2 absorbed and hence expired by the rat in the twenty minute test interval.

Since the heat production (or oxygen consumption) has been shown to be proportional to the surface area of the animal (Rubner 1902), the results were expressed as cubic centimeters of oxygen consumed per hour per 100 square centimeters of body surface. (The body surface in cm was obtained from the weight in grams by the formula $S = kW^{2/3}$ which Meeh worked out in 1879. The constant k was taken to be 11.2.) This value was termed the "basal metabolic rate" or B.M.R., as it will be referred to from here on. Although

the animals were not fasted as is generally the custom for B.M.R. measurements, the decrease of the metabolic rate in fasted animals was only slightly lower than in non-fasted ones (Grad 1949), and therefore use of this approximation was justified.

Value for Normal and Thyroidectomized Animals

The normal value of the oxygen consumption (cc./hr.-100cm^2) for 100 gram male albino rats, at standard laboratory conditions was found to vary between 70 and 85, while in thyroidectomized animals the value fell to approximately 60. The day to day variations in the same animals were found to be less than that between individual animals. This criterion was used extensively to determine whether the so-called "replacement" doses of thyroxine being administered to thyroidectomized animals were of the appropriate magnitude to just restore the B.M.R. to within a normal range.

TECHNIQUE USED FOR THE MEASUREMENT OF THE HEART RATE IN THE RAT (ELECTROCARDIOGRAPH MACHINE)

Since the albino rat, maintained at a temperature of 28 degrees centigrade, has a heart rate of about 350 beats per minute, some automatic means such as an electrocardiograph must be used to obtain an accurate measurement. In the apparatus (Grad 1949), the electrical impulse generated by the beating heart was picked up by means of safety pins inserted through the skin of the flanks of the animals which act as electrodes to which the leads of the electrocardiograph are clamped. The impulses are registered on light

sensitive time paper which subsequently is developed. The heart rate is then counted directly on the developed paper and the number of beats per minute calculated. The apparatus is so designed that it is possible to make determinations on thirty rats within one hour (Grad 1949).

Values for Normal and Thyroidectomized Animals.

The range of frequency for heart rate is fairly wide in normal animals, although the variations in a single rat from day to day is much smaller. The average value for a normal 100 gram rat is approximately 350 beats per minute, while for a similar thyroidectomized animal it would be about 300 beats per minute. However, at any rate, in all cases thyroidectomy significantly decreased the heart rate of individual animals, this effect being already observable on the second day after operation. The rate continued to fall gradually, until a new level was reached in about a week.

METHODS FOR STUDYING THE INTERACTION OF THYROXINE WITH OTHER HORMONES

ESTABLISHMENT OF BASAL CONDITIONS

In order to study the interaction of thyroxine with other hormones, (and insofar that we only desired to preserve the peripheral effects of these hormones on various organs, rather than their action on the endocrine glands themselves) a base line was established by removing the endocrines in question. Thus when it was desired to observe the action of thyroxine and testosterone, either alone or in combination,

on the epidermis, the animals were thyroidectomized and castrated. This procedure had the double advantage of (1) removing endogenous sources of these hormones, and thus making it possible to know the exact amounts present in the body and (2) eliminating any possible stimulation of the thyroid by the testis, and vice versa. This last advantage eradicated the complication of possible false conclusions being drawn as to the direct or indirect mode of action of these hormones.

When an indirect effect was suspected to be due to stimulation or inhibition of the adrenal, the suspicion was assessed by a further removal of the adrenal. In a later experiment it was found more expedient to use hypophysectomized animals, the base line being established by the absence of the three "trophic" hormones (thyrotrophin, gonadotrophin and ACTH). The assumption was made that any interaction of the hormones of the target glands on each other would be exerted through the hypophysis. A slight complication was the absence of growth hormone, thus introducing a further variable, but this will be discussed in a later section.

The hypophysectomized animals were already obtained in this condition from a commercial source. The technique of thyroidectomy was described in a previous section, and thus only the techniques of castration and adrenalectomy will be briefly described here. The general procedure, (anaesthesia, cleanliness, etcetera) is the same as for thyroidectomy.

CASTRATION (ORCHIDECTOMY)

An incision was made in the scrotum large enough to permit both testes to be extruded. A single ligature was tied around the differential vessels, the spermatic vessels and ducti deferentia of both testes. The testes together with the epidiymis were excised and removed. The incision in the scrotum was sutured.

No special post-operative treatment of castrated animals was necessary.

ADRENALECTOMY

There are two accepted ways of doing adrenalectomies in rats, one via the lumbodorsal route and the other via the abdominal. Both techniques were tried and the lumbodorsal one was found to be more satisfactory and therefore this technique will be described.

A single mid-dorsal longitudinal incision, about 1 inch in length was made just below the level of the ribs. Due to the looseness of the skin, the incision could be pulled either to the left or the right. A longitudinal incision was now made through the abdominal muscles of one side, just lateral to the intrinsic muscles of the back. The upper pole of the kidney, with the adrenal in close approximation now came into view and the latter could be easily removed, The same was done on the other side. The muscles on each side were sutured with linen thread. As the skin of the back was rather tough, it was found better to use metal clips rather than sutures to close the incision.

Post-operative treatment consisted of giving the animals a 0.9% NaCl solution instead of drinking water for as long as they survived. The length of survival varied with the treatment to which the animals were subjected, but it was found that most adrenalectomized-castrated-thyroidectomized animals succumbed within two to three weeks following adrenalectomy. (This last operation - adrenalectomy - was performed a considerable time after the other two which were done consecutively.)

DETERMINATION OF REPLACEMENT DOSES AND PREPARATION OF SOLUTION OF HORMONES

As the normal actions of the hormones were to be studied, it was essential that the doses given to the thyroidectomized-castrated (and one experiment also adrenalectomized) animals corresponded to the amount normally secreted by the excised glands. In order to determine the magnitude of these "replacement" doses, several criteria were used for each hormone.

DETERMINATION OF REPLACEMENT DOSES

Thyroxine

The criteria for determining the correct dose of thyroxine was the body weight, basal metabolic rate and heart rate. As the range between hypo- and hyperthyroidism is very narrow, in such animals, the dose had to be carefully adjusted and occasionally increased as the animals grew. The dose also had to be increased in the animals maintained in the cold. Thus while 3 micrograms of d,l - sodium thyroxine daily were sufficient for the maintenance of 50 gm rats at 27°C, the

dose had to be raised to 6 micrograms to produce the same effect at 16°C. Increasing the dose to 10 micrograms, however, again impeded the normal weight gain, thus indicating that the dose was too high.

Testosterone

The dose of free testosterone did not have to be adjusted too carefully. The main indications of its effectiveness, namely a great increase in size of the seminal vesicle as compared to castrated animals, could not be observed until autopsy. The dose commonly used was 500 micrograms daily.

Cortisone

It was very difficult to estimate the correct dose of cortisone, as this was a relatively new drug and little work had been done with small doses. A pilot experiment was set up in which one group of animals was given 100 micrograms, another 1 mg, and a third group 2 mg of cortisone daily. The animals were male albino rats weighing approximately 180 gm. A dose of 2 mg proved definitely toxic as most of the animals died within two or three days and the survivors showed abnormally small thymus and spleen and a large liver. The animals receiving 500 micrograms did not gain weight as well as those receiving 100 micrograms and none were as good as the non-adrenalectomized controls. Thus in future experiments it was decided to use 200 micrograms daily.

PREPARATION OF SOLUTIONS

As it was desirable to have as similar as possible condition in all the groups, a common solvent was used for all these

hormones. The most satisfactory solvent was found to be 0.9% saline to which was added sufficient Dupanol C - a deturgent - to give a final 1% concentration of this substance. The deturgent was necessary for a satisfactory suspension of testosterone, as well as gentle heating. It was necessary to make the solution slightly alkaline by the addition of 1 drop of 2N NaOh in order to dissolve the sodium thyroxine. Since cortisone was commercially obtained in the form of a solution, it was only necessary to dilute it with the solvent until the desired concentration was obtained. The concentration of the hormones was such that the amount of each injection would be 0.1 cc.

HISTOLOGICAL AND HISTOMETRIC TECHNIQUE

The routine techniques used to prepare most of the histological sections will not be described in detail. However, in our study of the influence of the testis and thyroid hormones on the epidermis, a number of histometric techniques were specially adapted for our convenience and these will be described extensively. A brief survey of the statistical techniques employed to evaluate the significance of our result will also be presented.

SACRIFICE, FIXATION, EMBEDDING, SECTIONING, STAINING

Unless mentioned otherwise all animals were sacrificed in chloroform, the organs removed immediately and fixed in Orth's solutions (for composition see Lillie 1948) The only exception was the hypophysis, which was put into Susa, as

the presence of acidophils indicating incomplete thyroidectomy can be observed better in sections prepared from this fixative. The skin, an organ especially studied, was taken either from the back of the animal or from the flanks, care being taken to avoid the injection site of the base of the neck. A piece measuring approximately 1 by 2 cm, the long side being parallel to the direction of the hair, was stuck onto a piece of cardboard (to prevent curling) before being placed in the fixative.

These tissues were allowed to stay in the fixative for 24 hours, then washed, dehydrated in dioxane and embedded in paraffin. The sections were cut at a thickness of approximately 5 μ and stained with haematoxylin and eosin.

WEIGHING OF ORGANS

As the hormones whose actions were investigated in this research, namely thyroxine, testosterone and cortisone, are all known to exert certain effects on the weights of a number of organs, it was standard practice to weigh all the organs which might be influenced and which were compact enough to permit accuracy. Thus when the animals were sacrificed, each of the organs was fixed in its entirety and no attempt was made to remove adhering connective tissue from the fresh tissues. After all the animals were killed, all the organs were removed from the fixative and spread out on a damp cloth. They were then carefully cleaned and weighed on an analytical balance, and only then trimmed for histological sections. The tissues commonly weighed included the

liver, spleen, kidneys, adrenals, hypophysis, testes, seminal vesicles, submaxillary gland, sublingual gland, cervical lymph nodes and sternomastoid muscle.

SUBJECTIVE MEASUREMENTS OF HISTOLOGICAL STRUCTURES

When the histological sections prepared as indicated above were examined, certain differences in the size of a number of structures was observed. The tissue studied most extensively was the epidermis, whose thickness was found to be influenced by both testosterone and thyroxine. These differences in thickness were obvious upon cursory examination, but to assess the results a systematic estimation of the thickness was carried out. The technique consisted simply of looking at each slide in turn, care being taken to first examine the first slide in each group, then the second and so on, instead of looking at all the slides in one group first. This practice was adopted as it seemed to somewhat reduce the subjective nature of this technique. The thickness of the Malpighian layers and cornified layers were then estimated separately and recorded as a number of "pluses" (+). The quantity of granules and various other structures were assessed in a similar manner.

Although this technique was necessarily rather crude, it was useful to establish a trend, which could then warrant more exact study, or to confirm results obtained in previous experiments. To test the reliability of this method, the same set of slides was examined by three different investigators and their estimations were found to be in reasonably good

agreement. The results of this test is given in Table 4.

TABLE 4

THE VARIATION IN THE THICKNESS OF THE VARIOUS LAYERS OF THE EPIDERMIS AS ESTIMATED BY THREE DIFFERENT INVESTIGATORS

	Thickness in (+)		
	Investigator 1	Investigator 2	Investigator 3
Malpighian layers	2.1	2.3	1.9
Cornified layers	3.4	2.2	2.6
Granules	2.0	2.0	2.1

PROJECTION AND MEASUREMENT OF HISTOLOGICAL STRUCTURES

Direct measurements of the thickness ("thickness" here refers to the height of the epidermis as it appears on the slide) of the epidermis with an ocular micrometer could not be made due to the large variation even within the same microscopic fields. Therefore a more precise technique, which will now be described, was employed.

Description and Operation of Apparatus

A schematic drawing of the apparatus is shown in Fig. 1. Essentially, it consistedof a stand on which a microscope and microscope lamp were fixed. The microscope was turned so that the body tube was horizontal and the mechanical stage vertical. As the lamp was free to slide in all directions it was adjusted so that its beam passed directly through the condenser of the microscope. It could thus be permanently fixed. Both microscope and lamp were enclosed in a tin box to keep extraneous light from the projected image. The entire apparatus was set up in a room which could be nearly completely blacked out.

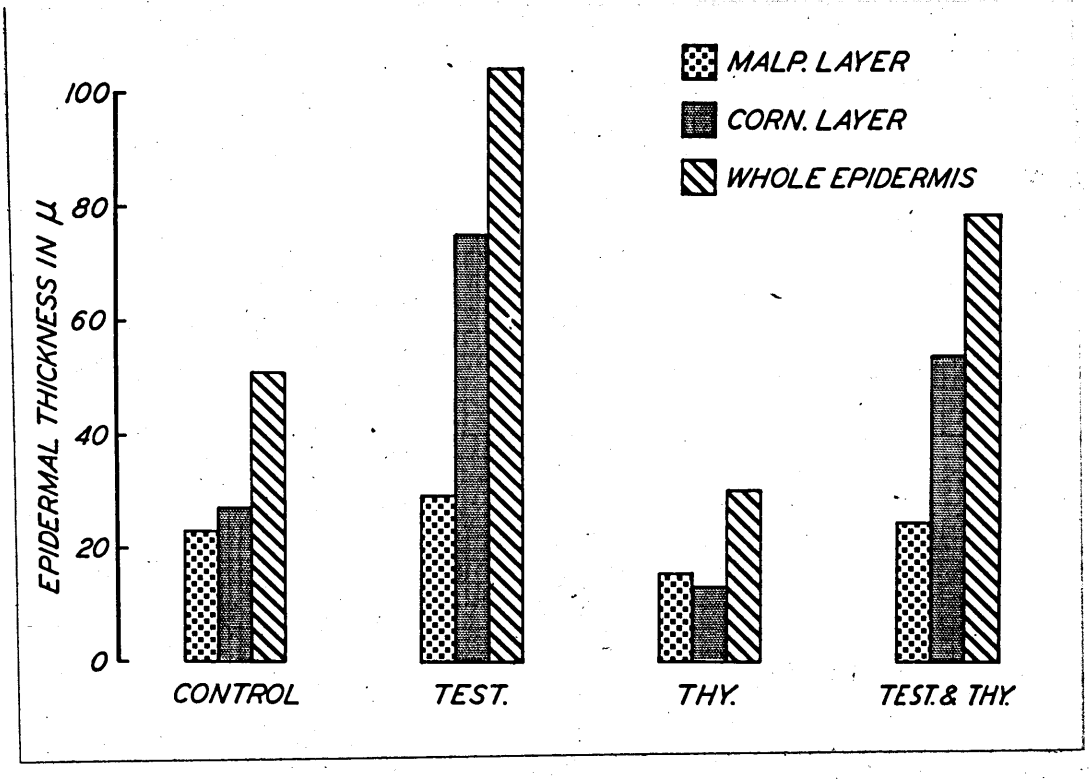
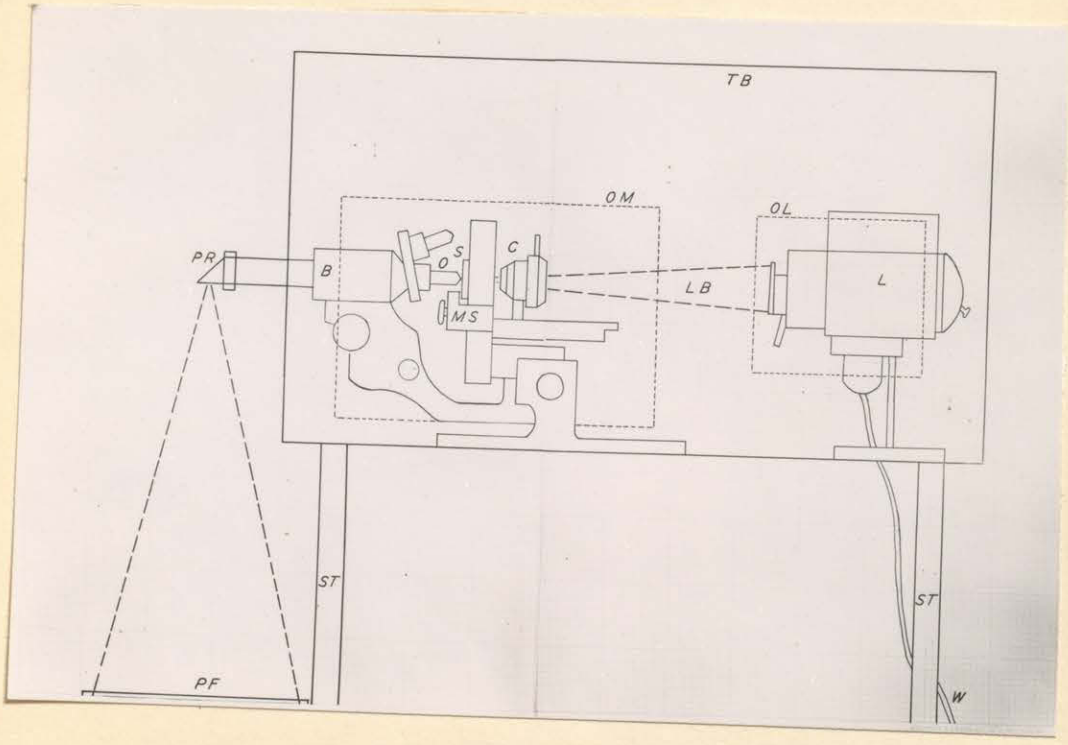

MALP. LAYER
CORN. LAYER
WHOLE EPIDERMIS
EPIDERMAL THICKNESS IN μ
100
80
60
40
20
0
CONTROL
TEST.
THY.
TEST.& THY.

Fig. 1 Diagram of projection apparatus used for the measurement of the thickness of the epidermis of rats subjected to various treatments.

Legend:

PF - Projected field
PR - 45° prism
ST - Stand
B - Body of microscope
O - Objective
S - Slide
MS - Mechanical stage
C - Condenser
LB - Light beam
L - Lamp
W - Wire
OM - Opening for adjustment of microscope
OL - Opening for adjustment of lamp
TB - Tin Box


TB
OM
OL
PR
B
O
S
C
LB
L
MS
ST
ST
PF
W

A forty-five degree prism placed in the eyepiece deflected the light coming through the microscope down onto a piece of white loose leaf paper lying on the table. The height of the stand was adjusted so that the diameter of the projected field almost equaled the width of the paper (20 cm). The optimum height was found to be approximately 13 cm. A tracing was made of the illuminated area. The slide to be studied was placed on the mechanical stage, and suitable fields for study were projected onto the paper.

For the measurement of the thickness of the epidermis, a 10 power ocular and a 4 mm. objective were used which gave a magnification of 430 times. The magnification was calculated by placing a haemocytometer on the mechanical stage in place of the slide, and the lines of its platform were projected onto a piece of paper. The distance between the images of the lines was measured, expressed in microns and divided by the actual distance between these same lines on the haemocytometer.

USE OF APPARATUS FOR MEASURING THE THICKNESS OF THE EPIDERMIS

Measurement of Relative Thickness:

The outlines of the magnified and projected epidermis in 6 consecutive fields were traced on the paper: that is, the basement membrane and the outer limit of the cornified layer was drawn. The limit between Malpighian and cornified layer was also traced. By cutting along the drawn lines, pieces of paper corresponding to the two main layers of the epidermis were obtained. These were then weighed on an analytical balance. The weights thus obtained were the relative weights

of the Malpighian and cornified layers and corresponded to the relative thickness of the two layers of the epidermis.

Calculation of Actual Thickness:

In order to obtain the actual mean thickness of the two epidermal layers, from the relative weights of their projections, several steps had to be taken. Firstly, the weights of the projections were re-expressed as surface area.

The detail of the calculations used to convert the weight of the paper projections of epidermal layers into the surface area of the projections was as follows:

Let w equal the weight of 1 cm^2 of paper ($\bar{w}$ = 5.669 mg), then 1 mg. of paper represents a surface of 1/w.

Let W equal the total weight of paper projections for each section in mg.
Let S equal the surface of these paper projections in cm^2.

Then $S = W/w$.

The surface area was determined by weighing pieces of paper of known area, and then calculating the surface area corresponding to 1 mg of paper.

Secondly, by dividing the square (the image had been magnified in two directions) of the magnification, the actual surface of each layer in the six fields drawn was obtained. The calculation used for this was as follows:

Let s equal the actual surface of the projected structures in cm^2.
Let m equal the magnification factor, (x430).

Then $s = \frac{S}{m^2} = \frac{W}{wm^2}$

Thirdly, the actual surface area was now re-expressed as mean thickness in the following manner. The total length of

the projected basement membrane was first measured. This was done by following the outline of its projection with a "map-reader". (A map-reader is an instrument consisting of a small wheel and a dial which registers the length of the line traced by the wheel.) This reading was then divided by the magnification in order to get the actual length of basement membrane. Assuming that the surface area of the projected Malpighian layer is a rectangle, the base of which is formed by the basement membrane, the mean thickness of this layer was readily obtained by dividing its actual area by the actual length of the basement membrane. For this the following calculations were used:

Let B equal the length of the projection of basement membrane for each section in cm.
Let b equal the actual length of this basement membrane in cm.

Then $b = B/m$

Assuming the surface measured to be a rectangle,
Let t equal the mean actual thickness of each section

Then $t = \frac{s}{b} = \frac{W}{wmB}$

But since w and ma are constants equal to 5.669 and 430 respectively,

Therefore $t = \frac{W}{5.669 \times 430 \times B} = 0.00041 \frac{W}{B}$ cm

Expressing this in microns, $t = 4.10 \frac{W}{B}$ microns.

RESULTS

PART I

In this section of experiments dealing with the attempt to isolate a number of factors which could influence the symptoms of thyroidectomy will be reported. The factors which were particularly investigated were diet and temperature. The experiments have been grouped in logical sequence in the following manner:

Influence of Diet on the Symptoms of Thyroidectomy

Combined Influence of Diet and a Cool Environment on the Symptoms of Thyroidectomy

Further Investigation of the Syndrome of Thyroid Deficiency

Evaluation of Methods and Results

General Summary

INFLUENCE OF DIET ON THE SYMPTOMS OF THYROIDECTOMY

This series of investigations was prompted by the observation that groups of thyroidectomized rats frequently continued growing normally after operation and showed few symptoms, while in other experimental series similar animals exhibited retardation of growth which was considered characteristic of thyroid deficiency.

It was at first thought that such differences could be attributed to microscopic remnants of thyroid tissue. However, careful examination of the thyroid region by serial cross-section proved this not to be true.

The diet, as a possible source of exogenous thyroxine, was next suspected when it was observed that during the last war thyroidectomized animals on a commercial diet, such as Purina Fox Chow, stopped growing while after the war similar animals, on the same commercial diet, grew fairly well. Since it was known that meat contained thyroxine, a change in the quality and quantity of the meat incorporated into this commercial feed could have been responsible for this phenomenon. To investigate this theory, thyroidectomized animals were fed a synthetic diet whose ingredients were chiefly of vegetable origin and whose composition is given under Methods. The iodine content of this diet was analyzed and found to be very low. Presumably this low iodine content indicated an equally low thyroxine content. The original supposition proved well founded when such thyroidectomized animals showed some of the symptoms of thyroid deficiency, namely growth retardation, low

B.M.R. and a somewhat shaggy coat. However, it was found necessary to keep the animals on this diet for a considerable length of time before such symptoms developed.

The positive results obtained with this so-called "iodine-deficient" diet seemed to call for further investigation on the influence of diet on the symptoms of thyroidectomy. Since the 2% pig liver used in the synthetic iodine deficient diet (the only animal protein present) was suspected of containing any residual thyroxine still present, a special diet was prepared in which this substance, that is the pig liver, was replaced by a presumably thyroxine-free animal protein. As previously indicated and described in the Methods, this protein was the pulverized dried carcass of thyroidectomized rats. Therefore the following experiment was set up.

EXPERIMENTAL SET-UP

A total of 72 male albino rats, weighing 40 - 60 gm (average 50 gm) were thyroidectomized and divided into four groups as follows:

Group 1 - 18 rats fed 5% rat protein diet and given 2 subcutaneous injections of 0.1 cc H_2O daily
Group 2 - 18 rats fed 30% rat protein diet and given 2 subcutaneous injections of 0.1 cc H_2O daily
Group 3 - 18 rats fed 5% rat protein diet and given 2 subcutaneous injections of 1.5 γ thyroxine in 0.1 cc H_2O
Group 4 - 18 rats fed 30% rat protein diet and given 2 subcutaneous injections of 1.5 γ thyroxine in 0.1 cc H_2O

These rats were kept in a room whose temperature was $27 \pm 3^{\circ}C$. They were given Purina Fox Chow (in the future referred to as Purina) until 2 days after operation, at which time the

was changed to that indicated above. A 1% Ca Lactate solution was given instead of drinking water from the day before until 12 days after the operation.

RESULTS

Growth

All animals were weighed weekly. From Fig. 2 it can be seen that compared to their controls receiving adequate doses of thyroxine (R5 + T and R30 + T), the animals on these diets (R30, R5) showed retardation of growth starting after the first week. This growth lag became progressively more pronounced, until, finally, around the third week, growth stopped completely. The initial and final average weights of the animals in the respective groups are given in Table 5.

(See following page for Table 5)

Footnote:
T stands for thyroxine, I stands for iodine deficient diet
R stands for rat protein diet, P stands for Purina. The numbers stand for the percentage of rat meat added to the iodine deficient diet in place of the pig liver in order to make up the rat meat diets used.

Fig.[2] The body weight of thyroidectomized rats receiving rat protein diet and kept at 27°C.

Note: (1). Animals receiving no thyroxine (R-5 & R-30) ceased gaining weight after two to three weeks, while those receiving 3 µgm thyroxine (R-5 & T and R-30 & T) continued growing.

(2). All animals receiving the 5% rat protein diet grew lesswell than those receiving the 30% rat protein diet.

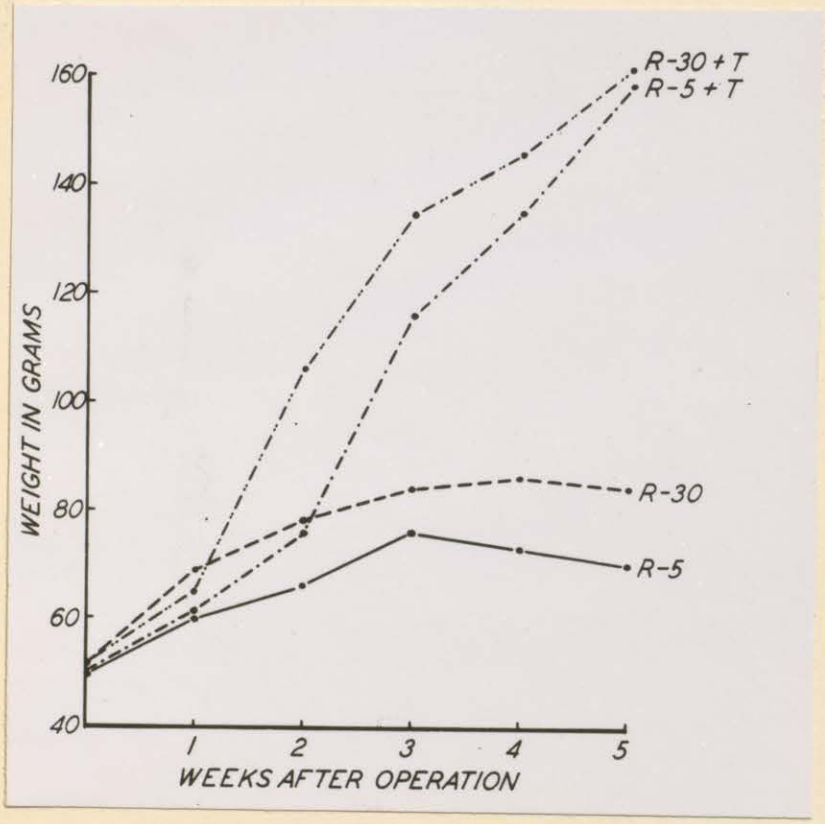

R-30 + T
R-5 + T
R-30
R-5
160
140
120
100
80
60
40
WEIGHT IN GRAMS
1
2
3
4
5
WEEKS AFTER OPERATION

TABLE 5

INFLUENCE OF DIET ON GROWTH AND WEIGHT OF SEMINAL VESICLES OF THYROIDECTOMIZED RATS

Diet	Daily dose of thyroxine	Average Body Weight (gm) Initial	5 Weeks after Operation	No. of Survivors
5% rat protein	0	49	70	3
30% rat protein	0	52	84	8
5% rat protein	3	50	158	6
30% rat protein	3	51	161	5

The observation that animals receiving the diet containing 30% rat meat consistently grew better than those receiving only 5% of this protein material could possibly be attributed to the lower protein level of the latter. Incidentally such an observation indicated that more than one variable could influence the appearance of the symptoms.

Survival

Survival of these thyroid deficient animals was about the same as for the controls receiving thyroxine. A total of 11 animals survived until 11 weeks after thyroidectomy.

General Appearance

No visible symptoms of thyroid deficiency were observable in the animals on the rat protein diet during the first two weeks after thyroidectomy. If, however, the animals were examined during the fifth week, a cursory glance was sufficient to identify them as typical cretins. The cretinoid appearance of these animals was further accentuated by the change in bodily proportions. Thus because there was retardation in the growth in length of the long bones, the animals appeared short and squat. One of the first symptoms to appear was a scaliness of the skin in general and of the tail in particular, which was indicative of hyperkeratosis. In spite of the observation that the skin became tight and adherent instead of being smooth, silky and pliable, there was no apparent myxoedematous swelling. An occasional patchy loss of hair gave the animals a shaggy appearance. The nails also appeared to be longer than normal. Paleness of the eyes indicated that the animals were also suffering from

anaemia.

RESULTS FOUND AT AUTOPSY

Organ Weights:

A number of organs were weighed and it was found that most of the organs of the animals receiving thyroxine weighed approximately twice as much as those of animals not receiving thyroxine (Table 6). This difference could, however, be accounted for by a similar difference in the body weight. Thus when the relative organ weights per 100 gm body weight were calculated, this difference virtually disappeared for most organs (See Table 7). The only organs which still showed a difference were (1) the hypophysis, whose weight was decreased by the administration of thyroxine, indicating that this organ was stimulated by thyroid deficiency and (2) the seminal vesicles which were undeveloped in thyroid deficient animals. The spleen weights showed great variability and the heart weight and adrenal weights seemed to be decreased by thyroxine. These observations were contrary to expectations and could not be explained. It should be emphasized that the animals were not sacrificed until 11 weeks after thyroidectomy, at which time only a small number of animals were still alive.

Histological:

Histological examination the skin revealed a marked increase in the thickness of the epidermis, especially of the cornified layers, confirming the initial observation that there was hyperkeratosis. Although specific stains for

TABLE 6

ABSOLUTE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 27°C

Diet	5% Rat Protein	30% Rat Protein	5% Rat Protein	30% Rat Protein
Dose of thyroxine	0	0	3 µgm daily	3 µgm daily
No. of animals	1	3	5	2
	Organ weights			
Organ	mg	mg	mg	mg
Adrenal (1)	9.0	8.2	11.2	16.4
Submaxillaries (2)	223.3	213.3	409.8	418.0
Kidney (1)	267.0	320.6	642.2	707.6
Spleen	153.6	255.7	660.4	922.6
Heart	360.6	408.1	687.1	616.7
Liver	2093.4	2907.5	6548.0	6922.1
Seminal vesicles (2)	17.5	35.0	627.1	886.2
Pituitary	5.7	5.3	8.2	8.7
Body weight at autopsy(gm)	76.0	87.6	244.0	224.0

Approximate age - 15 weeks
Time since thyroidectomy - 11 weeks

TABLE 7

RELATIVE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 27°C

Diet	5% Rat Protein	30% Rat Protein	5% Rat Protein	30% Rat Protein
Dose of thyroxine	0	0	3 µgm daily	3 µgm daily
No. of animals	1	3	5	2
	Organ weights (mgm) per 100 gm body weight			
Organ	mg	mg	mg	mg
Adrenal (1)	11.8	9.2	4.5	7.3
Submaxillaries (2)	293.8	243.4	167.9	186.6
Kidney (1)	351.3	365.9	263.3	315.8
Spleen	202.1	291.8	270.6	411.8
Heart	474.4	465.8	281.5	275.3
Liver	2753.0	3319.0	2683.0	3090.0
Seminal vesicles (2)	23.0	39.9	257.0	395.6
Pituitary	7.5	6.0	3.3	3.8
Body weight at autopsy (gm)	76.0	87.6	244.0	224.0

Approximate age - 15 weeks
Time since thyroidectomy - 11 weeks

mucopolysaccharides were not carried out, sections stained with haematoxylin and eosin gave no indication of mucoid infiltration of the dermis characteristic of myxoedema. No observable differences were found in any other organs.

SUMMARY

That the symptoms of thyroidectomy in the rat could be markedly influenced by variations in the diet was conclusively shown in this experiment. Thus, previous observations had indicated that thyroidectomized animals on Purina Fox Chow showed very few, if any, symptoms of thyroidectomy, those on the "iodine-deficient" (and presumably thyroxine deficient) diet showed some retardation of growth and hyperkeratosis. When, however, as in this experiment, the animals were fed a special diet, containing a presumably thyroxine-free animal protein they presented a typical cretinoid picture with considerable retardation of growth. Although these animals survived well, they remained stunted and lethargic, had dry scaly skin and scanty hair,,were anaemic and sexually undeveloped. It was concluded that the presence of thyroxine in the proteins of animal origin contained in commercial diets prevented the appearance of such symptoms in thyroidectomized rats.

THE COMBINED INFLUENCE OF DIET AND A COOL ENVIRONMENT ON THE SYMPTOMS OF THYROIDECTOMY

When such marked symptoms of thyroid deficiency were obtained in thyroidectomized animals fed a presumably thyroxine free diet, the possibility of further accentuating such

symptoms by increasing the need for thyroxine suggested itself. One mechanism for increasing the thyroxine requirements of an organism is exposure to cold (Ring1939). A series of experiments was therefore conducted, the purpose of which was to obtain the maximal syndrome of thyroid deficiency by appropriate control of the factors of diet and temperature. (It must here be emphasized that this is not necessarily the only way in which such a syndrome could be produced.) In this series of experiments the animals were therefore kept in a special room whose temperature was constant at $16 \pm 2^{\circ}C$.

METHODS

EXPERIMENTAL SET-UP

Male albino rats weighing between 40 - 60 gm (average 50 gm) were used throughout this series of experiments. All animals were given 1% Ca Lactate solution to drink from the day before until a week after operation. All the animals were thyroidectomized and immediately placed in a room in which the temperature was maintained at $16 \pm 2^{\circ}C$. The animals were also immediately given whatever diet they were to receive throughout the experiment. All injections were administered in 2 daily subcutaneous doses of 0.1 cc fluid. The animals were divided into the following groups:

(See following page)

Experiment	Group	Diet	No. of Animals Per Group	Injections	Temp.
A	1	5% rat protein	18	Duponol	16°C
	2	30% rat protein	18	Duponol	
	3	5% rat protein	18	3 Thyroxine	
	4	30% rat protein	18	3 Thyroxine	
B	1	Purina Fox Chow	6	None	16°C
	2	I-deficient diet	6	None	
	3	5% rat protein	30	Duponol	
	4	30% rat protein	10	Duponol	
	5	5% rat protein	15	6 Thyroxine	
C	1	Purina Fox Chow	10	Duponol	16°C
	2	I- deficient diet	10	Duponol	
	3	10% rat protein	15	Duponol	
	4	Purina Fox Chow	10	10 Thyroxine	
	5	I- deficient diet	10	10 Thyroxine	
	6	10% rat protein	15	10 Thyroxine	

Some difficulty was encountered in determining the exact requirements for thyroxine at this temperature. Thus 3 micrograms, the dose used in the experiment conducted at 27°C, was found insufficient at 16°C as indicated by the observation that animals receiving such a dose failed to grow normally (See Fig. 3, R-30 + T and R-5 + T). When, however, the thyroxine dose was raised to 10 micrograms the animals not only failed to grow as fast as expected but also developed diarrhoea - a typical symptom of thyrotoxicosis. (See Fig.5, P + T, I + T, R-10 + T). Thus it was concluded that 10 micrograms of thyroxine was a toxic dose under these conditions.

RESULTS

When the need for thyroxine was increased by subjecting the animals to a lower temperature (16°C), as shown by the

fact that a dose of 3 µgm daily which was sufficient for normal growth at 27°C was insufficient at 16°C. (See Fig. 3, R-5,&T and R-30 &T). The symptoms of animals in all groups were accentuated.

Purina

Thus in Experiment B (See Table 5 and Fig.4) Purina-fed animals (P) showed a significant decrease in the growth rate as compared to control animals (R-5 & T) receiving an adequate dose of thyroxine (6 µgm daily), yet they developed no other apparent symptoms of thyroid deficiency. In Experiment C (See Table 5) in which additional controls were set up, such Purina-fed animals grew at a slightly faster rate than those in the previous experiments. (See Fig.5 P as compared to Fig.4 P). In experiment C, however, no valid comparisons between the Purina-fed and the rat protein fed plus thyroxine (R-10 & T) or the Purina-fed plus thyroxine (P & T) controls could be drawn, because the increased dose of thyroxine (10 µgm as compared to 6 µgm) administered was found to be toxic. These Purina-fed animals also developed no other symptoms of thyroid deficiency.

Iodine-Deficient Diet

In Experiment B (See Table 5 and Fig. 4), whereas the animals on the iodine deficient diet grew less well than those on Purina (P), they did not grow any better than those on the rat protein diet (R-5 & R-30). In Experiment C (see Table 5 and Fig.5) similar animals (I) grew only slightly better than those on the rat protein diet (R-10).

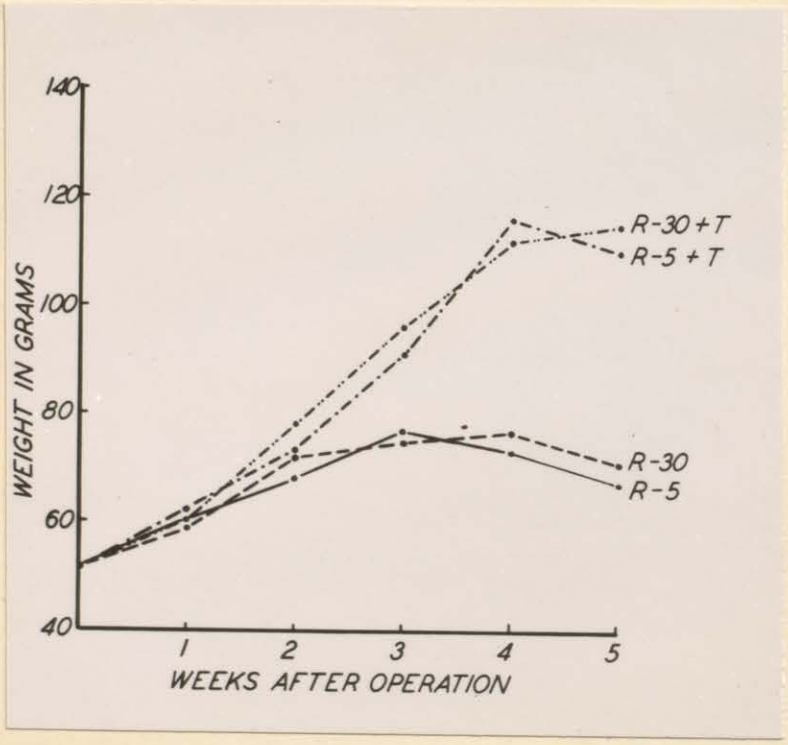

140
120
100
80
60
40
WEIGHT IN GRAMS
1
2
3
4
5
WEEKS AFTER OPERATION
R-30+T
R-5+T
R-30
R-5

Fig.3 The body weight of thyroidectomized rats receiving rat protein diet and kept at 16°C.

Note: (1). All animals receiving no thyroxine (R-5 and R-30) stopped gaining weight at the second week.

(2). All animals receiving 3 µgm of thyroxine daily (R-5 & T and R-30 & T) continued growing until the fourth week, but then these also showed a growth plateau. This dose of thyroxine was therefore insufficient.

(3). There was no difference in the growth rate of animals on the 5% rat protein diet and those on the 30% rat protein diet.

Fig. 4 The body weight of thyroidectomized rats receiving various diets and kept at 16°C.

Note (1). Animals on the 5% rat protein diet (R-5) showed the greatest retardation of growth. Next came the animals on the Iodine-deficient diet (I), then those on the 30% rat protein diet.

(2). Animals receiving Purina (P), although growing better than the others grew less well than the the controls receiving 5% rat protein diet and 6 µgm of thyroxine daily (R-5 & T).

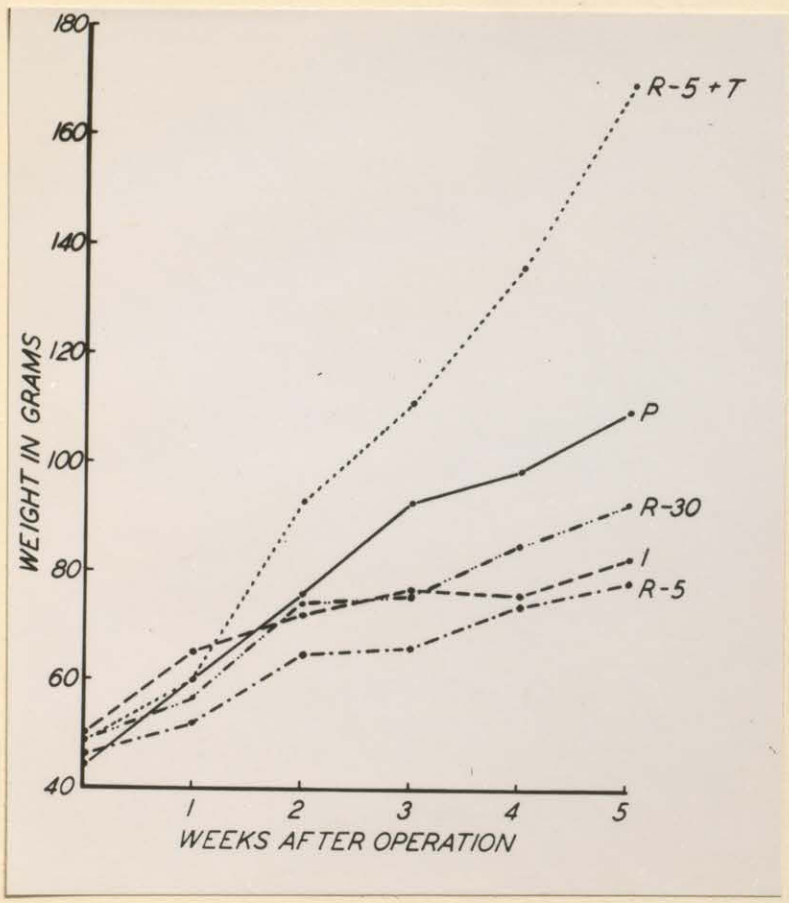
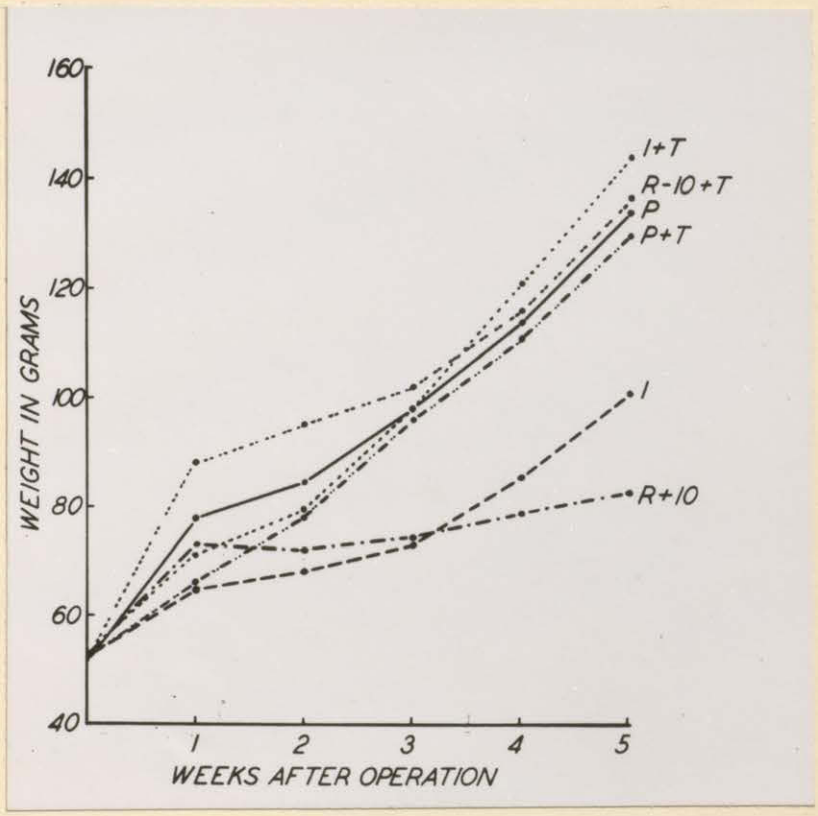

180
160
140
120
100
80
60
40
WEIGHT IN GRAMS
1
2
3
4
5
WEEKS AFTER OPERATION
R-5 + T
P
R-30
I
R-5

Fig. 5 The body weight of thyroidectomized rats receiving various diets and kept at 16°C.

Note: (1). Animals receiving 10% rat protein diet (R-10) showed the greatest retardation of growth. These were followed by animals on the iodide-deficient diet (I).

(2). The Purina fed animals (P) grew as well as the controls receiving 10 ugm of thyroxine (I & T, R-10 & T, P & T). This dose of thyroxine was found to be toxic.


160
140
120
100
80
60
40
WEIGHT IN GRAMS
1
2
3
4
5
WEEKS AFTER OPERATION
I+T
R-10+T
P
P+T
I
R+10

The animals on the iodine deficient diet, however, developed all the symptoms exhibited by animals on the rat protein diet kept at 27°C. (See page 44). Thus, by changing another variable, namely, temperature, the "typical" syndrome of thyroid deficiency was again obtained. At 16°C the animals on the iodine-deficient diet were, however, still in a relatively much healthier condition than those receiving the rat protein diet at the same temperature (16°C).

Thyroidectomized Rat Protein Diet

At 16°C, all the animals on the thyroidectomized rat protein diet either stopped gaining weight completely at the end of two weeks, or thereafter continued growing very slowly (See Table 5 and Figs 3,4,5). The maximum average weight reached by survivors of any one of these groups after 5 weeks was 93 gm (See Table 5 , B-4) as compared to 170 gms for the controls (See Table 5 , B-5). All these animals, were however, in very poor condition, and could at once be recognized as typical cretins whose symptoms had been even further accentuated.

After three to four weeks, these animals suddenly developed a new set of symptoms. The development of this acute phase was not peculiar to any one experiment, but was consistently observed in almost all the animals kept at 16°C and fed the rat protein diet. Furthermore, the onset was very sudden and definite, so that all animals developed this condition within one or two days of each other. This striking and curious syndrome resulted in death within two or three days. These symptoms appeared in approximately the following order.

1. A fall in body temperature - the animals felt cold when handled.

2. Progressive muscular paralysis, which first was recognized by a dragging of the hind legs and loss of the righting reflex. This phenomenon was very striking and the animals behaved in a curious and characteristic manner, namely, they persisted in walking backwards when placed on the table and in the more advanced stages, performed fly catching movements with their feet. Later still, the animal went into spastic rigidity when handled.

3. Loss of weight from the time paralysis set in - presumably due to inability of the animals to feed themselves.

4. Marked decrease in heart rate - from 400 to 200 beats per minute.

5. Decrease in respiratory rate.

6. Wetting of the genital region - indicative of diuresis.

The paralysis gradually spread upward from primary involvement of the hind leg musculature to that of the rest of the body. In time the corneal reflex disappeared and the animals lapsed into coma. Death which rapidly ensued seemed to be due to paralysis of the muscles of respiration although the possibility of food and water deprivation - due to the animal's inability to move could not be ruled out.

RESULTS FOUND AT AUTOPSY:

Organ Weights

The organs of all animals in experiments B and C were weighed at autopsy and showed substantially a close correlation with the body weights (See Tables 8,9,10,11).

The only organs showing a consistent difference between animals fed the various diets were the seminal vesicles which

TABLE 8

ABSOLUTE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 16°C

Diet	Purina	I-Def.	5% Rat Protein	30% Rat Protein	5% Rat Protein
Dose of thyroxine	0	0	0	0	6 γ daily
No. of animals	5	3	2	5	2
	Organ weights				
Organ	mg	mg	mg	mg	mg
Adrenal (1)	16	12	12	11	16
Submaxillaries (2)	266	221	255	249	457
Kidney (1)	510	335	390	404	772
Spleen	499	242	499	447	908
Heart	445	314	358	393	748
Liver	4555	3360	3240	3843	6659
Seminal vesicles(2)	184	123	31	42	457
Pituitary	5.2	5.0	4.8	4.9	4.5
Body weight at autopsy (gm):	118	86	77	93	170

Approximate age - 10 weeks
Time since thyroidectomy - 6 weeks

TABLE 9

RELATIVE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 16°C

Diet	Purina	I-Def.	5% Rat Protein	30% Rat Protein	5% Rat Protein
Dose of thyroxine	0	0	0	0	6γ daily
No. of animals	5	3	2	5	2
	Organ weights (mgm) per 100 gm body weight				
Organ	mg	mg	mg	mg	mg
Adrenal (1)	13	13	15	11	9
Submaxillaries (2)	225	256	331	267	268
Kidney (1)	432	389	506	434	454
Spleen	427	281	648	480	534
Heart	377	365	464	422	440
Liver	3860	3844	4207	4132	3917
Seminal vesicles(2)	156	146	40	45	268
Pituitary	5.8	4.4	6.2	5.2	2.6
Body weight at autopsy (gm):					
	118	86	77	93	170

Approximate age - 10 weeks
Time since thyroidectomy - 6 weeks

TABLE 10

ABSOLUTE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 16°C

Diet	Purina	I-Def.	10% Rat Protein	Purina	I-Def.	10% Rat Protein
Dose of thyroxine	0	0	0	10 µgm daily	10 µgm daily	10 µgm daily
No. of animals	9	7	10	7	4	5
			Organ weights (mgm)			
Organ	mg	mg	mg	mg	mg	mg
Adrenal (1)	17.8	14.2	13.7	14.9	14.0	13.4
Submaxillaries (2)	227.0	228.7	245.3	235.7	238.7	224.0
Kidney (1)	675.1	522.3	540.4	623.9	623.5	734.2
Spleen	1070.0	1113.3	824.9	1256.8	1334.6	1327.9
Heart	579.6	605.2	584.8	802.3	706.5	858.0
Liver	7386.9	5895.8	5201.4	7122.0	7089.1	7696.2
Seminal vesicles(2)	98.8	75.6	39.1	134.5	68.6	89.9
Pituary	6.0	7.3	4.6	7.0	5.2	6.1
Body weight at autopsy (gm):	136	104	89	128	126	152

Approximate age - 8 weeks
Time since thyroidectomy - 4 weeks

TABLE 11

RELATIVE WEIGHTS OF ORGANS OF THYROIDECTOMIZED ANIMALS FED VARIOUS DIETS AND KEPT AT 16°C

Diet	Purina	I-Def. T	10% Rat Protein	Purina	I-Def.	10% Rat Protein
Dose of thyroxine	0	0	0	10 µgm daily	10 µgm daily	10 µgm daily
No. of animals	9	7	10	7	4	5
			Organ weights (mgm)			
Organ	mg	mg	mg	mg	mg	mg
Adrenal (1)	13.1	13.6	15.2	11.9	11.2	8.8
Submaxillaries (2)	167	219	275	184	189	147
Kidney (1)	445	500	615	490	535	483
Spleen	787	974	926	983	1040	875
Heart	426	581	659	626	560	565
Liver	5440	5660	5840	5560	5620	5060
Seminal vesicles(2)	72.5	72.6	43.9	104.5	54.5	59.1
Pituary	4.4	7.0	5.2	5.5	4.2	4.0
Body weight at autopsy (gm):						
	136	104	89	128	126	152

Approximate age - 8 weeks
Time since thyroidectomy - 4 weeks

differed in their degree of development (See Tables 8, 10).

Animals on the rat protein diet showed completely undeveloped seminal vesicles in all experiments. In Experiment C, the seminal vesicles of the animals on the iodine deficient and Purina diets were intermediate in weight while those of the animals receiving thyroxine were fully developed. On the other hand, the animals which were getting a toxic dose of thyroxine (10 µgm), also had underdeveloped seminal vesicles.

In general the hypophysis varied as the degree of thyroid deficiency. Contrary to the previous experiment, thyroid deficiency did not seem to increase the relative weight of the heart.

Histology

Histological examination of the various organs showed:

(1) the typical absence of acidophils and presence of "thyroidectomy" basophils in the hypophysis (Leblond 1943). This well-established fact was used as a check on the completeness of thyroidectomy.

In addition,

(2) an increased ratio of cartilage to bone in the tibia and tail

(3) an increased cornification of the epidermis, tail

(4) an increase in the number of glomeruli per unit area - presumably indicating a decrease in the length of the proximal convoluted tubules of the kidney. (The reduction in absorbing surface may explain diuresis).

(5) a decrease in the size of the zone fasciculata and glomerulesa of the adrenal (This may partly explain the decreased resistance to cold and other damaging agents).

(6) no change in the muscles (No morphological basis was

found to explain the paralysis).

were also noted on cursory examination of the slides.

SUMMARY

Typical cretinoid symptoms, similar to those exhibited by thyroidectomized animals on the rat protein diet maintained at a normal temperature (27°C), could also be elicited in animals on the so-called "iodine-deficient" diet if the need for thyroxine of such animals was increased. This was done by placing them in a cool environment (16°C).

The syndrome of thyroid deficiency could be further accentuated in thyroidectomized animals on the rat protein diet if these were also placed in the cold. Up to the third or fourth week after operation, these animals showed the same, though more severe, symptoms than they did at 27°C. During the fourth week, however, a very striking acute syndrome developed, resulting in 100% deaths within two or three days. Low body temperature, loss of weight, diuresis, slow and feeble heart and respiratory rate and a progressively severe muscular paralysis preceded a comatose death.

FURTHER INVESTIGATION OF THE ACUTE SYNDROME OF THYROID DEFICIENCY

The striking symptoms obtained in thyroidectomized rats, under the combined influence of a controlled diet and temperature merited further consideration. The suspicion that the acute syndrome was due to exhaustion of thyroxine stores was investigated by observing whether the length of time before the onset of the acute syndrome after exposure to cold could be shortened by thyroidectomizing the animals one or more weeks before subjecting them to the reduced environmental temperature. The animals then would presumably enter the cold already in a state of partial thyroid deficiency and the decreased thyroxine stores should therefore be depleted faster under these conditions of increased stress.

In order to observe whether the symptoms could simply be due to a sudden failure of the mechanism of homothermy resulting in a sharp drop in body temperature, which in turn could affect the nervous system, rectal temperatures were taken daily.

The methods used to determine the nature of the paralysis were first of all designed to determine whether this paralysis was reverisble. For this purpose some animals were injected with thyroxine. Others were injected with prositigmine which inhibited choline esterase activity and thus presumably would relieve paralysis, if it was due to such a block at the neuro-muscular junction. An attempt was also made to revive the comatose animals by placing them in a warm environment.

EXPERIMENTAL SET-UP

EXPERIMENT A

26 male albino rats weighing 130 gm on the average were thyroidectomized, placed on an iodine deficient diet and kept at 27°C. Three weeks later the 14 surviving animals were removed to the cold room (16°C) and their diet was changed to 5% thyroidectomized rat protein.

EXPERIMENT B

Four rats which had been thyroidectomized four months previously and subsequently kept on an iodine deficient diet at 27°C, were found to exhibit marked symptoms of thyroid deficiency. Their average weight at this time was 118 gm. It was decided to place these animals in the cold (16°C) on a 5% thyroidectomized rat protein diet, in order to observe how soon such already severely thyroid deficient animals would develop the acute syndrome.

Body temperatures were taken rectally at intervals throughout these experiments. In addition the reversibility of the paralysis was tested in a number of animals, which had developed this paralytic syndrome. The following procedures were adopted:

(1) 2 paralyzed animals were given 3 micrograms of thyroxine subcutaneously
(2) 2 paralyzed animals were given 2 micrograms of prostigmine subcutaneously
(3) 2 paralyzed animals were removed to a warm room
(4) 9 animals which showed only the first signs of paralysis were removed to a warm room and subsequently fed an iodine deficient diet.

RESULTS

Survival

In Experiment A, where the animals were thyroidectomized 3 weeks before being placed in the cold, 5 of the 14 survivors (the rest having died of tetany), developed the paralytic syndrome on the fourth or fifth day in the cold and died one or two days later. The rest were removed to the warm room, as soon as the syndrome first became apparent. On the other hand, in Experiment B, where the animals had been thyroidectomized four months before being placed in the cold, the syndrome appeared within one or two days. Death followed in a matter of hours. The onset of the symptoms was sudden and dramatic in all cases and the paralysis spread rapidly.

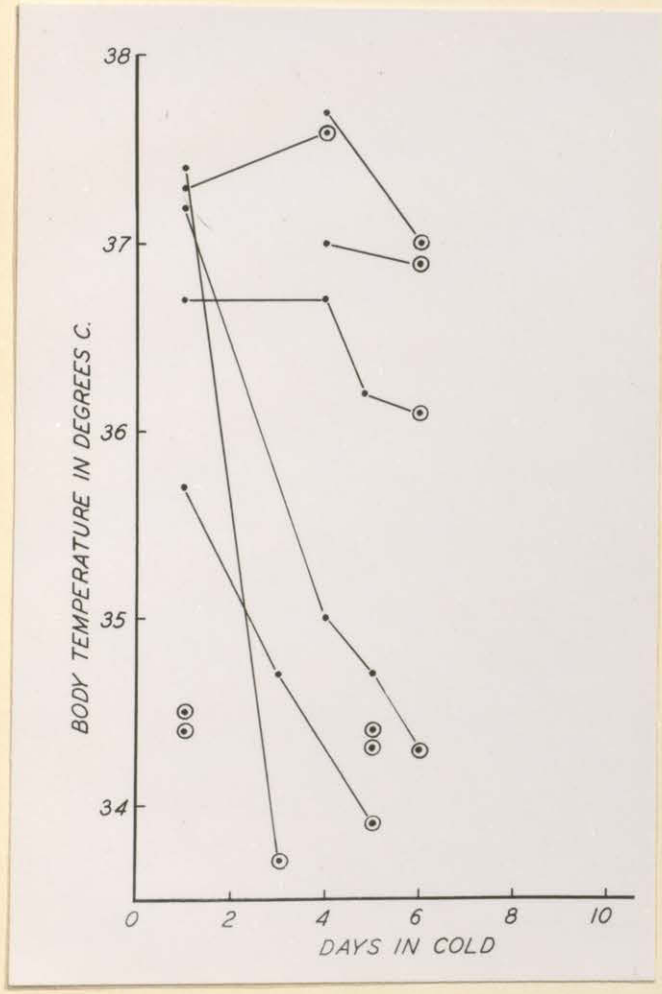
Thus, the longer the animals had been thyroidectomized before being placed in the cold, the sooner they developed the paralytic syndrome upon exposure to the reduced temperature.

Body Temperature

Examination of Table 12 and Fig. 6 showed that in all cases except two where the animals died of paralysis and its associated symptoms there was a marked fall in body temperature, beginning usually one or two days before death. Thus when the animals were moribund their rectal temperature fell as low as 33.7°C, while healthy animals kept at the same temperature managed to maintain their body temperatures between 36.7 and 38.8°C.

Fig. 6 The body temperatures of thyroidectomized rats fed a thyroxine deficient diet and kept at 16°C.

Note: The body temperatures of all animals except one fell preceding death, (indicated by a circle).


38
37
36
35
34
BODY TEMPERATURE IN DEGREES C.
0
2
4
6
8
10
DAYS IN COLD

Reversibility of the Paralytic Syndrome

Once the stage of general paralysis was reached, death usually occurred within a few hours. The injection of thyroxine (which has a latent period of about twenty-four hours) thus had no effect in reversing or attenuating the symptoms of such moribund animals.

The injection of prostigimine also had no effect. However, since this drug was supposed to act immediately, indications were that the paralysis was not of the same nature as Myasthenia Gravis, which is due to excessive choline esterase activity at the neuro-muscular junctions.

Removal of the animals to a warm room was ineffectual when these animals were moribund but was successful in reversing the syndrome in its initial stages.

SUMMARY

1. The longer an animal had been thyroidectomized before being placed in the cold, the shorter the time it survived once it was put in such an environment on a thyroxine deficient diet. However at least four weeks following thyroidectomy were required before the appearance of the paralytic syndrom in all cases.

2. The onset of the paralysis was always accompanied by a sharp fall in the body temperature. Thus all the evidence seemed to point to the symptoms being due to animals' inability to maintain normal body temperature under these conditions.

3. The paralysis was not relieved by an injection of thyroxine, probably because the animals died before its latent period was over.

4. The paralysis was not relieved by prostigmine thus indicating that it was not due to a block at the neuro-muscular junction.

5. If not too advanced the paralysis could be relieved by removing the animals to a warm room, but this was ineffective once coma had set in.

(See Table 12 - following page)

TABLE 12

BODY TEMPERATURE OF THYROIDECTOMIZED RATS FED A THYROXINE DEFICIENT DIET AND KEPT AT 16°C

Rectal Body Temperature of Individual Animals (degrees C)

		EXPERIMENT A Animals							EXPERIMENT B Animals			
		A	B	C	D	E	F	G	A	B	C	D
Days in Cold	1			36.7	37.2	37.3			34.4	34.5	37.4	35.7
	2								X	X		
	3										33.7	34.7
	4			36.7	35.0	37.6	37.0	37.7			X	
	5	34.4	34.3	36.2	34.7	X	36.9	37.0				33.9
	6	X	X	36.1	34.3		X	X				X
	7			X	X							

X - indicates death of animal.

EVALUATION OF METHODS AND RESULTS

The aim of these experiments was to determine what factors could influence the symptoms of thyroidectomy in the rat. Diet and temperature were believed to be two such factors. Accordingly an attempt was first made to increase the symptoms of thyroidectomy by modifying the diet. The first step in this direction was to decrease and possibly eliminate any exogenous supply of thyroxine in the diet. The influence of temperature was also examined. It was hoped to increase the need for thyroxine by lowering the environmental temperature. The results supported the assumptions made.

In all cases, thyroid deficiency was obtained by complete surgical removal of the thyroid. Thyroidectomized animals were then subjected to various influences which enhanced or decreased the symptoms of thyroid deficiency. Thyroidectomy was done with care to prevent regrafting of pieces from the removed gland. The forceps were dipped into alcohol every time thyroid tissue was touched to kill any adhering tissue which might "take" and regenerate. Thus, 100% removal could be effected in almost every case. Incomplete thyroidectomy could be suspected if one animal in a group grew at a faster rate than the others. The thyroid region was examined for any thyroid remnants at autopsy and as a further check the hypophysis of all animals was examined for the presence of acidophils, this being diagnostic of successful thyroid ablation (Leblond 1944). If

acidophils were found in an animal, the results obtained were omitted from the average.

The only disadvantage of this method was the unavoidable concomittant parathyroidectomy. Although administration of 1% calcium lactate as drinking water greatly reduced the incidence of tetany, this factor still caused a considerable number of deaths. Thus a loss of one-third of the animals during the first week was not unusual. There may also be other unknown side-effects of para-thyroidectomy which may influence the symptoms of thyroidectomy.

The general survival of thyroidectomized animals was only fair, as such animals are easily susceptible to infection. Differences in diet and even administration of thyroxine had little influence on the incidence of deaths from causes other than the paralytic syndrome.

In regard to diet, 100 grams of Remington's modified iodine deficient diet was found to contain approximately 10 to 20 µgm of free or bound iodine. The effectiveness of the diet in increasing the symptoms of thyroidectomy was probably due not so much to the low level of free iodine as to the presumed low level of thyroxine-bound iodine.

Another factor which had to be considered was that this diet was very low in animal protein (2%); if the observation that animals receiving 30% rat protein diet grew better than those receiving 5% rat protein diet was an indication of a protein deficiency in the latter, some of

the growth-inhibiting action of the iodine-deficient diet also might be attributed to the iodine deficient diets' low protein level.

The thyroidectomized rat protein diet, which was prepared in an endeavour to increase the symptoms of thyroid deficiency by further curtailing or even eliminating the supply of exogenous thyroxine, succeeded in its purpose. Symptoms of marked thyroid deficiency such as cessation of growth, lethargy, hyperkeratosis, anaemia, etc. occurred within a few weeks in animals fed any one of the diets containing various percentages of this thyroidectomized rat protein. Similar animals fed the iodine deficient diet did not develop such symptoms for several months while Purina-fed animals never developed them. As previously remarked, the difference in the growth-inhibiting properties of the 5 and 30% rat protein diet was possibly due to a protein deficiency in the former and such an observation served as a warning that results should not be ascribed to any single factor. The diet proved generally satisfactory as it was readily eaten by the animals without causing diarrhea or other observable direct deleterious effects. The main disadvantage was the number of animals as well as the time and labour involved in its preparation. Thus the animals used for the diet had to be thyroidectomized at least three weeks before sacrifice and the subsequent processing of the carcasses to reduce these to a dry powder required at least another week. Once a batch of diet was

prepared, it could, however, be kept almost indefinitely in a closed container.

When a positive state of thyroid deficiency had been produced by feeding thyroidectomized rats this rat protein diet, the question was raised as to whether further symptoms could be elicited by increasing the need of such animals for the missing thyroxine by exposure to mild cold. This added stress proved even more successful than expected, since not only did the well-known symptoms previously observed appear in an even more severe form, but in addition a new and striking syndrome developed, which always resulted in death. Mild cold increased the severity of symptoms of animals fed the iodine deficient diet, although not to the same degree. Such animals developed symptoms similar to those shown by the animals on the rat protein diet at 27°C.

The temperature chosen, 16°C proved satisfactory for this moderately cool temperature permitted the symptoms to evolve slowly. When as it had been shown by Leblond and Gross (1943) that in thyroidectomized animals exposed to temperatures near 0°C death occurred so quickly (sometimes within a few hours), that the exact cause of mortality could not be ascertained. In considering the effects of mild cold on the symptoms of thyroidectomy, the influence of the adrenal, which is an important agent in cold adaptation also had to taken into consideration. Thus some of the effects of thyroidectomy could have been mediated through the adrenal. For instance the sudden drop of body tempera-

ture accompanying the paralytic syndrome could have been due to a failure of the adrenal, rather than to a direct exhaustion of thyroxine, and only secondarily to a failure of the thyroid mechanism to take over. In 1938 Ring indicated that adrenalectomized animals could not maintain their body temperature at 20°C while in 1942, he established that the thyroid was also necessary. Failure of adrenal action as the factor responsible for the drop in body temperature in the present investigations was felt to be excluded by the fact that the syndrome could be precipitated within one or two days in the cold in animals previously made thyroid deficient.

The animals used for the experiments varied in weight, from 40 to 150 gm. All animals developed the paralytic syndrome given the rat protein diet and exposed to mild cold, developed the paralytic syndrome. Age therefore did not seem to be influential in the etiology of this syndrome.

As noted before, some difficulty was encountered in determining the exact dosage of thyroxine required to restore normal conditions at various temperatures. Increase in body weight was found to be a reliable guide as to whether this dose was too high or too low and it was adjusted accordingly in subsequent experiments. (Since cold was known to enhance both the metabolic and the heart rate, these guides which were otherwise used for the determination of the correct replacement dose of thyroxine could not be used at 16°C, without an extensive number of control experiments to

determine the normal rate at this temperature. Practical consideration also made removal of the oxygen consumption apparatus to the cold room impossible.

Occasional heart rate measurements were, however, made in order to obtain a general indication of the level reached by thyroidectomized rats under these conditions. Thus the great drop in heart rate accompanying the paralytic syndrome was detected.

The taking of rectal body temperatures with a clinical thermometer was generally satisfactory, and could be carried out without difficulty.

The results obtained from the methods used to test the reversibility of the paralysis were sufficient to give an indication that the syndrome could not be reversed by the administration of either thyroxine or prostigmine and could be reversed in its early stages by removal of animals to a warm room. However, such results could not be regarded as conclusive, due to the small number of animals upon which these tests were performed and to their moribund condition.

SUMMARY AND CONCLUSIONS

1. The symptoms of thyroidectomy could be influenced by alteration of the diet.

Thus, thyroidectomized animals on an iodine (and presumably also thyroxine deficient) diet developed more pronounced symptoms than similar animals on Purina diet.

Furthermore, the thyroidectomized rat protein diet increased the severity and the time of onset symptoms as compared to the iodine deficient diet.

2. The symptoms of thyroidectomy could be influenced by temperature. Thus, the thyroid deficiency of all animals, irrespective of diet was increased at a moderately cool temperature (16°C, as compared to the normal temperature for the rat, namely 27°C).

3. These 2 factors (thyroxine-deficient diet and cold) were found to influence the symptoms of thyroidectomy in a synergistic manner, so that when they were combined, not only were all the well-known symptoms of thyroid deficiency increased but additional, acute symptoms were produced.

4. This acute syndrome was characterized by a marked drop in body temperature and progressive muscular paralysis.

5. Following thyroidectomy four weeks was the minimum time required for the development of the symptoms. If, however the animals were kept at 27°C for a period of time after thyroidectomy, and then placed in the cold, the time of onset in the cold of the acute syndrome was shortened in proportion to the length of time the animal had been thyroidectomized.

6. Age was found to have little, if any, influence on the development of the acute syndrome since it could be produced in animals weighing 130 gm at the time of thyroidectomy as well as in those weighing only 50 gm.

Incidentally, thyroid deficiency caused lack of development of the seminal vesicles and stimulation of the hypophysis. The replacement dose of thyroxine was found to be around 3

micrograms daily at 27°C and around 6 micrograms daily at 16°C.

PART II

THE INFLUENCE OF OTHER HORMONES ON THE ACTION OF THYROXINE

In the previous experimental series it was noted that an equal dose of thyroxine - namely three micrograms - had quite different effects on thyroidectomized animals kept at 16°C than on similar animals kept at 27°C. Or in other words, such factors as cold could influence the action of thyroxine. Ershoff in 1949 [a&b] reported that certain dietary factors could also modify the action of this hormone, thus extending the known series of affecting factors. Furthermore, since there was also known to be a close correlation between all endocrine organs, it was decided to investigate the possibility of the combined action of other hormones on some of the peripheral organs shown by Grad to be under the influence of thyroxine. Grad had shown that testosterone and thyroxine were necessary for the maintenance of normal weight and histological appearance of a number of organs other than the accessory sex organs. Thus in the submaxillary gland normalcy was reestablished through synergistic action of these two hormones while in the case of the hypophysis a similar state was achieved by the additive action of these two hormones. Finally the results observed in the epidermis suggested the possibility that the combined action of these two hormones was antagonistic in this location.

A further investigation of this last phenomenon by a more intensive study of the histological sections of epidermis was undertaken in order to make sure of the facts and determine the mechanism of action of thyroxine in this case.

Other experiments were then carried out to determine whether this action of thyroxine and testosterone on the epidermis was direct or whether it was mediated through one or more glands. At the same time the influence of the absence or presence of other hormones on various other targets of throxine action were also studied.

The experiments will be reported in the following order:

1. The Influence of Testosterone on the Action of Thyroxine in Castrated-Thyroidectomized Rats (With Particular Emphasis on the Epidermis).
2. The Influence of Cortisone and Testosterone on the Action of Thyroxine in Adrenalectomized-Castrated-Thyroidectomized Rats (With Particular Emphasis on the Epidermis).
3. The Influence of Hypophysectomy on the Action of Thyroxine, Testosterone and Cortisone (With Particular Emphasis on the Epidermis and on Growth).

THE INFLUENCE OF TESTOSTERONE ON THE ACTION OF THYROXINE IN CASTRATED-THYROIDECTOMIZED RATS (With Particular Ephasis on the Epidermis)

EXPERIMENT A

EXPERIMENTAL SET-UP

Since some more work was done on the material of Grad's original experiment, this will be briefly described. 40 male albino rats weighing between 136 and 194 grams were thyroidectomized and castrated on the same day - and subsequently fed the iodine deficient diet. These experimental animals were divided into the following groups.

Group (1) 10 thryoidectomized-castrated rats receiving 2 daily subcutaneous injections of 0.1cc 1% Duponol

(2) 10 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of 0.25µmg of free testosterone in 0.1cc 1% Duponol

(3) 10 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of 3 µgm of d,l sodium thyroxine in 0.1cc 1% Duponol

(4) 10 thyroidectomized-castrated rats receiving both hormones in the doses indicated, care being taken to inject the testosterone into one side of the body and the thyroxine into the other.

The animals were sacrificed on the 52nd day of the experiment. A piece of skin measuring approximately 1 x 2 cm was taken from the lumbar region of each animal and fixed in Orth for histological study. The sections were cut at 5 µ and stained with haematoxylin and eosin.

RESULTS

Epidermal thickness

Microscopic examination of the epidermis indicated that testosterone increased the size of the Malpighian and cornified layers (Fig. 8) over that in the unoperated controls (Fig. 7) while tyroxine decreased the size of these layers (Fig. 9). Both hormones produced an intermediate effect (Fig. 10). As can be seen from these same pictures the number of granules of the stratum granulosum was influenced in the same direction as the epidermal thickness by these two hormones.

This impression was subsequently semi-quantitatively substantiated when the thicknesses of the Malpighian and cornified layers were subjectively assessed as a number

PLATE I

Epidermis Orth fixation Haematoxylin-eosin stain

Fig. 7 Control castrated-thyroidectomized male rat.
Note thickness of Malpighian and cornified layers, as well as the presence of keratohyalin granules in the stratum granulosum.

Fig. 8 Castrated-thyroidectomized male rat treated with testosterone.
Note that great stimulation of both cornified and Malpighian layers as well as an increase in the number of granules.

Fig. 9 Castrated-thyroidectomized male rat treated with thyroxine.
Note great decrease in thickness of both cornified and Malpighian layers, as well as virtual absence of granules.

Fig. 10 Castrated-Thyroidectomized male rat treated with testosterone and thyroxine.
Note the compomise effect produced here, in the thickness of the cornified and Malpighian layers as well as in the number of granules.

(From Grad 1949)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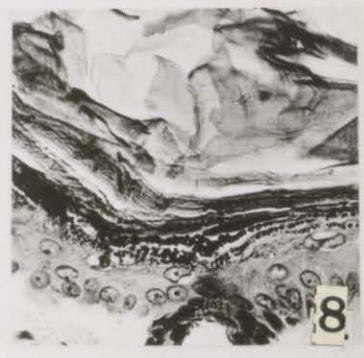
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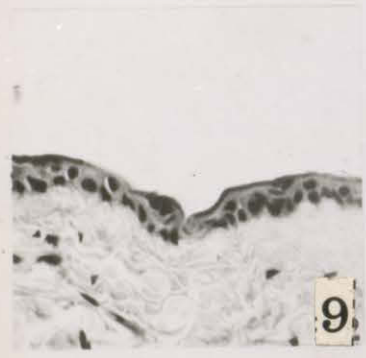
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of pluses. (See Methods) From Table 13 it could be seen

TABLE 13

EFFECTS OF TESTOSTERONE AND THYROXINE ON THE THICKNESS OF THE EPIDERMIS IN CASTRATED-THYROIDECTOMIZED RATS (SUBJECTIVE RESULTS

	Control	Testosterone	Thyroxine	Testosterone and Thyroxine
No. of animals in group	4	5	7	8
Mean Thickness (in pluses)				
a) Malpighian layers	2.2	3.4	1.0	2.1
b) Cornified layers	2.5	4.2	1.2	3.1

that testosterone increased the thickness of the Malpighian layers from the control value of 2.2 to 3.4 and of the cornified layers from the control value of 2.5 to 4.2. Thyroxine, on the other hand decreased the thickness of the Malphighian layers from the control value of 2.2 to 1.0 and of the cornified layers from the control value of 2.5 to 1.3. Both hormones together gave an intermediate thickness comparable to that of the controls, namely 2.1 as compared to 2.2 for the Malphighian layers and 3.1 as compared to 2.5 for the cornified layers. This data was also expressed graphically in Fig. 11 (top row).

There was however sufficient variation within slides to make it necessary to confirm these results by objective histometric studies. This was done by the projection technique as described in Methods. The mean thickness of the whole epidermis as well as of each layer separately was

ig. 11 The effect of testosterone and thyroxine on the epidermis of castrated-thyroidectomized rats - thickness (subjective results).

Note: (1). In all three series testosterone caused an increase in both layers of the epidermis, especially in cornified layers.

(2). In all three series thyroxine caused a decrease in both layers of the epidermis, especially the cornified layers.

(3). Both hormones together produced an intermediate thickness of the epidermis.

(4). The sham-operated animals showed a great variability in the thickness of the epidermis.

expressed in microns. A statistical analysis was then carried out on this data. P values smaller than 0.05 were considered to be statistically significant while values of 0.02 and 0.05 were considered to be on the verge of significance.

Examination of Table 14 showed that testosterone increased the thickness of the Malphighian layers from the control value of 23.2 μ to 28.9 μ while thyroxine decreased it to 16.4 μ. Both hormones together gave a value of 25.2 μ which could be seen to be comparable to that of the controls of 23.2 μ. In the cornified layers the effects were even more striking. Thus testosterone produced a thickness of 75.0 μ as compared to 27.3 for the controls while thyroxine decreased the thickness to 14.9 μ. The value for both hormones was somewhat higher than that found for the controls, namely 54.4 μ versus 27.3 μ. These results are also expressed graphically in Fig. 12

Statistical analysis of these data by the method of analysis of variance showed that in the Malpighian layers thyroxine alone as compared to the controls significantly decreased the thickness - while testosterone versus the controls here showed no statistical difference of the Malpighian layers. However testosterone versus thyroxine on the Malpighian layers was very highly significant. There was no statistical difference between the controls and the testosterone and thyroxine group.

TABLE 14

EFFECT OF TESTOSTERONE AND THYROXINE ON THE THICKNESS OF THE EPIDERMIS IN CASTRATED-THYROIDECTOMIZED RATS.

	Control	Testosterone	Thyroxine	Testosterone and Thyroxine
No. of animals in group	4	5	7	8
Mean body weight (gm.)				
Initial	176	180	168	175
Final	168	163	213	206
Mean Thickness (u)				
Whole epidermis	50.7	103.9	31.3	79.6
Malpighian layer	23.2	29.9	16.4	25.2
Cornified layer	27.3	75.0	14.9	54.4

Statistical Analysis: P Values

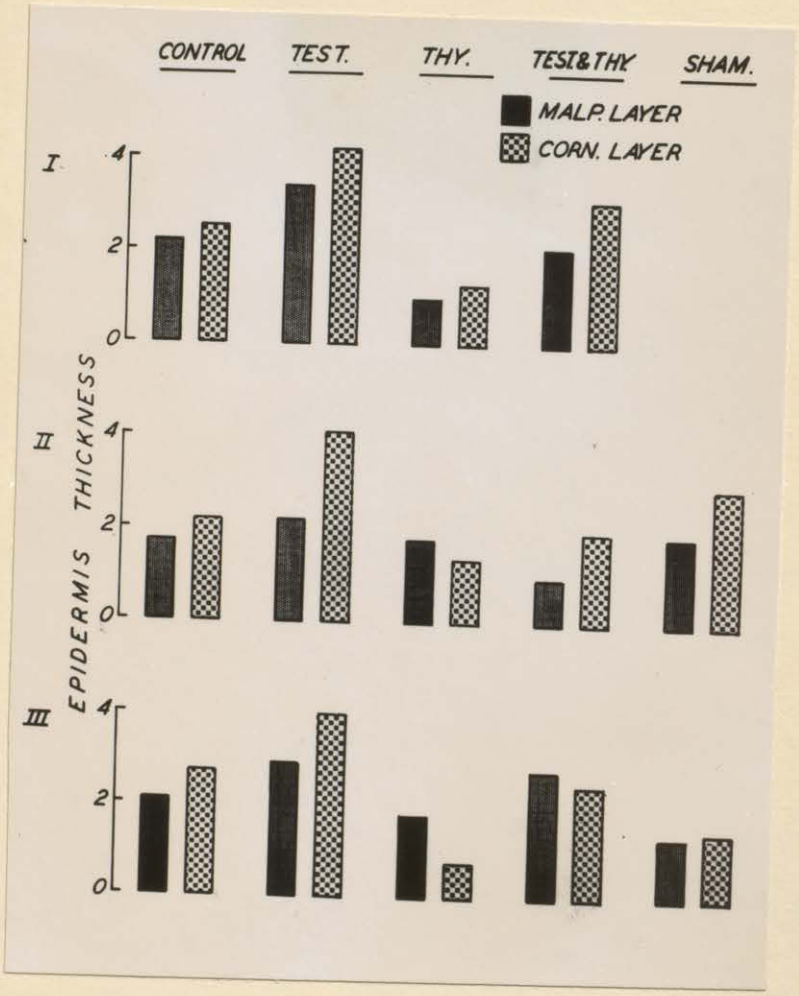
	Control vs. Testosterone	Control vs. Thyroxine	Control vs. Testosterone and Thyroxine	Testosterone and Thyroxine	Testosterone vs. Testosterone and Thyroxine	Thyroxine vs. Testosterone and Thyroxine
Whole epidermis	0.01	0.10	0.02	0.001	0.05	0.001
Malpighian layer	0.10	0.05	0.90	0.001	0.10	0.01
Cornified layer	0.01	0.02	0.02	0.001	0.05	0.001

Fig. 12 The effect of testosterone and thyroxine on the epidermis of castrated-thyroidectomized rats- thickness.

Note: (1). Testosterone increased the thickness of both layers of the epidermis, especially the cornified layers.

(2). Thyroxine decreased the thickness of both layers of the epidermis, especially the cornified layers.

(3). Both hormones together produced an intermediate Thickness.


CONTROL
TEST.
THY.
TEST&THY
SHAM.
MALP. LAYER
CORN. LAYER
I
II
III
EPIDERMIS THICKNESS
4
2
0

In the cornified layers on the otherhand the difference between the controls and the testosterone groups was highly significant while that between the controls and thyroxine was not significant. Testosterone versus thyroxine was again very highly significant while the control groups versus the testosterone and thyroxine groups was on the verge of significance. In addition testosterone versus testosterone and thyroxine was also significant and thyroxine versus thyroxine and testosterone was also very highly significant.

When the whole epidermis was considered, control versus testosterone was highly significant, controls versus thyroxine was not significant, controls versus testosterone and thyroxine was on the verge of significance and testosterone versus thyroxine was very highly significant, testosterone versus testosterone and thyroxine were very highly significant.

In conclusion testosterone increased the thickness of the epidermis particularly by increasing the cornified layers while thyroxine decreased the thickness of the epidermis but not to the same extent as testosterone increased the thickness.

Mitotic Counts

The question naturally arose as to whether the differences in thickness were due to a difference in the number of cells. Thus an attempt was made to determine the mitotic rate of the epidermis in the various groups.

Metaphases and anaphases were counted, as described in Methods and were expressed as number of mitoses per centimeter basement membrane. Examination of Table 15 shows that testosterone increased the number of mitoses from 67 in the controls to 122 while thyroxine decreased the number to 32. Both hormones together gave a value of 45. Statistical analysis revealed that the only significant difference was between thyroxine and testosterone and between testosterone versus both hormones together.

However, when the number of mitoses was plotted against the epidermal thickness it was found that a regression line could be drawn which proved to be highly significant, as tested by the "t" test. Thus, the epidermal thickness seemed to vary directly as the mitotic activity. (See Fig. 13).

Since the body weight increased in the animals given thyroxine it was deemed possible that the whole skin had been stretched thus causing a relative decrease in the number of mitoses per centimeter of basement membrane. Therefore, the mean number of mitosis per body surface (see Methods) was calculated (this value being strictly an approximation). It was found that the decrease persisted for thyroxine while the increase in number of cells in mitosis in the testosterone treated animals also was still apparent. Incidentally it was found that some 38.6×10^6 cells would be in mitosis at one time in the controls. This value was increased to 70.1×10^6 for the testosterone

TABLE 15

EFFECT OF TESTOSTERONE AND THYROXINE ON THE MITOTIC ACTIVITY OF THE EPIDERMIS IN CASTRATED-THYROIDECTOMIZED RATS

	Control	Testosterone	Thyroxine	Testosterone and Thyroxine
No. of animals in group	4	5	7	8
Mean body weight (gm.)				
Initial	176	180	168	175
Final	168	163	213	206
Mean number of mitoses per cm. of basement membrane (metaphases and antaphases only)	67	122	32	45
Mean number of mitoses per body surface ($x10^6$)	38.6	70.1	24.8	35.7
Ratios of:				
Thickness of epidermis to number of mitoses	0.76	0.85	0.97	1.76
Thickness of Malpighian layers to number of mitoses	0.35	0.24	0.51	0.56
Thickness of cornified layers to number of mitoses	0.41	0.62	0.45	1.20

Statistical Analysis: P Values

	Control vs. Testosterone	Control vs. Thyroxine	Control vs. Testosterone and Thyroxine	Testosterone and Thyroxine	Testosterone vs. Testosterone and Thyroxine	Thyroxine vs. Testosterone and Thyroxine
Mean no. of mitoses per cm. of basement membrane	0.02	0.10	0.30	0.001	0.001	0.40

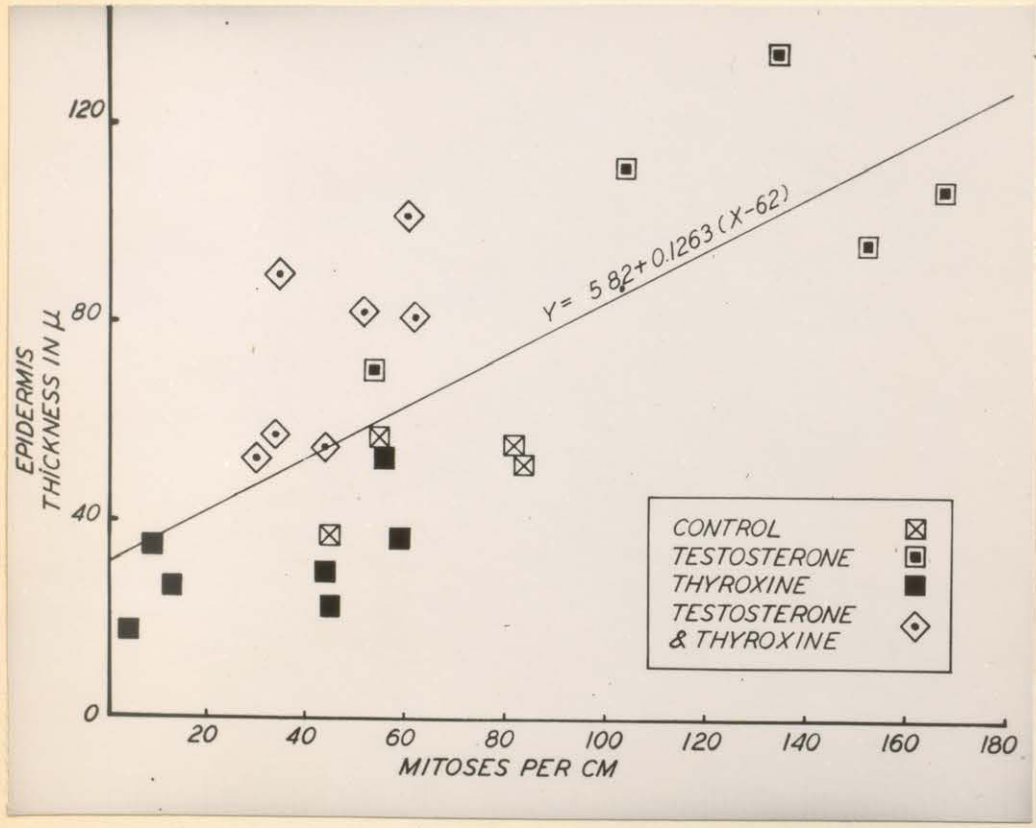
Fig. 13 The effect of testosterone and thyroxine on the epidermis of the castrated-thyroidectomized rat.— regression of epidermal thickness to number of mitoses.

Note: (1). Epidermal thickness varied as the number of mitoses per centimeter.

(2). The formula for the regression line is

$$Y = \bar{y} + \phi (X - \bar{x})$$

where $\underline{Y}$ is any value of y
$\bar{y}$ is the arithmetical mean of the y's
ϕ is the regression coefficient of y on x
$\underline{X}$ is any value of x
$\bar{x}$ is the arithmetical mean of the x's


EPIDERMIS THICKNESS IN μ
120
80
40
0
20
40
60
80
100
120
140
160
180
MITOSES PER CM
Y = 5.82 + 0.1263 (X-62)
CONTROL
TESTOSTERONE
THYROXINE
TESTOSTERONE & THYROXINE

treated animals and reduced to 24.8 x 10^6 for the thyroxine treated animals and when both hormones were given the values was found to be 35.7 x 10^6 indicating that although the animals were considerably larger the number of mitoses was not markedly different from that found in the smaller controls. All this has been expressed graphically - (Fig. 14)

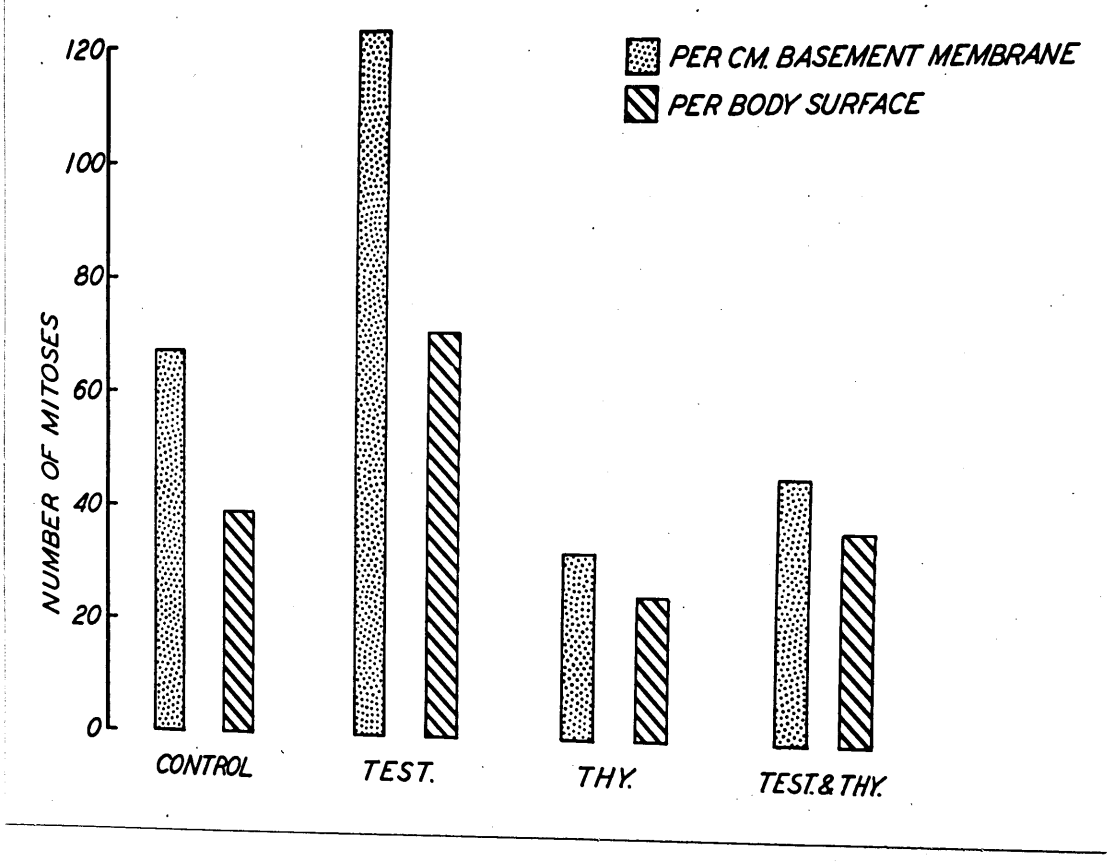
The ratio of the mean number of mitoses per centimeter of basement membrane was next calculated for the various groups. It was found that these ratios for the whole epidermis were not the same - but that they showed a consistent rise as one went from the control through the testosterone and thyroxine groups to the thyroxine-testosterone treated group - and were of the order of 0.78,0.85, 0.97 and 1.76 (See Table 15). This finding was contrary to expectation since if changes in mitotic rate were the sole factor involved the thickness of the epidermis should be proportional to the number of mitoses. An attempt at explaining these surprising results was made by examining the ratio of the thickness of the Malpighian layer to the number of mitoses and also that of the cornified layer thickness to the mitotic number separately. When this was done it was observed that the ratio of the Malpighian layer thickness to their number of mictoses was increased in both groups of animals receiving thyroxine (i.e. the third and fourth groups) which had values of 0.51 and 0.56 as compared to 0.35 for the controls and 0.24 for testosterone - treated animals. Thus, it was concluded that thyroxine had decreased the thickness of the Malpighian layers less than

Fig. 14. The effect of testosterone and thyroxine on the epidermis of castrated-thyroidectomized rats - number of mitoses.

Note: (1). Testosterone caused an increase in the number of mitoses - whether these were expressed per cm. basement membrane or per body surface.

(2). Thyroxine caused a decrease in the number of mitoses.

(3). Both hormones together produced an intermediate number of mitoses.


PER CM. BASEMENT MEMBRANE
PER BODY SURFACE
NUMBER OF MITOSES
120
100
80
60
40
20
0
CONTROL
TEST.
THY.
TEST.& THY.

it should have according to mitotic number found. Therefore the cells must have been larger - or they must have stayed longer in this layer. To attempt to settle this point - the cell size was next investigated and it was found that no difference could be detected between the cells in the Malpighian layers of any of the animals. Thus apparently the cells in the Malpighian layers of animals treated with thyroxine stayed longer in this layer - which would lead one to think that thyroxine had in some way interfered with the cell differentiation prior to passage into the cornified layers. This then explains why the effect of thyroxine on the Malpighian layers was less clear cut initially than the effect of this same hormone on the cornified layers.

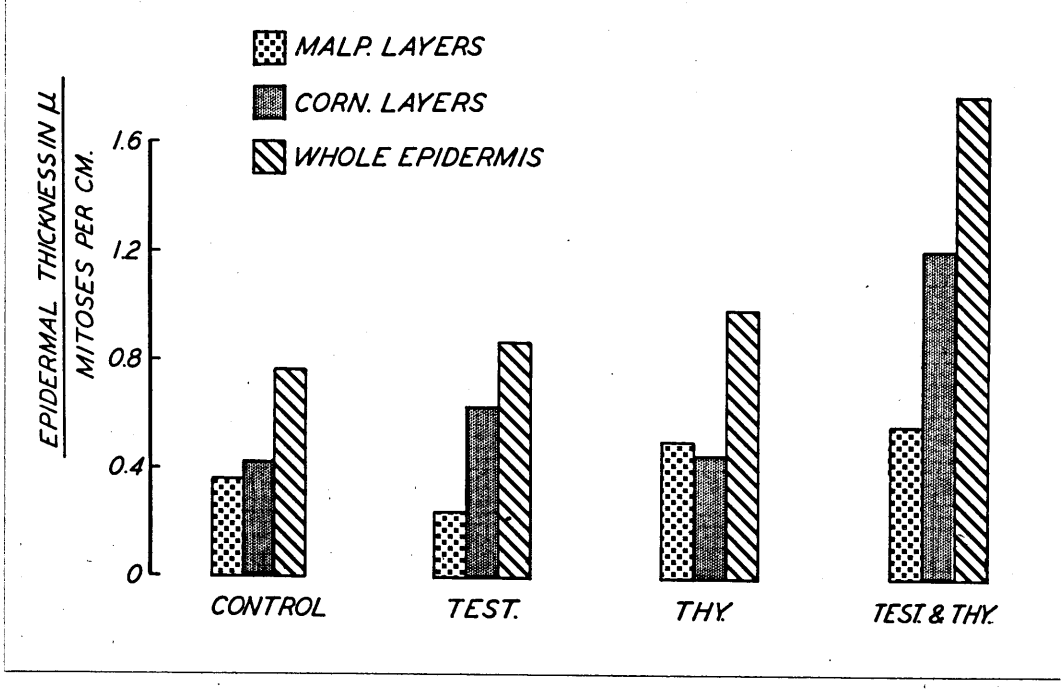
In the cornified layers, on the other hand, the ratios of the thicknesses to that of the mitotic rate was greater in the two testosterone-treated groups than in the other groups. Thus in the second and fourth groups the values were 0.62 and 1.20 as compared to 0.41 for the controls and 0.45 for thyroxine-treated animals (see Table 15). It was concluded from these findings that testosterone in some unexplained way had interfered with desquamation process. This lack of desquamation of the cornified layers combined with the increase in the number of new cells formed through mitosis in the Malpighian layer made the over all thickness of the epidermis of testosterone-treated animals especially striking. (See Fig.14 and Fig.15)

Fig. 15. The effect of testosterone and thyroxine on the epidermis of castrated-thyroidectomized rats - ratio of epidermal thickness to number of mitoses per cm.

Note: (1). Both groups receining testosterone (Groups 2 and 4) showed an increase in the ratio of the thinkness of the cornified layers to the number of mitoses.

(2). Both groups receiving thyroxine(Groups 3 and 4) showed an increased in the ratio of the thickness of the Malpighian layers to the number of mitoses.

(3). The group receiving both hormones (Group 4) showed great increase in the ratio of the thickness of the whole epidermis to the number of mitoses.


MALP. LAYERS
CORN. LAYERS
WHOLE EPIDERMIS
EPIDERMAL THICKNESS IN μ
MITOSES PER CM.
1.6
1.2
0.8
0.4
0
CONTROL
TEST.
THY.
TEST. & THY.

Hair Growth

At the same time in the same slides the hair follicles were also studied subjectively and it was found that thyroxine increased both the number and size. It was therefore concluded that thyroxine increased the rate of hair growth. Since increased hair growth implies increased mitotic activity of the hair follicle this result was contrary to expectation in the light of the findings on the epidermis - for hair is an outgrowth of the Malpighian layers and might have been expected to also show decreased mitotic activity. In the light of the known shedding of hair in thyroid deficient animals, this finding was to be expected. Consequently the action of thyroxine on hair growth was now a paradox.

Sebaceous Glands

In confirmation of Grad's findings (1949) testosterone was found to greatly stimulate the sebaceous gland and, while thyroxine had no effect by itself, in conjunction with testosterone it had a marked synergistic action on the sebaceous glands.

Summary:

1. It has been shown that thyroxine decreased the thickness of both the Malpighian and cornified layers of the epidermis in the castrated-thyroidectomized rat.. This effect was more striking in the cornified than in the Malphighian layers.

2. Testosterone on the other hand decreased the thickness of both layers in similar animals - the effect also being most striking in the cornified layers.

3. Both hormones combined gave an intermediate thickness of the epidermis - which was somewhat greater than that of the controls.

4. The main mechanism responsible for these differences in thickness was found to be the effect of these two hormones on mitosis as determined by the number of cells in mitosis in the Malpighian layers.

5. An additional mechanism responsible for the change in thickness was a decrease in the rate of desquamation due to testosterone and a decrease in the rate of cornification due to thyroxine.

6. In addition, thyroxine was found to increase the rate of hair growth - as shown by an increase in the number and size of hair follicles - and testosterone increased the size of the sebaceous glands. Thyroxine also was noted to have a synergistic action on these glands when given in combination with testosterone.

EXPERIMENT B

In order to test the constancy of the occurrence of this striking antagonistic action of thyroxine and testosterone on the epidermis, the experiment was repeated. To determine whether the combined action of the two hormones produced a normal condition, an additional control - sham operated - group was included for comparison. At the same time various other interactions of these two hormones such as that on the body weight, metabolic and heart rates and on the weight of various organs were also investigated.

EXPERIMENTAL SET-UP

75 male albino rats weighing from 105 to 135 gm were divided into 5 equal groups. The animals were fed the iodine deficient diet. Their basal metabolic and heart rates were taken twoice weekly. After two weeks, when the animals weighed from 150 to 180 gms they were thyroidectomized and castrated, and then divided into the following groups:

Group (1) 15 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of 0.1cc 1% Duponol

(2) 15 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of 250 µgm of free testosterone in 0.1cc 1% Duponol

(3) 15 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of 3 µgm of dl - sodium thyroxine in 0.1cc 1% Duponol

(4) 15 thyroidectomized-castrated rats receiving 2 daily subcutaneous injections of both hormones in the doses indicated

(5) 15 sham operated rats receiving 2 daily subcutaneous injections of 0.1cc 1% Duponol

The animals were given calcium lactate instead of drinking water for 10 days following operation. 38 days later all animals were shaved along an inch-wide strip from the middle of the back to the middle of the abdomen on the right side. Thus observations of the rate of re-growth of the hair was made possible. A week later all animals were sacrificed with chloroform, their organs fixed in Orth. Later these organs were weighed, and subsequently prepared for histological study. In particular, pieces of skin were again taken from the lumbar region of the unshaven side of the animals. Sections were cut at 5 μ and stained with haematoxylin and eosin.

A subjective estimation of the thickness of the various layers of the epidermis was made (Fig.11). Since the results were in close agreement with those of the previous experiment, no detailed study was made of this organ.

Statistical analyses were carried out on heart rates averages of the last 9 experimental days, at which time the heart rates were fairly well stabilized. Similarly statistical analyses were also carried out on the average basal metabolic rates of the last 16 days.

RESULTS

Epidermis

Epidermal Thickness

A subjective examination of the thickness of the epidermis (See Table 16 and Fig.11 , Series 2), confirmed the results of the previous experiment.

TABLE 16

EFFECTS OF TESTOSTERONE AND THYROXINE ON THE THICKNESS OF THE EPIDERMIS OF CASTRATED-THYROIDECTOMIZED RATS (SUBJECTIVE RESULTS)

	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals in group	8	8	5	3	15
Mean Thickness (in pluses)					
a) Malpighian layers	1.7	2.2	1.8	1.0	1.9
b) Cornified layers	2.2	4.1	1.4	2.0	2.9

Here again thyroxine decreased and testosterone increased the thickness of both layers. The sham operated animals and controls tended to have a skin of intermediate thickness. Results were more clear-cut in the cornified than in the Malpighian layers - as was also true in the previous experiment.

Rate of hair growth

Examination of the amount of hair which had regrown in the shaved area of the animals during the period of a week revealed that the rate of hair growth was accelerated in the thyroxine treated animals and somewhat retarded in the testosterone-treated group. The action of thyroxine was predominent in both the doubly treated and the sham operated animals. (The well-known fact that the hair of the rat grew in cycles was also observed here.) Upon examination of the sections of the epidermis there appeared to be a parallel increase in the number of hair follicles in the thyroxine treated animals. Thus similar observation in the previous experiment was confirmed.

Sebaceous Glands

The stimulation of the sebaceous glands by testosterone and the striking synergistic action of testosterone

and thyroxine together was again observed by examination of the sections of the epidermis.

Body Weight

Both the control animals and those receiving testosterone (see Table 17) ceased gaining weight or even lost weight soon after operation, although the testosterone-treated animals stabilized their body weight at a slightly higher level than the controls. (It should be noted that these animals were much older than those fed the rat protein diet (Part I) which continued to grow for two weeks before reaching a plateau). Testosterone, however, exerted an antagonistic action to that of thyroxine in the doubly treated animals, so that these animals gained less weight than those animals receiving only thyroxine. The sham operated animals grew best, a fact which may be explained as a failure to give a dose of thyroxine sufficient to maintain the oxygen consumption of thyroxine and thyroxine and testosterone treated animals at a level equal to the sham-operated animals.(See Table 18)

Oxygen Consumption

The oxygen consumption was very variable during the first few weeks (See Table 18) although a sharp drop of the basal metabolic rate became apparent during the first week after operation in all animals not receiving thyroxine, i.e. the first two groups. The animals receiving testosterone had, however, a consistently higher metabolic rate than the controls. Statistical analysis of the average oxygen consumption during the last 16 days revealed that this increase

was very highly significant (See Table 18) and paralleled the stimulating effect of testosterone on the body weight. (See Table 17) This time, however, testosterone did not depress the action of thyroxine. Such results were contradictory to those reported by Grad, who observed no effect of testosterone on either the body weight, oxygen consumption or heart rates.

Heart rate

Great variability was also characteristic of the heart rates (See Table 19), and the drop following operation was not as immediately apparent as the fall in the oxygen consumption. Testosterone increased the heart rate over the controls during the first two and a half weeks post-operatively and then suddenly it reversed its action, so that the controls now had a higher rate than the testosterone-treated group. Statistical analysis of the heart rates during the last 11 days revealed this effect to be very highly significant, although no explanation could be found. Testosterone had no effect on the action of thyroxine. Both groups receiving thyroxine had heart rates which were not significantly different from those of the sham-operated animals, indicating that this is a less sensitive index than either the oxygen consumption or the body weight for determination of replacement doses of thyroxine.

Organ weights

On examination of the organ weights (See Table 20 & 21) it appeared that thyroxine increased the weight of the adrenals

TABLE 17

BODY WEIGHTS OF THYROIDECTOMIZED-CASTRATED RATS RECEIVING REPLACEMENT DOSES OF THYROXINE AND/OR TESTOSTERONE

Treatment	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
	Body weights (gm)				
Weeks					
0	116	116	117	118	118
1	134	134	132	133	133
2	153	153	153	153	160
	Thyroidectomized and castrated				
3	162	158	161	156	180
4	157	168	177	170	201
5	150	173	191	178	221
6	156	168	202	190	237
7	155	171	217	201	250
8	156	168	226	206	258

TABLE 18

OXYGEN CONSUMPTION OF THYROIDECTOMIZED-CASTRATED RATS RECEIVING REPLACEMENT DOSES OF THYROXINE AND/OR TESTOSTERONE

Treatment	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals	11	8	5	3	15
	Oxygen Consumption (cc/hr/cm^2)				
Days					
1	79.9	78.8	82.1	76.6	87.0
4	79.4	71.3	74.0	84.6	84.2
7 Thyroidectomized and castrated					
11	62.8	74.6	89.4	82.8	89.1
14	52.0	58.4	80.4	72.2	80.8
18	52.9	64.5	80.6	76.4	81.2
20	57.6	64.4	75.2	78.1	82.0
25	53.9	59.3	75.6	75.1	76.2
26	56.0	60.6	79.6	68.0	76.9
32	66.6	63.0	75.5	85.9	72.9
33	57.5	65.4	75.1	72.0	77.4
39	60.9	68.4	78.5	79.1	76.9
40	61.3	68.3	73.8	79.0	82.1
46	64.8	65.3	73.4	72.8	76.8
Average of days 33 - 46	61.1	64.0	73.2	73.9	78.1

Statistical Analysis

	Average of days 33 - 46 P
Control vs. Testosterone	0.001
Control vs. Thyroxine	0.001
Control vs. Both	0.001
Control vs. Sham	0.001
Testosterone vs. Thyroxine	0.001
Testosterone vs. Both	0.001
Testosterone vs. Sham	0.001
Thyroxine vs. Both	-
Thyroxine vs. Sham	0.01
Both vs. Sham	0.02

TABLE 19

HEART RATES OF THYROIDECTOMIZED-CASTRATED RATS RECEIVING REPLACEMENT DOSES OF THYROXINE AND/OR TESTOSTERONE

Treatment	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals	11	8	5	3	15
			Heart Rate (Beats/min.)		
Day					
1	396	410	405	385	417
2	320	351	362	371	349
7 Thyroidectomized and castrated					
11	367	381	425	435	408
14	358	389	388	444	403
18	355	369	430	380	405
19	309	367	410	394	400
24	322	323	417	394	380
25	337	313	381	375	385
31	340	303	396	423	383
32	325	298	386	371	373
38	312	310	409	390	373
39	318	312	361	400	381
46	321	295	385	360	369
Average of days 38 - 46	319	302	378	382	375

Statistical Analysis

	Average of days 38 - 46 P
Control vs. Testosterone	0.001
Control vs. Thyroxine	0.001
Control vs. Both	0.001
Control vs. Sham	0.001
Testosterone vs. Thyroxine	0.001
Testosterone vs. Both	0.001
Testosterone vs. Sham	0.001
Thyroxine vs. Both	-
Thyroxine vs. Sham	-
Both vs. Sham	-

TABLE 20

ABSOLUTE WEIGHTS OF ORGANS OF THYROIDECTOMIZED-CASTRATED RATS RECEIVING REPLACEMENT DOSES OF THYROXINE AND/OR TESTOSTERONE

Treatment	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals	11	8	5	3	15
			Organ weights (mgm)		
Adrenals	26.0	19.9	37.1	29.8	31.3
Submaxillary glands(2)	271.9	263.6	415.1	410.6	525.2
Heart	567.9	529.6	777.7	853.6	888.5
Kidneys (2)	1065.6	989.8	1660.0	1548.0	1756.3
Spleen	552.0	459.6	964.0	755.6	913.6
Liver	5680.2	5765.3	8166.3	6211.6	9214.4
Seminal vesicles (2)	70.9	439.9	87.0	277.6	393.5
Hypophysis	12.4	9.2	13.3	9.6	8.4
Body weight (gm)	161.6	163.0	241.4	214.0	269.1

Time after thyroidectomy and castration - 45 days

Statistical Analysis

	Adrenal Weights P	Spleen Weights P
Control vs. Testosterone	0.02	0.5
Control vs. Thyroxine	0.001	0.02
Control vs. Both	0.3	0.4
Control vs. Sham	0.02	0.001
Testosterone vs. Thyroxine	0.02	0.01
Testosterone vs. Both	0.01	0.1
Testosterone vs. Sham	0.001	0.001
Thyroxine vs. Both	0.1	0.4
Thyroxine vs. Sham	0.05	0.8
Both vs. Sham	0.6	0.4

TABLE 21

RELATIVE WEIGHTS OF ORGANS OF THYROIDECTOMIZED-CASTRATED RATS RECEIVING REPLACEMENT DOSES OF THYROXINE AND/OR TESTOSTERONE

Treatment	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals	11	8	5	3	15
			Organ weights (mgm)		
Adrenals	16.1	12.3	15.4	13.9	11.6
Submaxillary glands (2)	167.1	162.6	173.0	193.0	196.1
Heart	350.0	325.1	323.0	399.0	330.4
Kidneys (2)	654.0	606.1	688.0	725.9	654.8
Spleen	342.0	282.1	360.0	354.4	339.7
Liver	3500.6	3544.1	339.1	2910.3	3412.6
Seminal vesicles (2)	43.8	271.3	87.0	130.0	146.5
Hypophysis	7.7	5.6	5.5	4.5	3.1
Body weight (gm)	161.6	163.0	241.4	214.0	269.1

Time after thyroidectomy and castration - 45 days

while testosterone decreased and both gave an intermediate weight approximating that of the sham-operated animals. These effects proved to be significant and were still apparent when corrections were made for the increase in body weight produced by thyroxine. Similarly thyroxine was found to increase the weight of the spleen while testosterone decreased it, and this again was found to be statistically significant and also still apparent after corrections for body weight. The absolute and relative weight of the seminal vesicles was increased by testosterone. These were found to be relatively larger in the small animals which did not receive thyroxine, than in normally growing animals (thyroxine treated or sham). Both thyroxine and testosterone decreased the weight of the hypophysis and the effect was additive when both hormones were administered together. Thyroxine and testosterone had only a slight synergistic action on the weights of the heart, kidneys and submaxillary gland (as previously claimed by Grad 1949).

SUMMARY

1. The antagonistic action of testosterone and thyroxine on the epidermis was confirmed.
2. Thyroxine was found to increase the rate of hair growth.
3. Testosterone was found to increase the size of the sebaceous glands, and thyroxine was found to exert a synergistic effect on this structure.
4. Testosterone seemed to increase the body weight when acting alone, but to depress the growth-stimulating action of thyroxine.
5. Testosterone also seemed to increase the oxygen consumption when acting alone, but had no effect when acting with thyroxine.

6. Testosterone had no consistent effect on the heart rate.

7. Thyroxine increased the weight of both the spleen and the adrenals, while testosterone decreased the weight of both these organs.

8. Both thyroxine and testosterone decreased the weight of the hypophysis and the effect was additive when both hormones were administered together.

(See Tables 17,18,19,20,21)

THE INFLUENCE OF CORTISONE AND TESTOSTERONE ON THE ACTION OF THYROXINE IN ADRENALECTOMIZED-CASTRATED-THYROIDECTOMIZED RATS (With Particular Emphasis on the Epidermis)

In previous experiments it was found that thyroxine increased the weight of the adrenal of thyroidectomized-castrated animals while testosterone decreased it. (See Tables 20,21.)

In the last two experiments it had been observed that testosterone increased the thickness of castrated-thyroidectomized rat epidermis, while thyroxine decreased it. Simultaneously these hormones were found to have an antagonistic effect to yield the normal picture.

Moreover, it had been shown that cortisone decreased the thickness of both the epidermis and dermis either when injected or applied locally (Baker and Whitaker, 1948, Castor and Baker, 1950). The thyroid and adrenal were known to exert their actions through certain reciprocal relationships. Thus, the possibility was raised as to whether the actions of thyroxine and testosterone on the epidermis and possibly on other structures and functions were mediated through the adrenals.

To test this hypothesis an experiment was carried out, using castrated-thyroidectomized-adrenalectomized animals treated with physiological doses of testosterone, thyroxine and cortisone in all possible combinations.

Since it has been observed that cortisone caused a thinning of the epidermis (Baker, 1948, Castor, 1950) and the thyroid and the adrenal have been shown to exert

certain reciprocal influences on one another (see Introduction Pg. 10), it was thought that perhaps the action of the thyroid and possibly also the testis, were exerted indirectly through stimulation and inhibition of the adrenal respectively.

In order to investigate this aspect of the problem, we decided to repeat the previous experiment, but using animals which were adrenalectomized as well as thyroidectomized and castrated and some of which received replacement doses of cortisone, either alone or in various combinations with the other hormones.

EXPERIMENTAL SET-UP

A pilot experiment was run in order to estimate the degree of survival and the approximate dosage of cortisone. This latter was found to be very difficult to determine, but as 2 mg. per day proved definitely toxic (the animals died within two to three days and showed atrophic thymus, spleen and enlarged liver) and 1 mg dose also seemed too large, while 100 micrograms seemed to have little effect, it was decided to use 200 micrograms. However, this dose was still arbitrary. The animals were given 1% saline to drink and survived fairly well, even when treated with thyroxine. However, it was observed that the adrenal regenerated very easily and thus in the main experiment all the animals showing regenerated adrenal tissue at autopsy had to be discarded and omitted from the results which reduced the experiment.

Main Experiment

200 animals were divided into thirteen groups of 150 gram male rats such that four major groups each containing

several subgroups were set up. All animals were thyroidectomized and castrated and four major groups were then set up on the basis of the following injections which were administered twice daily subcutaneously in o.1cc

Group 1 - 1% Dupanol - 46 rats
2 - 3 µg Thyroxine in 1% Dupanol - 48 rats
3 - 250 µg Testosterone in 1% Dupanol - 46 rats
4 - 3 µg Thyroxine in 1% Dupanol and 250 µg Testosterone in 1% Dupanol - 48 rats
5 - Sham-operated - 12 rats 1% Dupanol

25 days after the first injections 132 animals were left, of these 100 were adrenalectomized. The groups were then redivided in the following manner.

A. Thyroidectomized and castrated
(1) Dupanol (8) (+ adrenal)
(2) Thyroxine (8)
(3) Testosterone (8)
(4) Both (8)

B. Thyroidectomized, castrated and adrenalectomized
(1) Dupanol (10) (- adrenal)
(2) Thyroxine (10)
(3) Testosterone (12)
(4) Both (12)

C. Thyroidectomized, castrated and adrenalectomized
(1) Dupanol (11) (- adrenal + cortisone 200 micrograms daily)
(2) Thyroxine (11)
(3) Testosterone (12)
(4) Both (9)

D. Unoperated shams - Dupanol (9)

Electrocardiographic and basal metabolic studies (see graphs) were carried out at intervals during the experimental period which lasted 10 days.

Footnote: The numbers in ( ) indicate the number of animals in each group)

Many of the adrenalectomized animals died soon after operation despite the use of 1% NaCL as drinking water. All survivors were sacrificed on the tenth day in order to have a statistically significant number of animals left. At this time various organs were taken, fixed in Orth, weighed and then stained with haematoxylin-eosin. These tissues were later studied histologically. At time of autopsy a careful check was made for signs of adrenal regeneration and all animals showing such were eliminated from the results.

RESULTS

Epidermis

Epidermal Thickness

A subjective examination of the various layers of the epidermis (see Fig. 16) indicated at first glance that the characteristic antagonistic actions of thyroxine and testosterone were apparent whether or not the adrenal was present. (The result from the plus adrenal groups are also shown in Table 22 and Fig. 11,

TABLE 22

EFFECT OF TESTOSTERONE AND THYROXINE ON THE THICKNESS OF THE EPIDERMIS CASTRATED-THYROIDECTOMIZED RATS (SUBJECTIVE RESULTS)

	Control	Testosterone	Thyroxine	Testosterone and Thyroxine	Sham
No. of animals in group	7	7	5	5	6
Mean Thickness (in pluses)					
a) Malpighian layers	2.1	2.9	1.8	2.7	1.4
b) Cornified layers	2.7	4.0	0.8	2.5	1.5

Series 3, since they again confirm the results of the first experiment). Differences in thickness were again most clear

Fig. 16 The effect of adrenalectomy and replacement doses of testosterone, thyroxine and cortisone on the skin and its appendages and on the adrenal weights of castrated-thyroidectomized rats.

Note: (1). Thyroxine caused a decrease and testosterone an increase in all layers of the epidermis, while both hormones produced an intermediate effect. This action was most clear cut in the cornified layers.

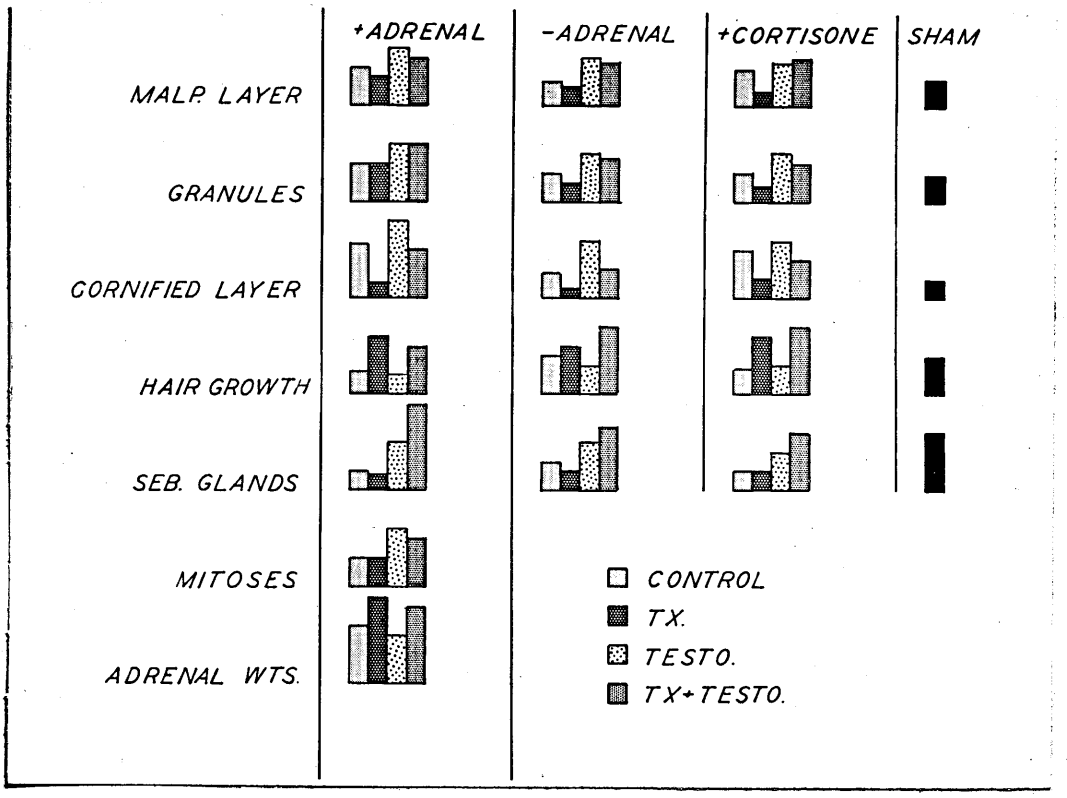
(2). Thyroxine caused an increase in the rate of hair growth.

(3). Testosterone caused an increase in the size of the sebaceous glands and while thyroxine had no effect of its own, both hormones together acted synergistically.

(4). Testosterone increased the number of mitoses in the epidermis.

(5). Thyroxine increased and testosterone decreased the weights of the adrenals.

(6). Neither adrenalectomy nor cortisone had any observable effect on these organs.


+ADRENAL
-ADRENAL
+CORTISONE
SHAM
MALP. LAYER
GRANULES
CORNIFIED LAYER
HAIR GROWTH
SEB. GLANDS
MITOSES
ADRENAL WTS.
CONTROL
TX.
TESTO.
TX+TESTO.

cut in the cornified layers although the antagonistic action of the hormones was also very apparent in the Malpighian and granular layers. The action of thyroxine and testosterone on the epidermal thickness thus seemed to be independent of the adrenal.

The incidence of mitoses was counted in the first series (in animals with intact adrenals) and although the mitotic activity in the control groups seemed to be very low, so that the effect of thyroxine was not apparent, testosterone definitely increased the number of mitoses and testosterone and thyroxine together gave an intermediate count. This again confirmed previous results.

Sebaceous Glands

Similarly testosterone was again found to increase the size of the sebaceous glands, and thyroxine to exert a synergistic action, all this being independent of the adrenal or of cortisone.

Body Weight

Results previously obtained were partially confirmed in this experiment. Thus in the animals with intact adrenals (See Fig.17), testosterone again seemed to increase the weight when administered alone and depress the effect of thyroxine, when given in combination with this hormone. In the other two series (- adrenal, - adrenal + cortisone) testosterone again increased the body weight when given alone, but its depressing action on thyroxine was not apparent. In fact the animals receiving testosterone and thyroxine had a higher growth rate than those receiving thyroxine alone. Adrenalec-

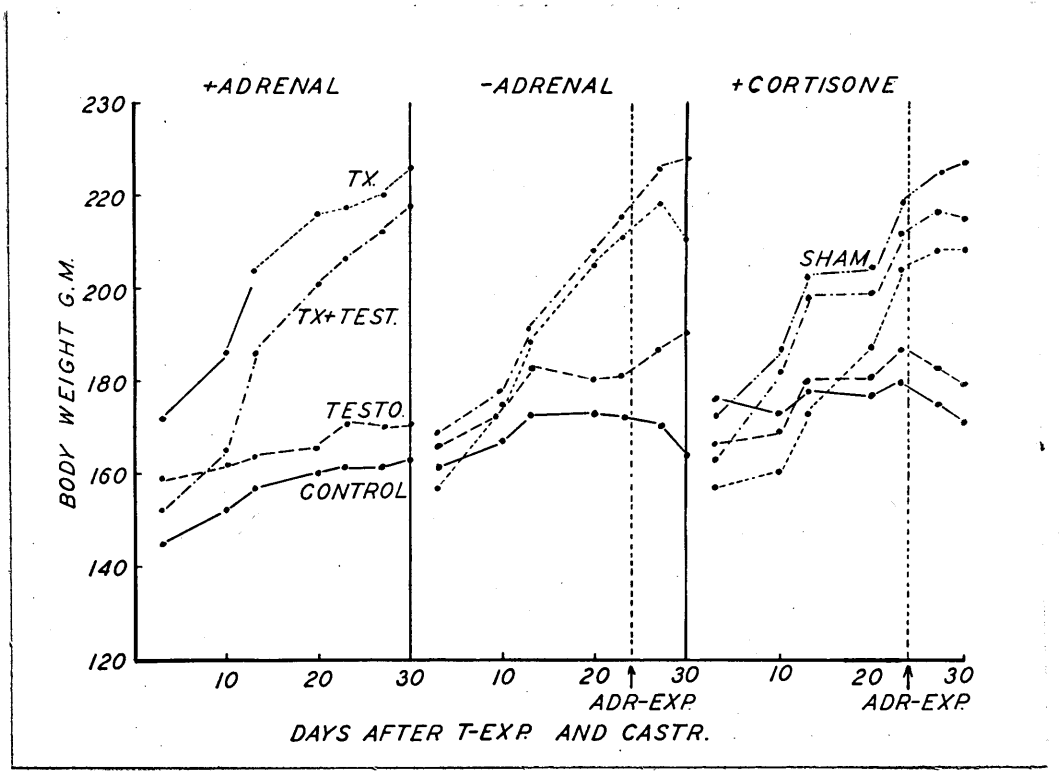
Fig.17 The effect of adrenalectomy and replacement doses of testosterone , thyroxine and cortisone on the body weight of castrated-thyroidectomized rats.

Note: (1). Adrenalectomy was not performed until the twenty-fifth day after thyroidectomy and castration in the minus adrenal and plus cortisone groups.

(2). Animals receiving thyroxine (Tx and Tx and Testo) showed a greater increase in body weight than animals not receiving thyroxine (Control and Testo).

(3). Animals receiving testosterone weighed consistently less than the controls.

(4). Adrenalectomy had no consistant effect on body weight.


+ADRENAL
-ADRENAL
+CORTISONE
230
220
200
180
160
140
120
BODY WEIGHT G.M.
TX.
TX+TEST.
TESTO.
CONTROL
SHAM
10
20
30
ADR-EXP.
DAYS AFTER T-EXP. AND CASTR.

tomy at the twenty fifth day with or without cortisone, caused a fall in body weight in all groups except for thyroxine in both cases and testosterone in the first case. Validity of this finding is questioned due to the high death rate during this period.

Oxygen Consumption

The basal metabolic rates were very variable (See Fig. 18) and the only clear-cut effect was the fact that all animals receiving thyroxine had a significantly higher oxygen consumption that those not receiving thyroxine. There was no significant effect of testosterone, but adrenalectomy with or without cortisone seemed to cause a slight fall in oxygen consumption.

Heart Rates

Heart rates (See Fig. 19) again showed great variability. The difference between thyroxine treated animals and those not receiving thyroxine were not as clear cut as those with the basal metabolic rates, again indicating that the latter was a better guide for the determination of a replacement dose of thyroxine. The diurnal variations were due to differences in room temperature.

Organ Weights

Examination of absolute and relative (i.e. per 100 gm body weight) organ weights (Table 23) gave no indication of any peripheral effect due to adrenalectomy or to cortisone when given in this dosage for this length of time.

However the effects of thyroxine and testosterone on

Fig.18 The effect of adrenalectomy and replacement doses of testosterone, thyroxine and cortisone on the oxygen consumption of castrated-thyroidectomized rats.

Note: (1). Adrenalectomy was not performed until the twenty fifth day after thyroidectomy and castration in the - adrenal and plus cortisone groups.

(2). All animals receiving thyroxine(Tx and Testo and Tx) constantly had a higher oxygen consumption than those not receiving thyroxine (control and Testo).

(3). Adrenalectomy, with or without cortisone produced a falll in oxygen consumption.

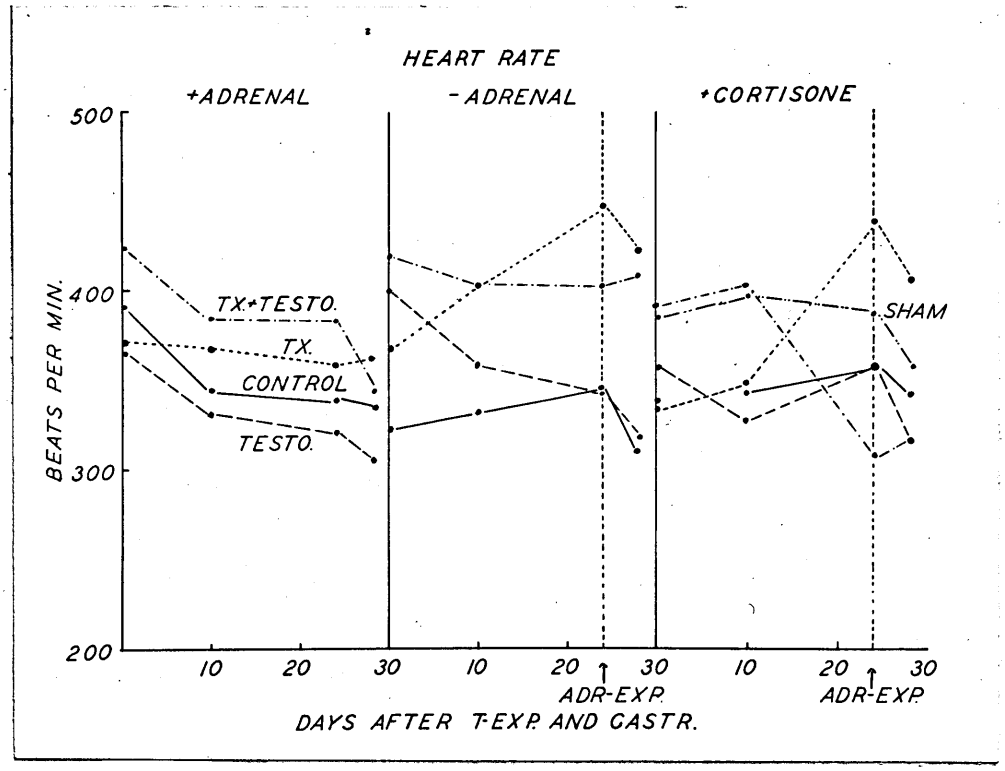

HEART RATE
+ADRENAL
-ADRENAL
+CORTISONE
500
400
300
200
BEATS PER MIN.
TX.+TESTO.
TX.
CONTROL
TESTO.
SHAM
10
20
30
10
20
30
10
20
30
ADR-EXP.
ADR-EXP.
DAYS AFTER T-EXP. AND CASTR.

Fig.18 The effect of adrenalectomy and replacement doses of testosterone, thyroxine and cortisone on the oxygen consumption of castrated-thyroidectomized rats.

Note: (1). Adrenalectomy was not performed until the twenty fifth day after thyroidectomy and castration in the - adrenal and plus cortisone groups.

(2). All animals receiving thyroxine(Tx and Testo and Tx) constantly had a higher oxygen consumption than those not receiving thyroxine (control and Testo).

(3). Adrenalectomy, with or without cortisone produced a falll in oxygen consumption.

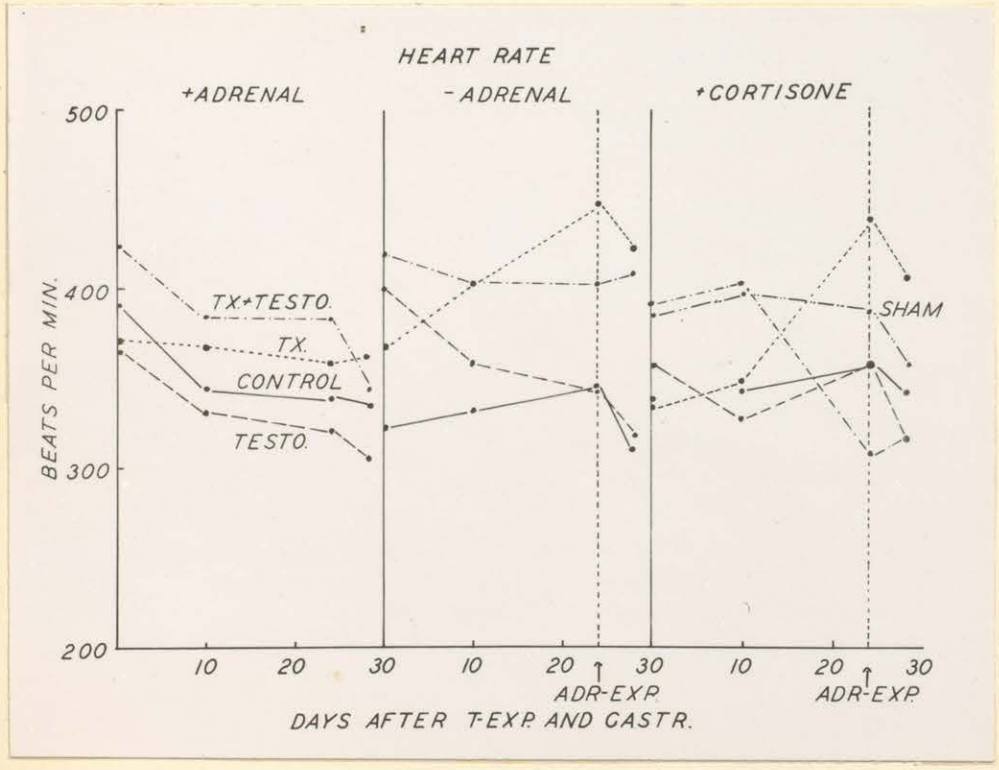

HEART RATE
+ADRENAL
-ADRENAL
+CORTISONE
500
400
300
200
BEATS PER MIN.
TX+TESTO.
TX.
CONTROL
TESTO.
SHAM
10
20
30
10
20
30
10
20
30
ADR-EXP.
ADR-EXP.
DAYS AFTER T-EXP. AND CASTR.

Fig.19 The effect of adrenalectomy and replacement doses of testosterone, thyroxine and cortisoneon the heart rate of castrated-thyroidectomized rats.

Note: (1). Adrenalectomy was not performed until the twenty-fifth day after thyroidectomy and castration in minus adrenal and plus cortisone groups.

(2). Animals receiving thyroxine (Tx and Tx and Testo) had a higher heart rate than animals not receiving thyroxine (Control and Testo).

(3). The fall in heart rate caused by adrenalectomy was accompanied by a parallel fall in animals which had not been adrenalectomized and was therefore probably not significant.

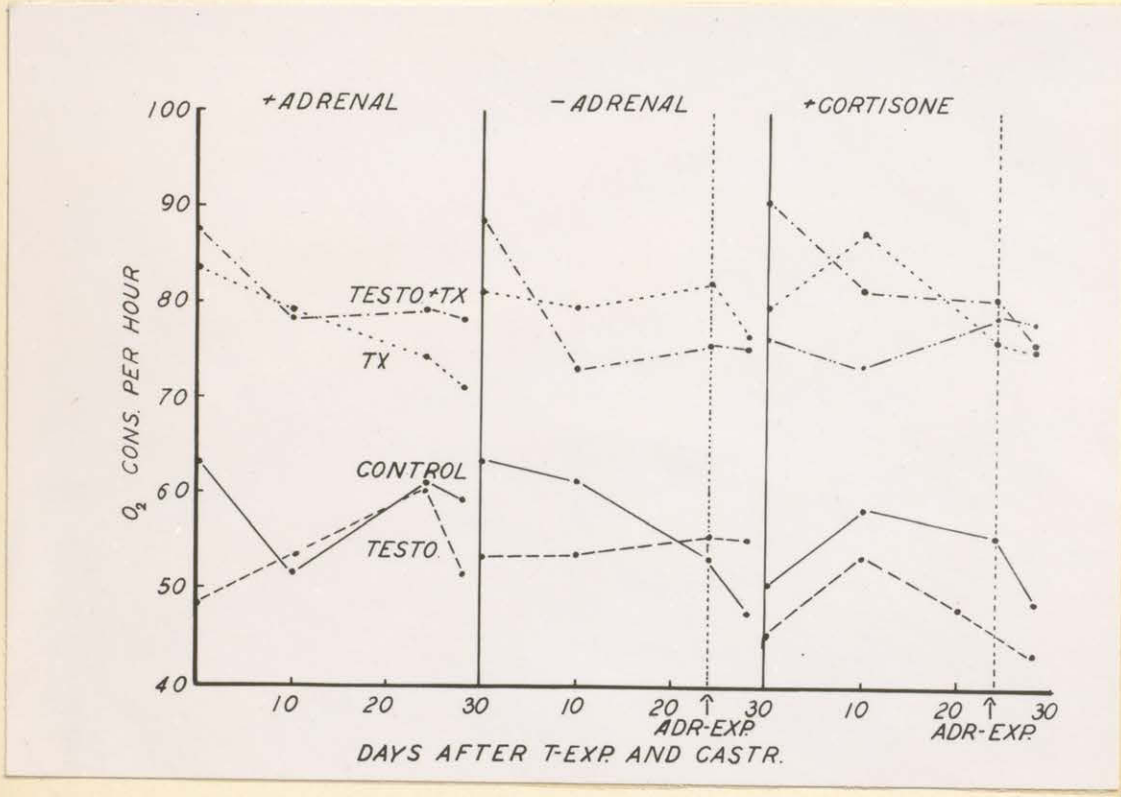

+ADRENAL
−ADRENAL
+CORTISONE
100
90
80
70
60
50
40
O_2 CONS. PER HOUR
TESTO+TX
TX
CONTROL
TESTO
10
20
30
10
20
30
10
20
30
ADR-EXP.
ADR-EXP.
DAYS AFTER T-EXP. AND CASTR.

TABLE OF ORGAN WEIGHTS -23

Organ		Plus Adrenal				Minus Adrenal				Plus Cortisone				SHAM
Wt. in mg		Control	Tx	Testo	Both	Control	Tx	Testo	Both	Control	Tx	Testo	Both	
Hypophysis	Abs.	8.3	7.7	8.0	6.2	9.0	8.1	8.0	6.1	9.6	8.1	7.7	6.1	5.5
	per 100	5.2	3.4	4.7	2.9	5.3	3.9	4.2	3.2	5.7	3.9	4.3	2.8	2.2
Adrehals	Abs.	31.5	42.4	25.4	40.0									
	Rel.	17.7	19.4	14.8	18.4									
Submaxillary	Abs.	307	381	268	429	196	454	358	441	300	424	358	487	513
	Rel.	192	182	158	195	106	209	185	193	173	214	183	225	211
Cervical	Abso.	106	124	93	110	73	122	102	111	78	128	72	119	114
Lymph Nodes	Rel.	62	55	55	50	41	63	56	50	52	63	37	55	48
Thymus	Abs.	263	333	293	222	272	439	266	389	314	428	226	287	303
	Rel.	159	257	164	92	150	212	136	169	180	210	113	131	296
Spleen	Abs.	744	1249	996	1649	1301	1543	932	1858	754	1751	844	647	836
	Rel.	452	555	579	750	717	785	507	830	425	853	437	763	355
Heart	Abs.	689	809	640	843	605	793	687	976	592	750	689	801	798
	Rel.	403	359	399	343	519	357	436	344	371	366	371	371	337
Kidneys	Abs.	1141	1749	1317	1856	1166	1711	1423	2134	1175	1869	1548	2307	1820
	Rel.	700	783	722	852	646	856	739	964	834	921	816	1073	757
Liver	Abs.	6236	9579	6354	9618	6065	8394	5917	9496	5985	8992	6040	9180	9309
	Rel.	3804	4290	3218	4415	3316	4096	3060	4291	3452	4438	3107	4265	3910
Seminal	Abs.	71	72	840	707	52	67	949	896	64	107	894	870	556
Vesicles	Rel.	42	32	495	322	30	31	504	410	37	61	475	405	234
Body Weight -Init.		145	172	159	152	170	156	168	166	174	151	165	147	173
	Final	163	226	170	218	180	210	193	228	171	208	189	215	227
Total No. of Animals		8	7	7	6	2	4	6	5	7	6	7	2	8
No. of Animals with Adrenal Remnants							2	3	1		3	4	1	

organ weights previously observed by Grad(1949) and in the experiments just described were confirmed in this experiment. Thus, in addition to causing an increase in the total body weight, thyroxine also increased the absolute and relative weights of the adrenals,(See Table 23) submaxillary glands, cervical lymph nodes, thymus, spleen, heart, kidney and liver, while it caused a decrease in the weight of the hypophysis.

Testosterone only caused a significant increase in the weight of the seminal vesicles, and a slight increase in the submaxillary gland, but it caused a marked decrease in the weight of the hypophysis.

A slight synergistic effect was observed in the absolute weights of the heart and kidney.

Adrenalectomy had no striking effects whatsoever, but it showed a tendency to cause a slight decrease in the weight of the submaxillary gland and cervical lymph nodes, both remedied by cortisone, and a slight increase in the weight of the spleen.

The previously observed effects of thyroxine and/or testosterone were thus apparently not modified by adrenalectomy or by supplimentation in these adrenalectomized animals by daily injections of small 200 µg doses of cortisone. Thus, from this data it could be said that the changes in adrenal weights observed in thyroxine and testosterone treated animals (which had been previously castrated and thyroidectomized) were just one of the many peripheral effects of these two hormones and not indicative of an indirect action on such peripheral organs mediated via the adrenal.

SUMMARY

1. The antagonistic action of thyroxine and testosterone persisted in spite of adrenalectomy. These actions therefore seemed to be direct.

2. The actions of thyroxine and testosterone were not affected by the administration of cortisone, which did not seem to have any effects.

3. The validity of these results was however impaired because of the short duration of the adrenalectomy (10 days) as compared to the castration and thyroidectomy, and also by the great incidence of adrenal regeneration.

4. Incidentally the slight growth-stimulating action of testosterone observed in the previous experiment was confirmed.

INFLUENCE OF HYPOPHYSECTOMY ON THE ACTIONS OF THYROXINE, TESTOSTERONE AND CORTISONE (with particular emphasis on the epidermis and on growth)

Results of the last experiment were somewhat ambiguous due to the short time, i.e. three weeks, sufficient adrenalectomized animals survived while the last and the previous experiments had been allowed to run at least five weeks. However, the high death rate of adrenalectomized animals and the speed of regeneration of adrenal tissue made a long-term experiment along these lines impractical. It was therefore decided to use hypophysectomized animals, which would be deprived of a considerable proportion of their endogenous thyroxine, testosterone and cortisone, due to the absence of the "trophic hormones". To these animals could be given replacement doses of the three hormones.

EXPERIMENTAL SET-UP

The twenty male albino hypophysectomized rats, weighing approximately 130 grams, were obtained from a commercial firm. These animals were divided into the following groups:

Group 1. Six hypophysectomized rats receiving 2 daily s.c. injections of 2 to 4 micrograms of thyroxine in 0.1 cc. of 1% Dupanol, the dose being adjusted throughout the experimental period so as to maintain an approximately normal metabolic level.

Group 2. Seven hypophysectomized rats receiving 2 daily s.c. injections of 100 micrograms of testosterone in 0.1 cc of 1% Duponol.

Group 3. Seven hypophysectomized rats receiving 2 daily s.c. injections of 100 micrograms of cortisone in 0.1 cc of 1% Duponol.

The animals were kept at a constant temperature of 28°C, for hypophysectomized rats are known to be very sensitive to the hypoglycemin induced by cold. They were fed Remington's iodine deficient diet. No saline was given in place of drinking water, - for supposedly the mineral-corticoid function of the adrenal is relatively independent of the hypophysis.

All the animals survived and were in excellent condition during the first two weeks. Heart rates were taken daily, and oxygen consumption measurements twice weekly. During the third week the animals receiving cortisone began to look sick and apathetic and started to die off. Thus the rats were sacrificed after twenty-one days, and the organs weighed and taken for histology. Pieces of dorsal and abdominal skin were taken for special attention.

RESULTS

Epidermis

Epidermal Thickness; Examination of the epidermis showed that thyroxine caused a decrease in all layers (See Fig. 20,21) and while testosterone caused an increase in thickness (See Fig.24,25). The cortisone picture was intermediate (See Figs.22,23), indicating that cortisone at this dose did not exert any influence on the thickness of the epidermis. (There were, however, no uninjected controls to use for comparison.) The dorsal and abdominal epidermis gave similar results, although in all cases the dorsal epidermis (See Fig.25 [21,23]) was thicker than the abdominal. (See Figs. 20,22, 24) Subjective estimations of the thicknesses of Malpighian and cornified

PLATE II

Epidermis Orth fixative Haematoxylin-eosin stain

Fig. 20 Hypophysectomized male rat treated with thyroxine. Abdominal. Note thinness of epidermis, especially the cornified layers.

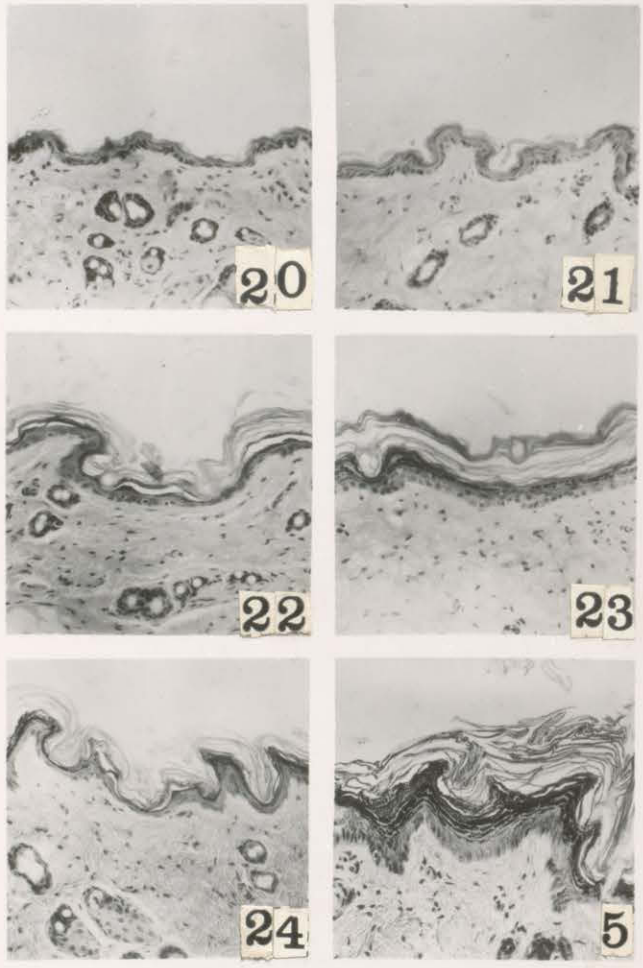
Fig. 21 Hypophysectomized male rat treated with thyroxine. Dorsal. Note similar thinness of epidermis, although thicker than in abdominal.

Fig. 22 Hypophysectomized male rat treated with cortisone. Abdominal. Note intermediate thickness of both layers of epidermis.

Fig. 23 Hypophsectomized male rat treated with cortisone. Dorsal. Note similar intermediate thickness of both layers of epidermis, although slightly thicker than abdominal.

Fig. 24 Hypophysectomized male rat treated with testosterone. Abdominal. Note increase in thickness of both layers of epidermis.

Fig. 25 Hypophysectomized male rat treated with testosterone. Dorsal. Note striking increase in thickness of both layers of epidermis. Especially note great increase in the number of keratohyalin granules.


20
21
22
23
24
5

layers are shown graphically in Fig. 26 . Here again it could be seen that in both abdominal and dorsal skin, testosterone markedly increased the thickness and thyroxine decreased the thickness of both layers, while cortisone gave an intermediate picture. It could also again be noted that the effect was more striking in the cornified than in the Malpighian layers. A conclusive proof was thus presented that the action of thyroxine and testosterone on the epidermis was direct and not mediated through the hypophysis.

Since thyroxine did not cause an increase, but even a decrease in the weight of the adrenal (See Table 20&21), it was also concluded that thyroxine's usual stimulating effect on adrenal weight was mediated through the hypophysis. The role of the adrenal as mediator in producing the changes in the epidermal thickness was therefore also ruled out.

Body weight

From Fig. 27 it can be seen that none of the animals showed any gain in weight. In fact some even lost. This finding is startling in the light of thyroxine's known effect on body weight in thyroidectomized animals. It can therefore be said that the growth stimulating action of thyroxine is mediated through the hypophysis. Since failure to grow as detected by changes in body weight is known to be a very sensitive index of thyroxine deficiency, the indirect action of thyroxine through the hypophysis on growth is all the more remarkable. The failure of thyroid deficient rats to grow can

Fig. 26 The effect of thyroxine, cortisone and testosterone on the epidermis of hypophysectomized rats - thickness of abdominal and dorsal epidermis (subjective results).

Note: (1). Similar results were obtained in dorsal and abdominal epidermis.

(2). Testosterone caused an increase in the thickness of both layers of the epidermis, especially the cornified layers.

(3). Thyroxine caused a decrease in the thickness of both layers of the epidermis, especially the cornified layers.

(4). Cortisone produced an intermediate thickness of the epidermis.

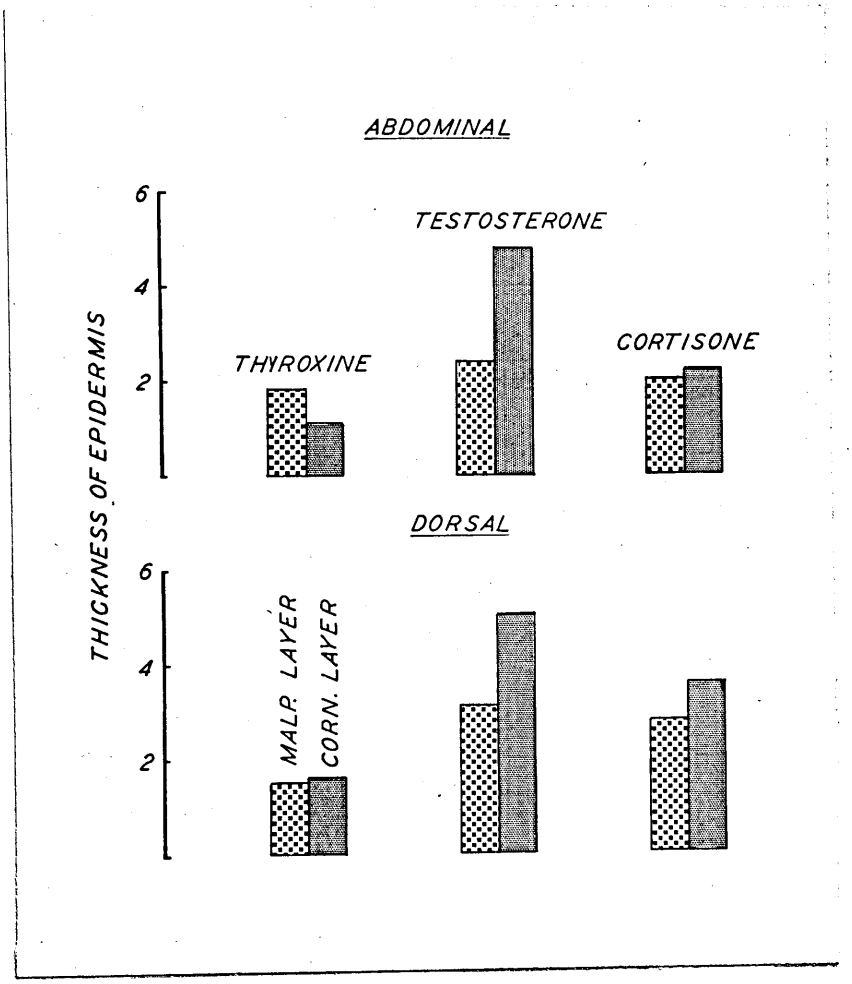
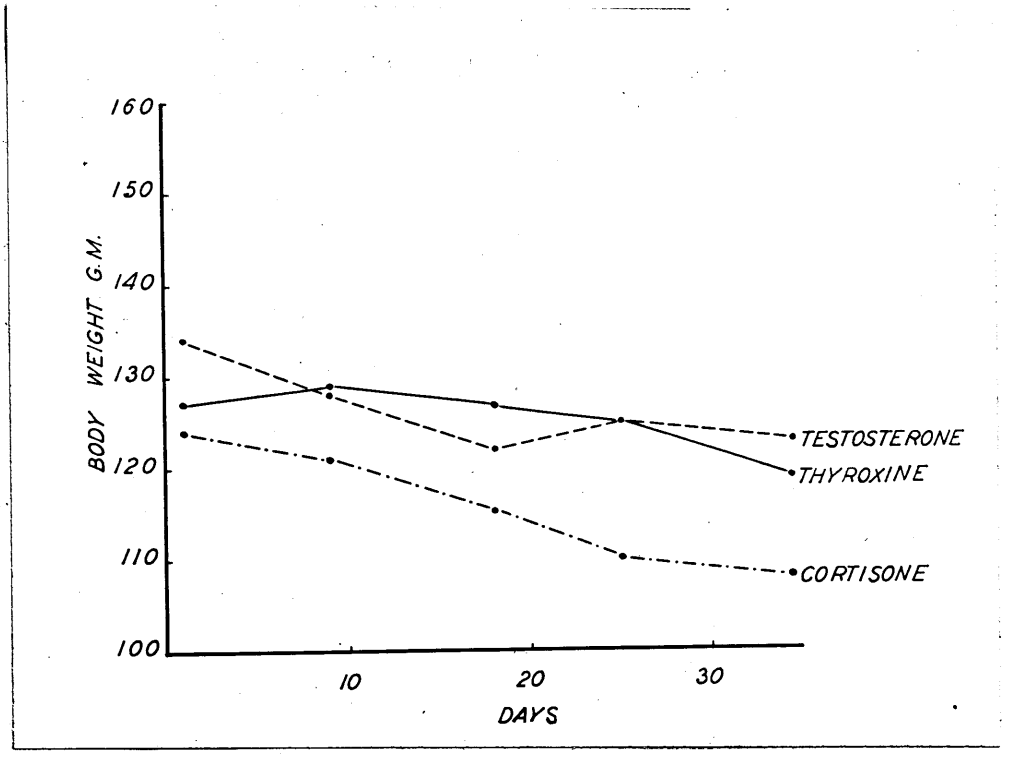

ABDOMINAL
6
4
2
THICKNESS OF EPIDERMIS
THYROXINE
TESTOSTERONE
CORTISONE
DORSAL
6
4
2
MALP LAYER
CORN. LAYER

Fig. 27 The effect of thyroxine, cortisone and testosterone on the body weight of hypophysectomized rats.

Note: Cortisone caused a loss of body weight.


160
150
140
130
120
110
100
BODY WEIGHT G.M.
10
20
30
DAYS
TESTOSTERONE
THYROXINE
CORTISONE

perhaps be explained by the lack of acidophiles in such animal's hypophysis for acidophiles are also known to be the parent cells of growth hormone, which here is presumed to be responsible for thyroxine's growth promoting effect in thyroidectomized rats. This latter phenomenon is presumably brought about by a reduction in the number of basophiles and a reappearance of the acidophiles.

For some unexplained reason, the animals receiving cortisone lost more weight than those receiving their thyroxine or testosterone, perhaps because the dose of cortisone used was slightly toxic.

Oxygen Consumption

As could be seen from Fig.[28], the metabolic action of thyroxine was still present in hypophysectomized animals. This effect therefore seems to be direct and not mediated through the hypophysis. Although cortisone seemed to produce a slightly higher oxygen consumption than testosterone, the difference did not appear to be significant. (Within the last year, a metabolism stimulating action was recently attributed to cortisone by Hill (1950), Beierwaltes, (1950), Wolfson, (1950)).

Heart rate

The cardio-accelerating action of thyroxine remained intact after hypophysectomy (See Fig.29), thus establishing this effect as a direct action of thyroxine, produced independently

Fig.28 The effect of thyroxine, cortisone and testosterone on the heart rate of hypophysectomized rats.

Note: The heart rate in the cortisone-treated group was intermediate between the thyroxine-treated and the testosterone-treated groups.

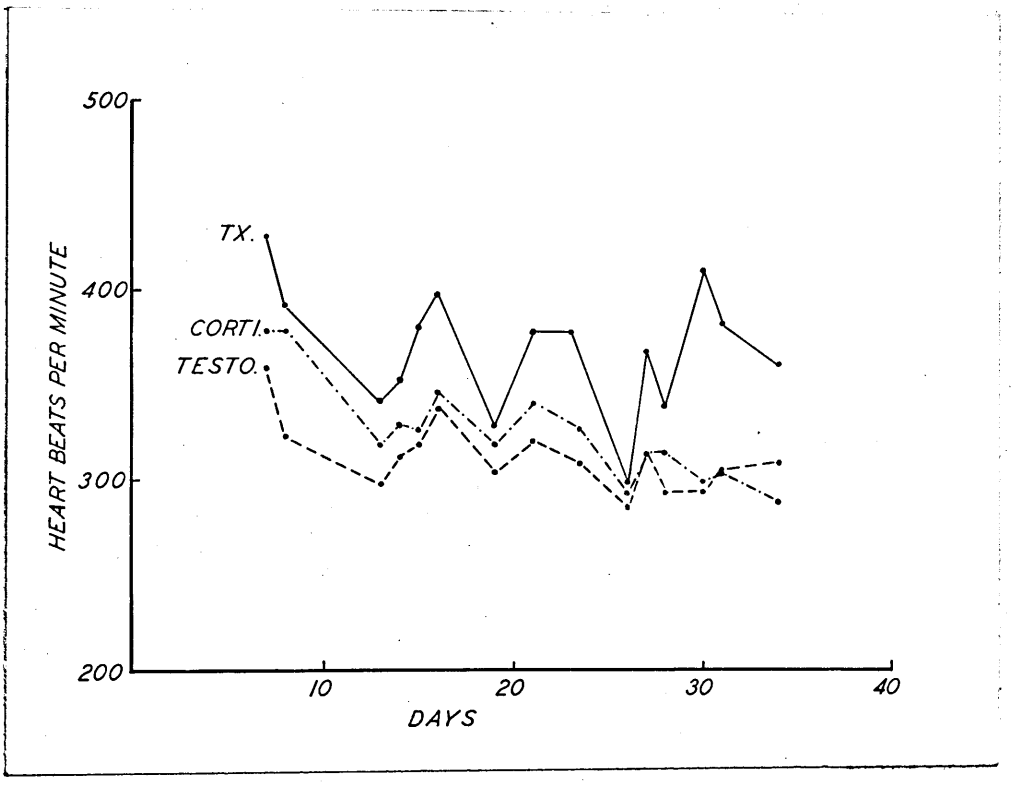
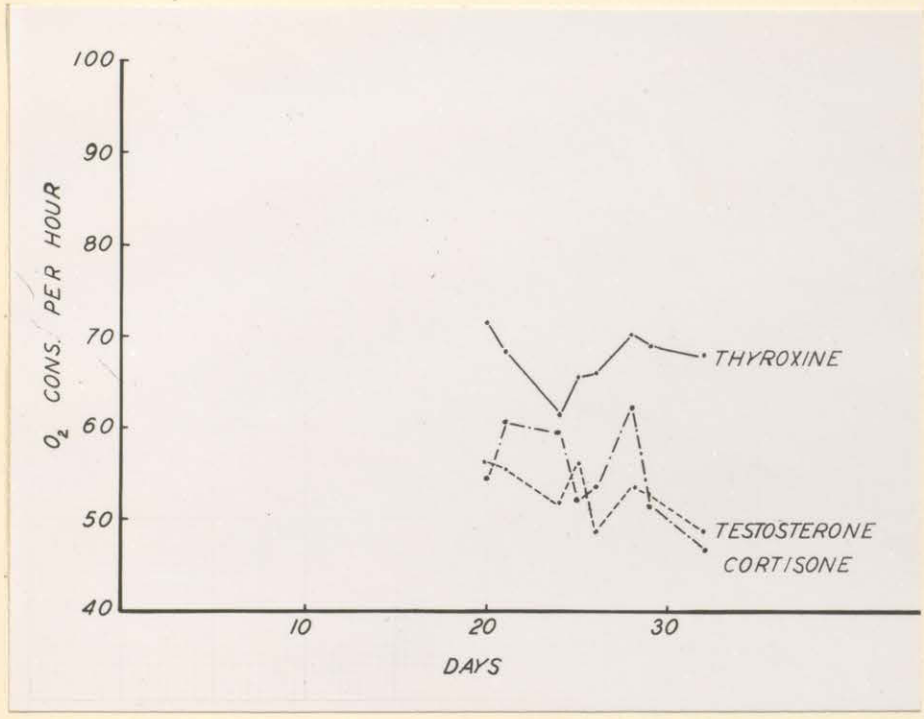

500
400
300
200
HEART BEATS PER MINUTE
TX.
CORTI.
TESTO.
10
20
30
40
DAYS

Fig. 29 The effect of thyroxine, cortisone and testosterone on the oxygen consumption of hypophysectomized rats.

Note: The heart rate in the thyroxine-treated group was much higher than in the other two groups.


100
90
80
70
60
50
40
O_2 CONS. PER HOUR
10
20
30
DAYS
THYROXINE
TESTOSTERONE
CORTISONE

of the hypophysis. A slight but constant stimulating effect on the heart rate could be attributed to cortisone in comparison to testosterone. Since daily variations seemed to be parallel in the three groups, probably they all could be attributed to changes in room temperature, Hypophysectomized animals being very sensitive to such changes.

Organ weights

Examination of the absolute organ weight (See Table 24) showed that in general there was no significant difference between the groups. The stimulating action of thyroxine and testosterone on such organs as the liver, heart and submaxillary gland were therefore shown to be mediated through the hypophysis, apparently through the growth-promoting principle. (The somewhat smaller weights of these organs in the cortisone-treated group corresponded to the smaller body weight of these animals). Thyroxine however did seem to stimulate lymphatic organs, such as thymus, spleen and cervical lymph nodes. A slight stimulating effect of testosterone on the testes was observed while its action on the seminal vesicles was very striking.

Summary

A group of animals were hypophysectomized and treated with replacement doses of thyroxine, testosterone and cortisone, in order to determine which of the effects of these hormones were mediated either through the hypophysis-adrenal

TABLE 24

ABSOLUTE WEIGHTS OF ORGANS OF HYPOPHYSECTOMIZED RATS TREATED WITH THYROXINE, TESTOSTERONE OR CORTISONE

Treatment	Thyroxine	Testosterone	Cortisone
No. of Animals	6	6	4
	Organ weights (mgm)		
Organ	mg	mg	mg
Submaxillary glands (2)	202	216	179
Lymph nodes (2 cervical)	82	77	46
Spleen	651	501	520
Thymus	247	179	201
Heart	488	468	444
Kidneys (2)	1030	844	843
Liver	4983	4921	4179
Seminal vesicles (2)	37	500	48
Testes (2)	156	217	175
Body weight (gm)	119	123	108

system or the hypophysis alone.

1. The antagonistic effects of thyroxine and testosterone on the epidermis were direct, and not mediated either through the hypophysis nor the adrenal, since they also appeared in hypophysectomized animals.
2. It was found that the general somatotrophic action of thyroxine was mediated through stimulation of the acidophils of the hypophysis which secrete the growth hormone. Hypophysectomized animals failed to grow in spite of thyroxine therapy.
3. The effects of thyroxine and testosterone on various organ weights were also mediated through the hypophysis, as no significant differences were observed between groups except for the kidney and immediate targets, e.g. seminal vesicles for testosterone.

EVALUATION OF METHODS AND RESULTS

An attempt was made to study the influence of other hormones, in particular testosterone and cortisone on the action of thyroxine. An intensive study was made of the action of these hormones on the epidermis and its appendages. A general investigation of the physiological action of these hormones was also undertaken.

METHODS

Operations

In general the technique of removing the organs responsible for the secretion of the hormones to be studied in order to establish a base line against which the action of replacement doses of these hormones could be compared, proved very satisfactory. The animals supported simultaneous thyroidectomy and castration well, so that out of seventy-five animals subjected to these operations, fifty-five survived for at least five weeks at which time they were sacrificed. These animals received 1% calcium lactate for one day before and approximately a week after the operation. They were fed the iodine deficient diet. The animals responded as expected to the injection of thyroxine and/or testosterone. The use of sham-operated animals as controls served as a final indication as to whether the doses of hormones injected were adequate to simulate a normal condition.

When such castrated-thyroidectomized animals were adrenalectomized four weeks later, 35 out of 87 survived for ten days at which time they were sacrificed. However, out of these, 14 showed some degree of adrenal regeneration. The delay between the castration and thyroidectomy and the adrenalectomy caused some ambiguity in the results and this method was therefore not deemed satisfactory. Since the administration of three hormones in various combination required thirteen separate groups, and if in addition, provision

had to be made for a high mortality following the triple operation, a great number of animals would be required, thus making the experiment a practical impossibility.

To overcome these difficulties, an attempt was made to use hypophysectomized animals, which would be functionally if not organically deprived of the hormones in question (thyroxine, testosterone and cortisone), and which could thus be used as a base-line to test the interaction of these hormones. This method proved very satisfactory since only two out of twenty animals died in twenty-one days and all the animals were in very good condition throughout the experiment. The functional absence of a significant amount of either thyroxine or testosterone was substantiated by the rise in metabolic rate due to a small dose of thyroxine and by the lack of development of the seminal vesicles in the absence of testosterone treatment. There was no objective standard by which the absence of cortisone could be judged.

Measurements of epidermal thickness

In the study of the epidermis, the subjective technique of estimating the relative thickness of this organ in various groups of animals proved very valuable as a first approximation and also as confirmation of results obtained in other experiments. The results obtained by this method were in remarkably close agreement with those obtained by the much more precise projection technique. This latter technique,

however, made it possible to express the results in absolute terms, i.e. thickness in microns which could then be subjected to a strict statistical analysis. This projection technique was deemed to be reasonably accurate since the main source of error was in the fact that the paper on which the projections were traced may not have been of uniform thickness, but such a variation would be very slight, since the same kind of paper was used throughout the experiment. This technique could be used to determine the absolute size of other histological structures.

Mitotic counts

The accuracy of the method of estimating the mitotic activity of the epidermis by counting the number of mitoses present at any one time along a certain length of basement membrane, suffered from the fact that the number of mitoses per section was very small. If done carefully, however, this somewhat crude method was sufficient to give a good indication of the general trend. The extrapolation of these results to yield the number of mitoses per body surface was thus strictly an approximation for two reasons, namely: (1) because of the small number of original counts from which the extrapolation was made and (2) because it was assumed that the mitotic activity was the same all over the body of the animal. Nevertheless, this method gave an indication not only that the decreased number of mitoses in thyroxine treated

animals was not due to stretching of the epidermis, but is also showed that the number of cells in mitoses at one time in the whole body surface was of the order of 1×10^8.

RESULTS

Epidermal Thickness

The results obtained from measurements and estimations of the epidermal thickness were very clear-cut in regard to the antagonistic action of thyroxine and testosterone. The validity of the results from the experiment designed to test the action of adrenalectomy with or without replacement doses of cortisone, was impaired by the fact that the animals had been thyroidectomized and castrated four weeks previously. Some of these animals had been treated with thyroxine and/or testosterone during this period of time and since the turn-over time of the skin was of the order of several weeks (the exact time for the dorsal skin was not known, but Storey in 1949 had shown that the turn-over time of the foot-skin of the rat was approximately four weeks,) a ten-day period following adrenalectomy was not a sufficient time to permit this new factor to alter an already established trend.

Hair growth

The stimulating effect of thyroxine on the hair was shown both by observing the amount of hair growth after

shaving the animals and by estimating the number of hair follicles in section of the skin. This was in agreement with the well-known shedding of hair characteristic of thyroid deficienty. As already discussed this action of thyroxine constitutes a paradox since growth of the hair is due to mitotic activity in the layers of the hair follicles which are derived from the Malpighian layers of the skin. This paradox will be further discussed in the General Discussion.

Sebaceous glands

The stimulating action of testosterone on the sebaceous glands and the synergistic action of thyroxine when given in combination with thyroxine was very clear-cut in all experiments and confirmed a similar observation by Grad (1949).

Body weight

In general, body weight served as a good guide to the effectiveness of a given dose of thyroxine to produce a normal condition. This close correlation was even more striking when it was found that this action of thyroxine was not direct but was mediated through the hypophysis since the response of the body weight to thyroxine was absent in hypophysectomized animals. In addition to this clear-cut phenomenon, testosterone showed a tendency to cause a slight stimulation of the body weight when given alone. When given with thyroxine, it did not exert such an effect, in fact,

occasionally testosterone was found to depress the growth-stimulating action of thyroxine.

Oxygen consumption

The oxygen consumption showed its usual response to thyroxine. This action proved to be independent of the presence of the adrenal. In addition, testosterone was generally found to slightly depress the oxygen consumption while cortisone had a slightly stimulating effect. The significance of the actions of these last two was not established.

Heart rate

In general, the heart rates were not as sensitive an index of the action of certain doses of thyroxine as either the oxygen consumption of the body weights. Nevertheless, thyroxine consistently increased the heart rate and this action again was found to be independent of the presence of the hypophysis. Testosterone had no consistent effect and cortisone seemed to cause a slight rise. Again the significance of this finding was doubted.

Organ weights

Grad's observations that thyroxine and testosterone exerted a synergistic action on the weights of the heart,

kidneys and submaxillary glands (their weight being increased) and on the hypophysis (its weight being decreased) were generally confirmed. This action was, however, absent after hypophysectomy indicating that it was exerted through the hypophysis. Similarly the antagonistic action of these two hormones on the adrenal (thyroxine increased and testosterone decreased its weight) was also mediated through the hypophysis. The only organs significantly affected after hypophysectomy seemed to be the lymphatic organs which were increased in weight by thyroxine, and the seminal vesicles which were the direct target organs for the action of testosterone.

SUMMARY AND CONCLUSIONS

1. Thyroxine decreased and testosterone increased the thickness of all layers of the epidermis, the two hormones together producing an intermediate condition, approximating that found in normal animals.
2. This action was direct, i.e., it was independent of the presence of either the hypophysis or the adrenal.
3. This difference in thickness was mainly produced by a change in the mitotic activity of the basal layers of the epidermis induced by thyroxine and testosterone. Thus thyroxine reduced the mitotic activity and consequently the number of cells, while testosterone had the opposite effect.

4. The direct relationship between mitotic activity and epidermal thickness was slightly modified by the fact that thyroxine decreased the rate of cornification (thus allowing the cell to stay longer in the Malpighian layers) while testosterone decreased the rate of desquamations (thus causing the cell to stay longer in the cornified layers.)
5. Thyroxine stimulated the growth of hair.
6. Testosterone increased the size of the sebaceous glands, and thyroxine, while having no effect of its own, acted in a synergistic manner on this structure, when given in combination with testosterone.
7. The growth-promoting action of thyroxine was mediated through the hypophysis, thyroxine having no effect on the body weight in the absence of the adrenal
8. The metabolism-stimulating and cardio-accelerating actions of thyroxine were direct and could be observed whether or not the hypophysis was present.
9. Most of the actions of thyroxine and testosterone on the organ weights, except for direct target organs such as the seminal vesicles for testosterone, seemed to be mediated through the hypophysis, there being no significant difference between groups in the absence of this gland, (except for the effect of thyroxine on kidneys).
10. Cortisone in hypophysectomized animals seemed to have a slight stimulating action on the heart rates and oxygen consumption, a slight depressing effect on the body weight

but no effect on the thickness of the epidermis or on organ weights.

GENERAL DISCUSSION

PART I

FACTORS MODIFYING THE MANIFESTATIONS OF THYROID DEFICIENCY

An attempt was made to evaluate the factors which could influence the manifestations of thyroid deficiency in the albino rat. Due to the wide scope of the field, the induction of thyroid deficiency was limited to thyroidectomy and the only modifying factors studied were diet and moderate reduction in temperature. Incidental observations were also made on the modifying effects of time and age. In this section the broader implications of the results obtained will be discussed.

A careful consideration of possible agents which could influence the symptoms of thyroid deficiency revealed a considerable number. These factors will be taken up in more or less detail, in the following order:

Diet,
Temperature,
Time,
Age,
Growth,
Methods of production,
Infection,
Other endocrines.

INFLUENCE OF DIET ON SYMPTOMS OF THYROIDECTOMY

DIET AS A SOURCE OF THYROXINE

Upon consideration of what factors in the diet could influence the symptoms of thyroid deficiency in a thyroidectomized animal, the presence of thyroxine itself merited first consideration. Such thyroxine could exist in a free state or possibly be linked to other molecules such as proteins. Since the sensitivity of thyroidectomized animals to minute amounts of thyroxine was shown by Andik in 1949 to be increased as much as 500 times as compared to the sensitivity of intact animals to a similar amount of thyroxine (the response studied was the oxygen consumption), it seemed likely that the presence of absence of small amounts of thyroxine could significantly influence the degree of thyroid deficiency. Support for such a theory was advanced by the finding of considerable amounts of thyroxine in muscle (meat) and an even greater concentration in liver and intestine (Gross and Leblond, 1950). The latter tissue was known to be protein constituents of most commercial feeds.

Thus, when it was observed that thyroidectomized animals, on an apparently similar commercial feed, grew less well during the war than after, a change in the quantity or quality of the protein incorporated into such a diet was suspected. A diet, therefore, was prepared in which almost all animal proteins were eliminated (Remington's modified iodine deficient diet),

and which, when analyzed was found to contain relatively little iodine, Such a low iodine content was also taken as indication of a low thyroxine content. It did not seem surprising therefore that thyroidectomized animals fed such a diet grew less well and showed more signs of thyroid deficiency than animals fed Purina Fox Chow, which showed virtually no symptoms.

When even the 1.9 % pig liver of this iodine-deficient diet was replaced by the pulverized dried carcass of rats thyroidectomized three weeks previously, thyroidectomized animals fed such a diet ceased gaining weight and rapidly developed a typical cretinoid syndrome, etc. Kommerall in 1931 described a similar phenomenon in thyroidectomized dogs, for when he fed such animals ordinary meat they did not appear thyroid deficient, but when he fed them meat from thyroidectomized dogs they also developed the typical cretinoid symptoms.

It would thus appear that the amount of thyroxine in the diet was an important factor which could markedly modify the severity of the symptoms of thyroid deficiency following surgical thyroidectomy.

DIET AS A SOURCE OF IODINE

The question next considered was whether the presence of free iodine in the diet could have any effects in thyroidectomized animals. It was realized that an abundant supply of iodine would facilitate the formation of thyroxine from thyroid

remnants. However, a careful examination of the thyroid region by serial sections revealed that no thyroid remnants remained if care had been taken during thyroidectomy to dip the forceps used on thyroid tissue into 70% alcohol, which killed any adhering thyroid tissue and prevented such tissue from being seeded back into the operative site by subsequent use of the forceps.

Extra-thyroidal secretion of thyroxine also had to be considered for an abundant supply of iodide would then enhance such a phenomenon. Such a possibility was suggested by Chaikoff and Taurog in 1947 who observed that small amounts of protein-bound iodine (with thyroxine-like activity) were still being synthesized from radio-active iodide twenty-five hours after thyroidectomy. Three days later such protein-bound iodine was still to be found in the plasma. Whether such findings indicated that there was extra-thyroidal production of thyroxine, or whether this was purely due to the fact that radio-iodine solutions often contained radio-I^2 (which would then react with tissue proteins to provide thyroxine) remained questionable.

Furthermore, at the acidic pH of the stomach, ingested iodide could be transformed into iodine which in turn could react with proteins to produce thyroxine.

Thus the possibility that the iodine deficient diet was effective in somewhat increasing the symptoms of thyroid deficiency because of its low iodide as well as its low thyroxine content, could, however, not be dismissed. Chapman in 1944 also observed that when thyroidectomized rats were fed an iodine-deficient diet,

they had fewer acidophils in the hypophysis (indicating a greater thyroid deficiency) than when they were fed a commercial diet.

PROTECTIVE FACTORS IN THE DIET

A change in the relative proportions of proteins, carbohydrates and fats could alter the efficiency of thyroxine as a respiratory activator by changing the respiratory quotient. Thus Schlotthauer (1929) observed that he could change the symptoms of a thyroidectomized pig by simply increasing the amounts of carbohydrates in the diet. He found that when these pigs were fed a balanced diet, they showed cutaneous myxoddema and gained weight as controls, while, if they were fed a high carbohydrate diet, they did not develop the oedema of myxoedema but were underweight. Since both myxoedematous infiltrations and a gain in weight indicate laying down of protein, these findings could be explained by assuming that protein was being stored on a balanced diet, while on a high carbohydrate diet (which would presumably contain a relatively smaller proportion of protein), such protein was being execreted or metabolized at a faster rate than it was being taken in .

Since liver was an important constituent of commercial feeds as well as of Remington's modified iodine deficient diet used in these investigations, the possibility of a protective factor in the liver also had to be considered. Ershoff in 1947 and Betheil in 1949 had shown that there was a factor in

the liver (an anti-pernicious anaemia factor) which decreased the toxic effects of high doses of thyroxine in mice. It was conceivable that such a factor could slow down the utilization of thyroxine and thus conserve any available supplies; an opposite action, namely, a destruction of thyroxine was however deemed more likely.

FACTORS INHIBITORY TO THYROXINE

Symptoms of thyroid deficiency could be increased by the presence in the diet of factors which could counteract or inhibit thyroxine. Such factors have been suggested by Ershoff (1949) who found a liver factor which prevented the toxic growth inhibiting action of thyroxine, and by Emerson who in the same year showed that vitamin B_{12} from yeast had the same effect. Certain goitrogens may also have similar actions.

FACTORS WHICH PRODUCE SYMPTOMS SIMULATING THYROXINE DEFICIENCY

It is well known that certain vitamin deficiencies produce scaliness of the tail - pantothenic acid is one example. Protein deficiency may inhibit growth and therefore care had to be taken not to confuse such nutritional deficiencies in the thyroxine deficiency.

INFLUENCE OF LOW TEMPERATURE ON THE SYMPTOMS OF THYROIDECTOMY

Exposure of thyroidectomized animals to a moderately cool temperature (16 °C as compared to the normal temperature for the rat of 27 °C) increased the symptoms of thyroidectomy, regardless of the diet. Thus animals on the thyroxine-free thyroidectomized rat meat diet were placed in the cold and their cretinoid condition was even further enhanced. If these animals were kept at this temperature for three or four weeks their thyroxine stores were presumably completely exhausted and they developed an acute syndrome, characterized by a fall in body temperature accompanied by a progressive muscular paralysis which lead to death within one or two days. Cold and diet were thus found to have a synergistic effect. In this section, an attempt will be made to explain why cold increased the symptoms of thyroid deficiency and then how this phenomenon was brought about.

WHY DID COLD INCREASE THE SYMPTOMS OF THYROID DEFICIENCY?

Increased Need for Thyroxine

That there was an increased need for thyroxine in the cold was shown by Ring, 1936, 1938 and others, because a higher metabolic rate was necessary in order to maintain a normal body temperature. This was especially important in small animals such as the rat and the mouse, whose large surface volume ratio caused a rapid dissipation of heat. The immediate reaction to cold was shivering and this mechanism

was highly effective in increasing the heat production. However, this could not be kept up long; the adrenal therefore took over with an increased secretion of adrenalin output which caused a rise in the heart and respiration rate and an increased oxygen supply to the tissues. In the long run, however, it was necessary for thyroxine to increase the actual tissue respiratory rate, thus raising the basal metabolic rate.

This could also be explained in another way. Increased caloric requirements to maintain body temperature could lead to a more rapid depletion of thyroxine stores. The ensuing failure in caloric production could then lead to a falling body temperature, which would subsequently cause paralysis and eventual death.

The increased need for thyroxine was well demonstrated in the present investigations since thyroidectomized animals which were fed a diet presumably containing enough thyroxine to prevent any deficiency symptoms from appearing, developed such symptoms when exposed to the cold. If such animals were fed a diet already very deficient in thyroxine, so that they had developed deficiency symptoms even in the warm room, their symptoms were markedly enhanced upon exposure to cold. Eventually the homeothermic mechanisms of the animals failed completely, their body temperature dropped and a paralytic death rapidly ensued. This paralytic syndrome could not be brought on by diet alone and therefore it was felt that cold was necessary as a precipitating factor.

Death might also occur if thyroidectomized animals were

fed a diet adequate in thyroxine, when the temperature was further decreased. Thus Leblond and Gross showed in 1942 that thyroidectomized animals subjected to temperatures near the freezing point succumbed within a few hours, while normal animals could survive such temperatures for a week, indicating that at this temperature even the relatively large amounts of thyroxine in the diet were not sufficient.

Increased utilization of thyroxine

Parallel to this increased need for thyroxine, there was also an increased utilization of thyroxine. This was shown in intact animals by the fact that the thyroid was stimulated in the cold (Riddle, 1932) which indicated that there was a decrease in the amount of circulating thyroxine, which in turn stimulated the secretion of thyrotrophic hormone and consequently the thyroid. It was also shown that the tolerance to large doses of thyroxine without toxic reaction was considerably increased below 20 °C and was especially striking at 4° - 6°C. (Bodansky, 1936).

Such an increased utilization of thyroxine would result in a more rapid exhaustion of available supplies, thus accelerating the onset of symptoms of thyroid deficiency. This proved to be the case in the present investigations. Cold generally accelerated the onset of all symptoms. Thus, while thyroidectomized animals on the iodine deficient diet kept at 27 °C took several months to show a full-blown cretinoid picture, similar animals kept at 16 °C developed such symptoms

within a few weeks. Also, the longer the animals were kept on the iodine-deficient diet at 27 °C before being placed in the cold, the lower their thyroxine stores and therefore the faster these stores were used up. At 16 °C it took only two days for the acute syndrome to develop if the animals had been thyriodectomized several months previously, while it took three to four weeks if the operation was performed immediately before placing the animals in the reduced temperature. Nevertheless, whether or not the animal spent the first week in the cold, it always took at least four weeks following operation for the acute syndrome to develop.

Another confirmation of the increased utilization of thyroxine in the cold was the fact that an equal degree of thyroid deficiency could be produced by feeding a thyroidectomized animal a very thyroxine deficient diet and keeping it at 27 °C, or by feeding it an only moderately thyroxine deficient diet and keeping it at 16 °C.

The train of symptoms observed upon exposure to moderate cold were probably similar to those produced in the thyroidectomized animals exposed to extreme cold described by Leblond and Gross (1942), but they were more spread out in the former case, due to a slower depletion of thyroxine stores, and therefore could be better observed.

HOW DID COLD INCREASE THE SYMPTOMS OF THYROID DEFICIENCY?

Nature of acute syndrome

An attempt was made to determine by the direct failure of what mechanism the acute syndrome was brought about. It seemed

apparent that the lack of thyroxine was the basic factor, since it could only be produced in thyroidectomized animals which had been fed a relatively thyroxine deficient diet. However, the action of cold as a precipitating factor also seemed necessary. This latter statement was not conclusively proved, since no attempt was made to keep the animals on the rat protein diet at 27 °C for longer than a few weeks. Perhaps they would have eventually developed the syndrome even at that temperature. Also, no other stressing agent was used which could have had an accelerating effect similar to cold.

The most characteristic signs of the acute syndrome were a progressive muscular paralysis and a fall in body temperature accompanied by a fall in all physiological activities measured. Examination of the muscles gave no visible morphological basis for the paralysis. Similarly the lack of reactivity to prostigmine seemed to clear the neuromuscular junctions of responsibility, at least so far as the acetyl choline-choline esterase mechanism was concerned. (Prostigmine was known to destroy choline esterase, thus allowing acetyl choline to act in transmitting nervous impulses from the nerves to the muscles.)

The only reference in the literature to a comparable syndrome associated with thyroid deficiency, was Astwood's communication in 1945. He described a similar paralytic syndrome (without mentioning any drop in body temperature) following the administration of dithiobiuret (an anti-thyroid agent) to rats. This paralysis could be reversed by removal of the drug, but did not react to any of the drugs known to

affect the nervous system, namely: prostigmine, strychnine, pilocarpine, atropine, epinephrine, ephedrin, nor a number of vitamins, namely crude liver extract, vitamin A, biotine, brewer's yeast nor biuret. He concluded that some new mechanisms were involved. However, the possibility was not excluded that the syndrome was due to a direct toxic effect of the drug, dithiobiuret, rather than to its anti-thyroid action.

A suitable theory could be worked out on the basis of the work of Minz and Cohen (1949). As previously mentioned, these workers attributed the action of thyroxine to its function as a respiratory enzyme activator particularly concerned with the conversion of Pantothenic acid to Co-enzyme A. This latter substance was shown to be necessary for all acetylations. A sudden depletion of thyroxine stores, leading to a cessation of synthesis of Co-enzyme A could thus cause a block in two locations, namely: (1) at the neuro-muscular junction, due to lack of Co-enzyme A for the synthesis of acetyl choline. Such a block should, however, be relieved by prostigmine, which would prevent the destruction of any acetyl choline which was still being released, provided any was there in the first place of course; (2) in the Krebs cycle of carbohydrate metabolism where Co-enzyme A was also necessary for the acetylation of oxal-acetic acid. This would cause a sudden drop in the general metabolic rate, thus leading to a fall in body temperature.

In addition, Minz and Thibault in 1948 claimed that thyroxine activated adrenaline by decreasing its rate of oxidation

and by further inhibiting the rate of oxidation of its even more active oxidation product, adrenochrome to inactive substances. Ring (1942) claimed that one of the main actions of thyroxine in cold adaptation was that it activated adrenalin. If such an action was interrupted the animals' mechanism for cold adaptation was impaired and the animals thus failed to maintain their body temperature.

Since, in the limited number of cases where it was tried, prostigmine had no effects at the neuro-muscular junction, it seemed more likely that the failure in the homeothermic mechanism was the primary symptom and that the paralysis was a secondary effect, namely, a direct result of the fall in body temperature. Thus Britton in 1922 described a progressive muscular paralysis in a cat subjected to gradual cooling. This was a direct effect of cold on the nervous system. The symptoms showed a strong similarity to anaesthesia, with consciousness disappearing between 27° - 25°C body temperature. (Due to the rapid heat dissipation in the rat, it was possible that such symptoms appeared at a higher temperature, since none of the animals had a temperature below 33°C). Britton described the following syndrome namely: The higher centers were put out of action first (e.g. walking) and then the more primitive automatic centers. Death seemed to be due to paralysis of the respiratory center, since this went out of action at a temperature much higher than that necessary to kill the skeletal and heart muscle, or the peripheral nervous system. He also found that spontaneous recovery could be

effected in ten to twelve hours.

In confirmation of the theory that similar factors were influential in the development of paralysis in thyroidectomized rats was Cassidy's observation in 1925 that hypoglycemia produced by insulin facilitated the appearance of paralysis and made the refrigerated animals appear to be in a state similar to hibernation. Since hypoglycemia was also associated with thyroid deficiency, it seems possible that this contributed to the development of the syndrome. Hypoglycemia could also have been in use in the paralyzed animals due to their inability to eat. The spastic paralysis into which the animals went upon handling could also have been hypoglemic convulsions, although Cassidy did not observe convulsions in hypoglycemic refrigerated cats.

Lack of myxoedematous infiltrations

When a state of presumably complete thyroid deficiency was produced in the rat, these animals presented a typical cretinoid picture, that is, they were stunted in growth, with abnormal body proportions, they were slow and lethargic and anaemic, had a dry skin and a patchy loss of hair. These animals, however, showed no signs of the myxoedematous infiltration of the skin which is so characteristic of human thyroid deficiency, and had also been observed in several of the higher mammals. It was therefore concluded that rats did not exhibit myxoedema as a symptom of thyroid deficiency. There were several possible explanations for this observation: (1) that

myxoedema was not necessarily a symptom of thyroid deficiency, but could be due to malfunctioning of the thyroid (Hertzler); (2) that the rat's metabolism differed from the human in its response to thyroid deficiency, or (3) that a subclinical myxoedema existed but was not detected,- even histologically; (4) that myxoedema was due to some wholly unrelated mechanism - made apparent by an interference with normal metabolism.

CONCLUSIONS: HOW THYROID DEFICIENCY SYMPTOMS WERE INCREASED IN THE PRESENT INVESTIGATIONS

A state of presumably complete thyroid deficiency was produced in thyroidectomized rats by a combination of the following factors, namely:

1. Removal of the endogenous source of thyroxine.- After a careful thyroidectomy, the animals were in addition fed an iodine deficient diet to prevent any extra-thyroidal production of thyroxine.

2. Removal of exogenous sources of thyroxine.- The animals were fed a diet which was made as thyroxine-free as possible. The only animal protein in this diet was the pulverized carcass of thyroidectomized rats.

3. Inactivation of any thyroxine still present.- Substances presumably having anti-thyroxine activity, such as yeast and liver were included in the diet.

4. Increased need and utilization of thyroxine available.- The animals were exposed to a low temperature in order to increase

the severity of the symptoms by increasing the need for thyroxine and accelerating the onset of the symptoms by a more rapid utilization of any thyroxine still available.

When these four factors were combined the animals not only showed all the classical symptoms of thyroid deficiency, but in addition developed an acute paralytic syndrome resulting in death. This condition could however not be produced in the absence of cold. The question was therefore raised, whether any thyroxine was needed for survival at ordinary temperatures. From all indications, the answer to this question was "no", but since no attempt was made to keep the animals on a thyroidectomized rat meat diet for a long period at 27°C, and since it was impossible to determine whether the animals were not still either receiving or producing minute amounts of thyroxine from some source, a positive answer to this question could not be given. Also since no other stressing agents were tried, it was not known whether cold did not act as general stress.

OTHER FACTORS WHICH COULD INFLUENCE THE SYMPTOMS OF THYROID DEFICIENCY

Time factor

It was found possible to considerably alter the symptoms of thyroid deficiency by varying the time for which a treatment was continued. Thus, it was found that a minimum of four weeks after thyroidectomy was required for the development of the

acute syndrome in the cold. Also, when animals were kept on an iodine deficient diet, they did not develop any symptoms for a period of several weeks but if they were kept on this diet for several months they became typically cretinoid in appearance. When such animals were now exposed to the cold they developed the syndrome much more rapidly than when they were placed in the cold immediately after thyroidectomy.

Age factor

Most of the rats used in these experiments were young, weighing only approximately 50 gm at the time of operation. Salmon (1936) and Scow (1944) and other workers had shown that the younger the animals at the time of thyroidectomy, the more acute were the symptoms produced. It was, however, possible to produce the acute syndrome also in older animals since 130 gm rats, thyroidectomized a considerable time before exposure to cold, also developed the syndrome. It was here shown that the body used any available amounts of thyroxine to its best advantage, in all cases growth was sacrificed for general well-being. Thus animals on the iodine deficient diet and on the thyroidectomized rat protein diet in the cold, showed a similar retardation of growth but the former were in much better general condition than the latter.

Factors associated with thyroid deficiency

Thyroidectomy was the only method used in this work to produce thyroid deficiency. The method suffered from the fact

that concomitant parathyroidectomy could not be avoided and the exact influence of the latter could not be assessed. The use of anti-thyroid drugs on the other hand, had the disadvantage that the drugs could exert direct effects which would then be ascribed to thyroid deficiency. Recently it has been shown by Asling et al (1951) that radio-active Astatine - At^{211} - was taken up by the thyroid in the same manner as radio-iodine, but that due to its higher energy emission and shorter radiation smaller doses of it could be used and would selectively destroy thyroid tissue, without damaging the parathyroids. The use of such a method could give some useful new indications on the influence of parathyroidectomy or the symptoms of thyroid deficiency.

Infections

Since thyroidectomized animals were very susceptible, the presence of a latent infection before the operation could easily become manifest following operation and this could influence the symptoms of thyroid deficiency. An experiment which was not reported had to be discarded because almost all animals died of infections.

Other hormones

It was well-known that there was a close interrelation between all endocrines, and it was therefore possible that thyroidectomy could so upset the endocrine balances as to produce symptoms due to excess or deficiency of other hormones

which would then be ascribed to thyroid deficiency. An attempt was therefore made to study the influence of other hormones on the symptoms of thyroidectomy and on the action of thyroxine. Results from such investigations will be described in the following section.

PART II

INFLUENCE OF OTHER HORMONES ON THE ACTION OF THYROXINE

The influence of castration and testosterone, adrenalectomy and cortisone, and hypophysectomy on the symptoms of thyroidectomy, and the action of thyroxine, were investigated. Particular emphasis was placed on the interaction of these various hormones on the epidermis and its appendages, although physiological and other morphological effects were also considered. The results of this series of experiments are summarized in Table 35 , and reference to this Table might aid in clarifying the discussion.

EFFECTS OF CASTRATION AND TESTOSTERONE

EFFECTS OF CASTRATION IN ABSENCE OF THYROXINE

In attempting to determine the effects of castration in thyroidectomized animals, one was handicapped by the lack of adequate controls to use for comparison. Thus these animals could hardly be compared to thyroidectomized animals, since

TABLE 25

EFFECT OF REMOVAL OF THYROID TESTIS ADRENALS AND HYPOPHYSIS AS WELL AS REPLACEMENT DOSES OF THYROXINE TESTOSTERONE AND CORTISONE IN THE ALBINO RAT.

	Thyroidectomy-5 weeks										Hypophysectomy-3 weeks			
	Alone	Castration-5 weeks				Adrenalectomy-10 days					Alone			
		No Hormone	Testo-sterone	Thyro-xine	Both	No Hormone	Corti-sone	Testo sterone with or without Cortisone	Thyro-xine	Both	No Hormone	Testo-sterone	Thyro-xine	Corti-sone
Group Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14
TARGETS														
Epidermis	//	0	////	----	0	0	0	////	----	0		////	----	0
Thickness														
Mitotic Rate		0	////	----	0	0	0	////	----	0				
Hair Growth	--	--	--	0	0	--	--	--	--	0				
Sebaceous Gls.	--	--	-	0	0	--	--	--	-	0				
Body Weight	---	---	--	0	0	---	---	---	--	0		---	---	---
B.M.R.	--	--	---	0	0	--	--	--	---	0		--	0	--
E.C.G.	--	--	--	0	0	--	--	--	--	0		--	0	--
Organ Weights.														
Heart, Kidney and Submax. Gl.	--	--	-	-	0	--	--	--	-	0		--	--	--
Adrenals	--	-0	-	/	0	0	0	-	/	0		-	--	--
Hypophysis	//	////	//	//	0	////	////	//	//	0				
Seminal Vesicles	--	--	0	--	0	--	--	0	--	0		0	--	--
Testis	--											-	--	--

Note: Column 5 = Column 3 plus Column 4 minus Column 2
Column 6 is the same as Column 2, Column 7 the same as Column 2, Columne 8 the same as Column 3
Column 9 the same as Column 4, and Column 10 the same as Column 5.
0 represents the condition in non-operated animals,
/ indicates an increase from normal and - indicates a decrease from normal.

thyroidectomy prevented sexual development and the animals were therefore functionally castrated. If normal sham operated animals were used as controls, the level of secretion of the various hormones varied between individuals and there was therefore a good deal of variation in all the targets observed.

Epidermis and Appendages

However, it was found that the thickness of the epidermis of castrated-thyroidectomized animals was approximately the same as in the shams operated animals. Whether or not it was thinner than in animals which were only thyroidectomized was not determined. The mitotic activity of the epidermis also seemed approximately equal to that in the sham operated animals.

Hair growth was retarded in these animals, as was also the case in thyroidectomized animals without castration. In humans, castration alone did not cause a loss of hair but rather seemed to prevent baldness. The retardation of hair growth thus seemed to be primarily an effect of thyroidectomy. Similarly the sebaceous glands were small and atrophied as compared to the shams, but these were not compared to animals which were only thyroidectomized.

Physiological processes.

The retardation of growth, the fall in oxygen consumption and heart rates, as well as the decrease in organs weights, except for an even greater atrophy of the seminal vesicles was the

same as in thyroidectomized animals. Castration has not been reported to have any influence on these functions.

EFFECTS OF REPLACING TESTOSTERONE

When testosterone was given in replacement doses, certain functions were restored to normal, other functions were not influenced and still others reacted in quite an unexpected manner.

Epidermis and Appendages

When replacement doses of testosterone were given to these castrated-thyroidectomized animals there was great increase in thickness in all layers of the epidermis, both as compared to the controls not receiving any hormones and the shams. A similar increase in the thickness of the epidermis of non-thyroidectomized rats was reported by Hooker in 1943 and by Graaf in 1946. This increase in thickness seemed to be brought about mainly by an increased mitotic activity of the basal layers of the epidermis, while, all other things being equal, would result in an increased number of cells. The mechanism of this action was unknown. In addition testosterone further increased the thickness by delaying the rate of desquamation, thus prolonging the life span of the cell. These observations were in general accordance with the popular concept that the skin of men was thicker and rougher than the skin of women and that eunuchs has a skin similar to the female. The increased

epidermal mitotic rate induced by testosterone could have possible clinical significance in the field of dermatology where a rapid growth of skin was desired, as for example, in the treatment of burns.

There was no change in hair growth, which however did not deny the claim that testosterone produced baldness, since these animals already showed a great decrease of hair growth due to thyroidectomy. Testosterone, however, produced a marked stimulation of the sebaceous glands, indicating that the atrophy of these structures following thyroidectomy might have been due indirectly to the lack of testosterone. Such stimulation of the sebaceous blands was also observed by Hamilton in 1941 and by Grad in 1949.

Physiological processes

It was generally believed that the sudden increase in the rate of growth at puberty was due to the action of the gonadal hormones. Noback (1949) denied that testosterone had any growth-stimulating action in the rat, and Grad (1949) confirmed this observation. In the present investigation testosterone was found to cause a slight but consistent increase in the rate of growth over that of the castrated-thyroidectomized controls. This was in agreement with Nyda's recent observation (1950) that physiological doses of testosterone propionate caused an increase of growth rate in the rat. (Excessive doses had the opposite effect.) The increase in growth was, however, only slight and thus could not account for the great

change at puberty. It seemed more likely that this was due to an increase in the secretion of growth hormones, accompanying its increased secretion of gonadotrophin, , than to a direct effect of the gonad hormones.

In the present investigations, testosterone seemed to cause a slight depression in the basal metabolic rate. No such depression was observed by Grad in 1949, nor by Meyer in 1942, who even observed a rise. No explanation could be given for the results obtained. The heart rate remained unaffected by testosterone.

On examination of the organ weights , Grad's previous observations were confirmed, when it was observed that testosterone caused a marked increase in the size of the seminal vesicles (being target organs of this hormone), a slight increase in the weight of the heart, kidneys and submaxillary glands, and decrease in the weight of the adrenal and hypophysis, the latter now not having to produce as much gonadotrophin to attempt to compensate for a lack of circulating testosterone.

EFFECTS OF REPLACING THYROXINE

When, instead of testosterone, thyroxine was replaced in castrated-thyroidectomized animals, a condition similar to simple castration was set up. Thyroxine was found to restore many bodily processes to normal, but in the absence of testosterone it had several remarkable effects. Testosterone therefore modified the action of thyroxine. Conversely, some symptoms following castration which were commonly attributed directly to

an absence of testosterone, could have been due to the uninhibited action of another hormone, such as thyroxine. An organ so affected was found to be the epidermis.

Epidermis and Appendages

In the absence of testosterone, thyroxine was found to cause a marked decrease in the thickness of all layers of the epidermis, but especially in the cornified layers. That thyroxine should cause a thinning of the epidermis was to be expected, since thyroidectomy was known to produce a thickening, and similarly a thin skin was characteristic of hyperthyriodism (also in humans). The remarkable fact was, however, that this reduction in the thickness of the epidermis with a physiological dose of thyroxine was in excess of normal. Since testosterone was found to bause an increase in the thickness of the skin, the question was raised whether the antagonistic action of testosterone was necessary to produce a normal thickness of the epidermis and this was indeed found to be the case. Thus, the thin skin of eunuchs may not only have been due to a lack of testosterone, but also to the unrestrained action of thyroxine.

Similarly to the action of testosterone, this decrease in the thickness of the skin due to thyroxine seemed to be mediated through a decrease in the mitotic activity, thus reducing the number of cells. A recent paper by Bullough and Eisa (1950) suggested a possible mechanism for thyroxine's action. These authors showed that the diurnal variation in the number of

epidermal mitoses was strictly parallel to the diurnal variation of the glycogen content of the epidermis and also of the liver. This indicated that the energy required for mitosis was supplied by glycogen. Now, thyroxine treatment was shown to decrease the glycogen level in liver (Defauw, 1930; Fieschi, 1933; Coggeshall and Greene, 1933; Sternheimer, 1939) and heart (McDonald et al. 1938; Moses, 1944). Should a similar lowering of the glycogen level be produced by thyroxine in the epidermis, it would presumably account for the decrease in mitotic activity. An investigation was made of why the Malpighian layers were less responsive to thyroxine than the cornified layers. It was observed that just as thyroxine inhibited the cells from entering into mitosis, thyroxine also seemed to delay the transformation of the Malpighian cell into the cornified cell. It was possible that glycogen was also needed for the differentiation of the cells, as well as for their division. This delay in keratinization resulted in the cells spending a longer time in the Malpighian layers, thus increasing the thickness of this layer, and partly counteracting the effect of the decreased mitotic rate.

In the cornified layers, on the other hand, the decreased number of cells entering this layer was reinforced by the absence of testosterone to which inhibited desquamation. This layer was therefore extremely thin.

In conclusion it seemed strange that thyroxine, which generally stimulated the growth probably by increasing the mitotic rate, should have the opposite effect on the epidermis. A possible

function of such a mechanism could be that a thinner skin would aid in the dissipation of excess heat produced by the higher metabolic rate, and conversely, that a thicker skin would help conserve bodily heat in thyroid deficiency.

Contrary to its action on the epidermis, thyroxine seemed to increase the rate of hair growth. This was to be expected from the knowledge that thyroid deficiency caused a loss of hair and decreased hair growth. Since an increased rate of hair growth involved an increased mitotic activity in those layers of the hair follicle which were derived from the Malpighian layers, a paradox was created.

In the case of the sebaceous glands, thyroxine did not seem to have any effect. This confirmed the view previously stated that perhaps the dryness of the skin associated with thyroid deficiency was only secondary to a decrease in testosterone due to a lack of sexual development.

Physiological Processes

Thyroxine was found to increase all physiological processes, such as body weight, oxygen consumption and heart rate to normal. The former two functions were even used as criteria to regulate the dose of thyroxine to a physiological level. Heart rate, although reacting to thyroxine in the same direction, i.e. by increasing, seemed to be a less sensitive index of thyroxine activity, since it showed a good deal of variation.

Beside causing an increase in the body weight in general,

thyroxine was found to exert an additional stimulating action on the heart, kidneys submaxillary, and lymphatic organs, confirming findings already made by Hammett in 1926, and more recently by Grad (1949). Thyroxine also decreased the weight of the hypophysis, the latter now not having to produce as much thyrotrophin to compensate for thyroxine deficiency. There was also an increase in the weight of the adrenal as found by Nelson in 1942. The seminal vesicles remained completely undeveloped in the absence of testosterone.

EFFECTS OF REPLACING BOTH TESTOSTERONE AND THYROXINE

When replacement doses of both testosterone and thyroxine were given to castrated-thyroidectomized animals, one would have expected these to be similar to the sham-operated animals. This, however, was not quite the case, firstly because it was difficult to obtain exactly the right dose of hormone, and secondly, because the latter showed great variation due to the variations in the amounts of hormones secreted by individual animals. Since the level of hormones in the doubly-treated experimental animals was the same in all, these made a better base for comparison of the combined effect of the two hormones to the effect of each hormone given separately, than did the sham-operated animals. Such animals were therefore considered to represent the normal picture.

Epidermis and Appendages

As was to be expected, when thyroxine and testosterone were administered simultaneously they counteracted one another to produce an epidermis of normal intermediate thickness. The antagonistic action of these hormones and the necessity of the presence of (or absence) of both to produce a normal thickness was established. It was also shown that the action of thyroxine could be modified by another hormone, such as testosterone.

Hair growth was the same as in thyroxine-treated animals, and testosterone had no modifying influence on the action of thyroxine on this structure.

In the sebaceous glands, however, although thyroxine had no effect by itself, it had a synergistic effect on this structure when given in combination with testosterone. Thyroxine thus reinforced the action of testosterone, showing that just as testosterone modified the action of thyroxine, thyroxine could also modify the action of testosterone. This synergistic action had also been observed by Grad (1949).

Physiological processes

Testosterone did not influence the action of thyroxine on the body weight, oxygen consumption or heart rate. It did, however, have a synergistic effect with thyroxine in increasing the weights of the heart, kidneys and submaxillary glands, and in decreasing the weights of the hypophysis. Thyroxine and testosterone had an antagonistic effect on the adrenal. (All these effects were also observed by Grad in 1949 and were fully

discussed in his thesis.)

EFFECTS OF ADRENALECTOMY AND CORTISONE

When it was observed that thyroxine increased the size of the adrenals, the question was raised whether perhaps some of the actions of thyroxine were mediated through the adrenal and some of the effects of thyroid deficiency might be really due to a secondary adrenal hormone deficiency. Similarly, the question was raised whether the actions of testosterone could be mediated through a decrease in adrenal function. Cortisone had been shown by Castor in 1950 and Baker in 1948 to cause a decrease in thickness of the epidermis and an increase in the growth of hair, thus simulating the action of thyroxine, and this supported the hypothesis that the adrenal was an intermediary for the actions of thyroxine and perhaps also for testosterone.

Neither adrenalectomy, nor replacement doses of cortisone during a ten day period seemed however, to have any effect on either the epidermis or its appendages, or on the other physiological processes (the slight fall in these functions merely indicated that the animals were moribund). As previously discussed, this period may have been too short to change a trend already established by a four week treatment of castrated-thyroidectomized animals with replacement doses of thyroxine and testosterone in various combinations. However, observations following hypophysectomy seemed to indicate that adrenalectomy

really did not have any effect on these organs and functions and probably was not the mediator of the actions of thyroxine and testosterone.

EFFECTS OF HYPOPHYSECTOMY

Hypophysectomy was considered functionally almost equivalent to removing the thyroid, testes and adrenals, due to the absence of the "trophic" hormones to stimulate these glands. In addition, there was also an absence of growth hormone which could, and indeed was found to modify the actions of thyroxine. Whether hypophysectomy could influence the action of testosterone and cortisone was also investigated.

ON THE ACTION OF THYROXINE

Epidermis

The epidermal thinning effect of thyroxine persisted in spite of hypohysectomy, thus clearly indicating that this was a direct action of thyroxine. This was a remarkable finding, since all the other morphological influences of thyroxine on the organ weights disappeared after hypophysectomy.

Physiological processes

The growth and metabolism-stimulating actions of thyroxine, which usually were so closely interrelated, were separated by hypophysectomy. It was thus found that hypophysectomized animals given thyroxine did not grow, but even lost weight,

yet the oxygen consumption and heart rates responded to thyroxine in the expected manner. A similar lack of growth of hypophysectomized animals was described by Evans in 1939. He found that growth hormone and thyroxine were synergistic, but whereas growth hormone could restore growth and even produce gigantism in the thyroidectomized animals (a greater dose being required to produce the same effect in the absence than in the presence of the thyroid), thyroxine could not restore growth in hypophysectomized animals. From the results of this experiment it seemed likely that the lack of growth of thyroidectomized animals was due to the absence of acidophils (to which cells the secretion of growth hormone has been generally attributed). The restoration of the acidophils was suggestive of thyroxine role in the pituitary's secretion of growth hormone and thus explained the growth-stimulating action of thyroxine. The synergism of growth hormone and thyroxine described by Evans and confirmed by Scow in 1949 could be explained by the fact that in thyroidectomized animals there was no endogenous growth hormone being produced to supplement that which was injected, while in the normal animals there was such endogenous secretion. The synersism between growth hormone and thyroxine could also lie in intermediate metabolism, the function of thyroxine being catabolic, i.e. to provide building blocks and the action of growth hormone being anabolic, i.e. to build up body proteins from these blocks.

The stimulating action of thyroxine on the organ weights was also absent in hypophysectomized animals, indicating that

this was also an indirect effect.

ON ACTION OF TESTOSTERONE

Epidermis

The thickening action of testosterone on the epidermis also persisted in hypophysectomized animals, indicating that in the case of testosterone, as in the case of thyroxine, the effect was direct.

Physiological Processes

Since normally testosterone had very little, if any, action on growth, oxygen consumption or heart rate, it was not surprising that these remained unaltered after hypophysectomy. The slight growth-promoting action of testosterone had disappeared, probably indicating that "castration" basophils similarly to the "thyroidectomy" cells seemed to crowd out the acidophils, but not nearly to the same degree.

Any effects of testosterone on organ weights, except for its direct action on the seminal vesicles, also disappeared.

ON ACTION OF CORTISONE

This experiment permitted observation of the effects of presumably physiological doses of cortisone on various targets, without the interference of any other hormones.

Epidermis

Cortisone did not seem to have any effect on the epidermis, since it appeared intermediate in thickness to that in similar animals treated with either thyroxine or testosterone. There did not seem to be a decrease in thickness as described by Baker in 1948, who, however, applied cortisone locally, and therefore in much more concentration.

Physiological processes

Cortisone caused a loss of weight in hypophysectomized animals, and these animals seemed to be in a slightly worse condition than similar animals receiving thyroxine and testosterone. This could be explained by the gluconeogenic properties of cortisone, i.e. cortisone caused proteins to be broken down to carbohydrates, and in hypophysectomized animals this was not counteracted by anabolic hormones such as the growth hormone.

It was noted that cortisone caused a slight but constant increase in the heart rates over animals receiving thyroxine, and a similar trend was indicated in the oxygen consumption. This was in agreement with recent findings by Hill (1950) and Bierwaltes (1950) who observed that in humans cortisone caused an increase in the basal metabolism whether thyroid function was subnormal or not. These workers attributed this action of cortisone to an increased efficiency of utilization of thyroxine. Since there still appeared to be a basal production of thyroxine

even in hypophysectomized animals, such an effect may have been operative in this investigation.

FACTORS INFLUENCING THE THICKNESS AND CORNIFICATION OF THE EPIDERMIS

It would here be of value to summarize factors which were found to influence the thickness and cornification of the epidermis.

An increase in the thickness of the epidermis could be brought about by any one of these interrelated factors, (the possibility of there being other factors not being excluded), namely: (1) Thyroxine deficiency, (2) testosterone excess, and (3) pantothenic acid deficiency.

A possible system of interaction could be as follows:

A.

PANTOTHENIC ACID $\xrightarrow{\text{THYROXINE}}$ Co-enzyme A

Co-enzyme A in Krebs Cycle leads to

Glycogen breakdown $\longrightarrow$ (1) Decreased mitoses in epidermis, (2) Decreasekeratinization of epidermis.

(1) Decreased mitotic avtivity $\longrightarrow$ Decrease in thickness of epidermis,

(2) Decreased keratinization $\longrightarrow$ Relatively smaller decrease in thickness of Malpighian layers as compared to cornified layers

B.

TESTOSTERONE ——?——> (1) Increased mitoses in epidermis; (2) Decreased desquamation rate

(1) Increased mitoses in epidermis
(2) Decreased desquamation rate ——> Increased thickness of all layers of epidermis.

If either pantothenic acid or thyroxine were removed, there would be a break in cycle A, leading to a deposition of glycogen in the epidermis, an increased number of mitoses and an increased keratinization rate. The increased number of mitoses would lead to an increase in the thickness of the epidermis, somewhat counter-acted in the Malpighian layers by the increased keratinization rate, (the cells thus spending less time in this layer.)

An additional increase in the thickness of the epidermis under either pantothenic acid or thyroxine deficiency would be produced due to the uninhibited action of testosterone. This latter would however be limited in the case of thyroxine deficiency due to the inhibition of sexual development accompanying such deficiency.

CONCLUSIONS.- THE INFLUENCE OF OTHER HORMONES ON THE ACTION OF THYROXINE

In conclusion it could be said that there was close interrelation between all endocrine organs and that the absence of other hormones could profoundly alter the action of thyroxine.

(1) Testosterone, by exerting an antagonistic action on the thickness of the epidermis, modified the thinning action of thyroxine. When both these hormones were given in replacement doses, a moderate picture resulted, but in the absence of testosterone, the uninhibited action of thyroxine produced an extremely thin epidermis.

(2) The dryness of the skin due to a lack of secretion of the sebaceous glands, often attributed to thyroid deficiency, was probably due to a secondary lack of testosterone, since thyroxine did not stimulate these organs in the absence of testosterone.

(3) The absence of the hypophysis profoundly influenced the actions of thyroxine, since it abolished the growth-promoting action of thyroxine which was always considered as one of its fundamental properties. Hypophysectomy separated the growth-promoting influence of thyroxine from its action on the oxygen consumption and heart rates.

The interactions of these various hormones are given in Table 25. Please note that after a ten-day adrenalectomy the responses of the target organs to thyroxine and testosterone were not altered.

SUMMARY AND CONCLUSIONS

Thyroidectomized male rats were exposed to various conditions of diet and temperature to see whether or not the intensity of the symptoms of the thyroid deficiency would be influenced.

In regard to the diet, an attempt was made to reduce the content of iodine and thyroxine present in many types of diet and expecially in commercial feed. The iodine deficient diet of Remington (No. 342) was used as a base, since preliminary work disclosed that thyroidectomized rats given this diet developed symptoms which were not observed if they were fed the commercial "Purina Fox" chow. Since Remington's diet contained 1.9% pig liver and liver had been shown to contain a fair concentration of thyroxine, this liver protein was replaced by various amounts of pulverized dried carcass of rats thyroidectomized three weeks previously and subsequently kept on the modified Remington's Iodine deficient diet. Thyroidectomized animals fed such a diet developed cretinoid symptoms within 5 weeks. Such animals failed to gain weight, developed abnormal body proportions, were lethargic and anaemic and had a dry scaly skin with patchy hair loss.

In case there was still some thyroxine left in the diet, an attempt was now made to even further increase

the deficiency by increasing both the animals requirements for, and their rate of utilization of thyroxine. This was done by exposing the animals to a relatively cool temperature, namely 16°C, as compared to the normal temperature for the rat, which was 27°C. As expected, exposure to cold increased the symptoms of thyroid deficiency in all animals regardless of the diet. When animals fed the thyroidectomized rat protein diet were exposed to this reduced temperature, they not only developed a severe cretinoid condition within two to three weeks, but around the fourth week they suddenly developed an acute syndrome. This was characterized by a sharp fall in body temperature and all physiological activities and the development of a progressive muscular paralysis. The animals lapsed into a coma within one or two days and died several hours later. The onset of this syndrome was very sudden and appeared at almost the same time in all comparable animals. A virtually thyroxine-free diet and a a cool temperature were thus synergistic in producing a condition which had not otherwise been obtained.

The onset of these symptoms was attributed to a break in the animals' metabolism resulting in a failure to keep up the body temperature, thyroxin being attributed the function of a metabolic catalyst. All other symptoms were considered secondary to this failure of the homothermic

mechanism.

In the second part of the investigation the influence of other hormones on the action of thyroxine were considered. The epidermis was made the object of special study. It was observed that thyroxine decreased the thickness of the epidermis but that under normal conditions this effect was modified by an antagonistic action of testosterone which increased the thickness. In the absence of either of these hormones, the uninhibited action of the epidermis of the other hormone could be observed. The increase or decrease in thickness of the epidermis was found to be mainly due to a change in the mitotic rate in the basal layers, although thyroxine also decreased the rate of keratinization and testosterone decreased the rate of desquamation. Since the mitotic rate of the epidermis was known to be related to the glycogen content of this organ and since thyroxine was also known to deplete the glycogen content of certain organs, the theory was advanced that the decrease in mitotic rate was due to a decreased glycogen content in the skin. The interrelation between pantothenic acid and thyroxine in the metabolism of carbohydrate and the resemblance between the symptoms (especially the scaliness of the skin) of the deficiencies of either of these substances were also considered to be linked with the glycogen content of the epidermis. The mechanism of action of testosterone on the epidermis was not known.

The actions of both thyroxine and testosterone on the epidermis were found not to be mediated through the hypophysis nor the adrenals since they persisted in the absence of these glands.

Incidentally, it was observed that thyroxine increased the growth of hair. Also, testosterone was found to increase the size of the sebaceous glands and while thyroxine had no effect of its own, it had a synergistic action on these sebaceous glands when given in combination with testosterone. The dryness of the skin associated with thyroid deficiency thus seemed to be due indirectly to a deficiency in testosterone associated with a lack in sexual development induced by the thyroid deficiency.

Another interesting finding was that it was possible to separate the usually intimately associated metabolism and growth stimulating actions of thyroxine, one being a direct effect and the other being mediated through the hypophysis. Thus it was found that hypophysectomized animals treated with physiological doses of thyroxine failed to show any increase in weight, yet showed the expected increase in oxygen consumption and heart rate. The increase in organ weights attributed to both thyroxine and testosterone were also found to be mediated through the hypophysis. The lack of growth of thyroidectomized animals was therefore attributed to the associated lack of acidophils in the hypophysis, the latter being replaced

by overactive "thyroidectomy" basophils. A synergistic action of thyroxine and growth hormone in promoting growth was also suggested, thyroxine providing the metabolic building blocks and growth hormone synthesizing these into body proteins.

Incidentally, cortisone was also found to have a slight calorigenic action associated with loss of weight in hypophysectomized animals probably due to its gluconeogenic properties.

In conclusion it was established that many factors could influence both the symptoms of thyroxine-deficiency and the action of thyroxine itself. These investigations were an attempt to define only a few of the many factors which would all merit separate consideration.

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