

SEPTEMBER, 1943

RESEARCH BULLETIN 377

UNIVERSITY OF MISSOURI

COLLEGE OF AGRICULTURE

AGRICULTURAL EXPERIMENT STATION

M. F. MILLER, *Director*

The Effects of Mild Hyperthyroidism on Growing Animals of Four Species

MARVIN KOGER AND C. W. TURNER

(Publication Authorized September 2, 1943)



COLUMBIA, MISSOURI

TABLE OF CONTENTS

	Page
Introduction	5
Effect of Mild Hyperthyroidism on Growth	6
Review of literature	6
Historical	6
Effects of hypothyroidism	8
Effects of hyperthyroidism	12
Thyroid activity and vitamin requirements	18
Experimental	19
Experiments with mice	20
Experiments with rats	28
Experiments with guinea pigs	43
Experiments with rabbits	50
Effect of Thyroprotein Feeding on Organ Weights	54
Review of literature	54
Experimental	56
Effect on pituitary	59
Effect on thyroid	60
Effect on Adrenals	60
Effect on thymus	60
Effect on the ovary	60
Effect on testes	60
Effect on heart, liver, and kidneys	61
Effect of Thyroprotein Feeding on Hypophyseal Thyrotropin and Gonadotropin	61
Review of literature	62
Experimental	62
Discussion	64
Summary	67
Bibliography	69

ABSTRACT

Growing animals of four species, including mice, rats, guinea pigs, and rabbits, were treated with thyroactive preparations varying in amounts from relatively large dosages which were toxic to very small dosage which apparently did not affect growth or were so small in amount that it appeared impractical to attempt further reduction in dosage.

The growth rate of mice was consistently and significantly increased by treatment with a rather wide range in dosage (0.01 to 0.04 mg. thyroxine-sodium daily or 0.04 to 0.32 per cent thyroactive iodocasein in the ration while maximum size attained by control and treated animals was unchanged. Feed intake of mice was increased by treatment. The treated animals stored more protein and more body weight per unit of feed consumed than controls while control animals were more efficient in storage of fat and energy.

The effect of feeding thyroactive iodocasein to rats was variable with strain and sex. There was some evidence of slight acceleration of growth in weight of a few females due to feeding thyroactive iodocasein, but for the most part body weight was unaffected or depressed. The nose-anus length of male rats of one strain (Sprague-Dawley) was increased due to treatment, but was not observed in any of the other animals. Male rats were less tolerant of thyroactive preparations than female.

The growth rate of male guinea pigs was slightly accelerated by mild treatment (0.0025 to 0.0075 per cent thyroactive casein in the ration) for a short period of time, but the same treatment later became toxic with increase in age and arrival of warm weather. Growth rate of female guinea pigs was not affected for a few weeks, after which time treated animals ceased growing and lost weight.

Small amounts of thyroactive casein (0.0025 to 0.02 per cent of the ration) apparently did not affect growth of rabbits while larger amounts caused a depression of growth.

The effect of thyroactive casein on the organ weight of rats was studied. Extremely small dosages given to males did not affect the weight of any of the glands or organs weighed, although the thyroids of treated animals showed histological evidence of inactivity. Larger dosages given to either sex resulted in hypertrophy of heart, liver and kidneys. The effects on other glands was variable with strain and sex of animals.

The thyrotropic potency of the pituitaries of animals was markedly lowered by feeding thyroactive iodocasein.

ACKNOWLEDGMENT

The writers wish to express their appreciation to Professor A. C. Ragsdale, Chairman, Department of Dairy Husbandry, University of Missouri, for his interest and encouragement; to Dr. E. P. Reineke, Assistant Professor of Dairy Husbandry, University of Missouri, for his cooperation in supplying certain thyroactive preparations, and for assaying the preparations for thyroid activity; to Dr. W. R. Graham, Cerophyl Laboratories, Kansas City, Missouri, for supplying one lot of thyroactive iodocasein; and to Victor Hurst, Research Assistant, Department of Dairy Husbandry, University of Missouri, for supplying data in Experiment 1 with mice.

The Effects of Mild Hyperthyroidism on Growing Animals of Four Species

MARVIN KOGER AND C. W. TURNER

It has long been known that the thyroid gland plays an important role in the metabolic processes which regulate growth and other biological transformations. During recent years, however, it has become evident that the pituitary gland occupies an even more important place, in that it appears to integrate the entire endocrine system.

The control of the pituitary gland over thyroid function by means of the thyrotropic hormone is well known. Likewise, pituitary function is influenced by the thyroid gland as evidenced by decreased thyrotropic hormone secretion resulting from excess thyroid activity.

Other interrelationships of the two glands have not received much attention, but there is considerable evidence that the effects of the thyroid hormone are mediated by way of the pituitary as well as by its effect on metabolism *per se*.

If the thyroid glands are removed from a growing animal, growth rate declines rapidly and eventually ceases completely. Growth in such an animal can be restored either by replacement of thyroid hormone or by administration of pituitary extracts. Conversely, removal of the pituitary results in immediate cessation of growth and thyroid has no beneficial effects on growth of such animals. Administration of pituitary extract causes restoration of growth which can be further augmented by simultaneous treatment with thyroid hormone.

From these observations it would appear that the thyroid influences the secretion by the pituitary of the growth hormone, or hormones. There are also reports, which are reviewed later, that the thyrotropic potency of the pituitary is greatest during periods of rapid growth when growth hormone secretion would presumably be high. The hypothesis that the thyroid hormone influences the organism in ways other than by a mere increase in metabolic rate is further supported by the fact that such agents as dinitrophenol elicit none of the features of thyroid treatment other than an increase in metabolism.

The concepts generally held concerning the relation of the thyroid gland to growth have arisen largely as a result of observations of changes which accompany absence of thyroid activity and to changes occurring when the thyroid becomes extremely hyperactive. The results of only a slight departure from normal activity of the gland, however, are not agreed upon and considerable confusion is evident in reports dealing with the effects of mild hyperthyroidism produced by administration of thyroid materials.

A few scattered reports, which will be reviewed in a later section, have indicated that mild hyperthyroidism is favorable in the sense

that rapid growth is induced, while many others have reported unfavorable effects. The incongruity of these reports appears to have arisen in part from a lack of appreciation, by most experimenters, of the sensitivity of organisms to thyroid materials and to the great variability in potency of thyroid preparations.

Consideration of these reports gave rise to the question of the consistency and nature of the effects of mild hyperthyroidism on growth of young animals. The question appeared to be of practical significance due to possible applications in animal husbandry. If certain types of animals should be found to respond uniformly to thyroid treatment by acceleration of growth, as certain experimental results would indicate to be possible, the recent discovery of cheap methods of producing thyroactive materials would make the treatment of animals on a commercial scale feasible.

Information concerning the effects of mild hyperthyroidism in different laboratory animals has been obtained by giving thyroid material in dosage ranging from very small amounts which did not affect the animals, to larger dosages which were inhibitory to growth. It was planned originally to determine, in addition to the effects on growth, the growth hormone content of pituitaries of the differently treated animals, but no assay sufficiently sensitive to determine growth potency of small amounts of pituitary was developed.

The investigations reported herein have been concerned mainly with the effects of different levels of thyroid treatment on the growth rate of young animals of four different species. Included also are observations on changes in certain organs due to the administration of varying amounts of thyroactive preparations.

EFFECT OF MILD HYPERTHYROIDISM ON GROWTH

Review of Literature

Historical.—Most writers prior to the fifteenth century, including Hippocrates and Plato, ascribed to the thyroid the function of lubricating the trachea, larynx and pharynx (Schneider, 1939).*

Thomas Wharton first published a good anatomical description of the thyroid in 1656, but he still attributed to the gland functions that seem rather fantastic. He listed its functions as (1) heating the hyoid cartilage which would otherwise be cold, (2) lubrication of the larynx, and (3) giving rotundity and beauty to the neck. He gave a careful description of the anatomy of the gland and placed emphasis on its great vascularity.

Little new information was added to that presented by Wharton until 1850 when Curling noted in two cases of typical cretinism in humans that the thyroid glands were atrophied or absent. This paper of Curling's was the first in a long series which contributed to a better understanding of thyroid function. In 1855, Claude Bernard published his epoch, "Lecons de Physiologie Experimentale," in which

*For list of references see page 69.

he differentiated between glands of external secretion and those of internal secretion. Four years later Bernard (1859) described the thyroid as a gland of internal secretion. One year after Bernard's first paper, Schiff (1856) published the results of the first attempt to study the function of the thyroid by surgical removal of the gland from dogs. A few of his operated animals lived for several weeks after operation, but most of them soon went into tetany and died. In all cases growth stasis occurred. It is now known that the lethal effects of thyroidectomy observed by Schiff were due to concomitant removal of the parathyroid glands.

The lethal effect of surgical removal of the gland from animals in these early attempts was a handicap to further progress since there was no known way to experimentally produce a hypothyroid condition. As a result, it remained for the next real progress to be made as a result of surgical removal of the gland from humans. Kocher (1878) was the first to practice surgical removal of the thyroid for treatment of goiter. A few years later, Kocher (1883) and Reverdin and Reverdin (1883) published papers dealing with pathological conditions arising a few months after total thyroidectomy. Kocher termed the condition "cachexia strumipriviã" and described a condition similar to myxedema. Reverdin and Reverdin described the condition as "operative myxedema." Neither of the authors apparently recognized the parallelism of myxedema and cretinism. During the same year Lombard (1883) reviewed the previous literature on the thyroid indicating that the gland was essential for proper development of children and that its integrity was essential for normal mentality and appearance of adults.

In 1884 Horsley reported that thyroidectomy of young monkeys led to myxedema and pointed out the similarity of the condition to that described by Schiff arising from thyroidectomy of dogs, the "cachexia strumipriviã" of Kocher and "operative myxedema" of Reverdin and Reverdin (Paget, 1919). He concluded further that the conditions arising from thyroid removal closely paralleled the condition found in clinical myxedema.

The pathological features appearing after the removal of the thyroid from human patients naturally led to attempts at replacement therapy. The first attempt reported, however, was made by the physiologist Schiff (1884). In 1884 he published an important paper reporting that part of the harmful effects of thyroidectomy could be relieved by transplanting a gland from another dog. He even ventured the suggestion that similar results might have been secured by injection of an extract or by ingestion of thyroid glands. Murray (1891), following the suggestion of Schiff, found that myxedema in humans was relieved by injection of an extract of sheep thyroids. The following year, Fox (1892) successfully treated a case of myxedema

by giving thyroid by mouth. The technique of studying the physiology of a gland by removal, followed by replacement therapy, was firmly established.

About that time Magnus-Levy (1897) added another method of approach. He reported upon the feeding of thyroid to normal subjects and studied the effect on oxygen and nitrogen metabolism. He also studied the metabolism of myxedematous patients and those who had been treated successfully by thyroid therapy. Moussu (1899) reasoned that if a small amount of thyroid is necessary for growth, would not a little extra result in more rapid growth? Acting upon this hypothesis, he fed fresh thyroid to dogs and observed rapid skeletal growth with early maturity.

Thus, knowledge of the physiology of the thyroid gland was beginning to expand rapidly. By 1900 publications on the gland were numerous and investigations extended into many branches of biology and medicine. The thyroid was beginning to attract the attention of chemists, also. As early as 1820 iodine was shown to relieve certain types of goiter (Harrington, 1933), and Baumann (1896) had shown that iodine was a normal constituent of the thyroid.

The developments along the different types of investigation have given rise to a multitude of reports, a review of which is quite beyond the scope of a dissertation such as this. This review will consequently be confined to subjects with which this investigation has been concerned.

Effects of Hypothyroidism. *Clinical Observations on Humans.*—The literature is in agreement that hypothyroidism, whether induced or spontaneous, is detrimental to growth. Curling (1850) first associated the absence of the thyroid with the characteristic condition known as cretinism in humans. In such individuals body growth in general is subnormal with characteristic disproportions of the body. The individual is extremely short with coarse features, dry, rough skin and hair, and usually a typical "pot belly". There is an accumulation of fluid in the intercellular spaces of the body giving the characteristic myxedema of hypothyroidism (Lerman, 1941).

If the deficiency develops early in life the skeleton is also affected in a striking manner. The long bones are shorter and frequently more dense than normal (Aub, Bauer, Heath and Ropes, 1927). There is a marked delay in appearance of the ossification centers and in epiphyseal unions, resulting in a subnormal "bone age." If the deficiency develops after growth has been attained, skeletal derangements are not necessarily noticeable but myxedema, along with mental derangement, rough, dry skin, and coarse hair, are soon manifest (Kocher, 1883; Reverdin and Reverdin, 1883).

Experiments with Laboratory Animals.—The features of hypothyroidism in animals are analogous to those observed in humans. Schiff (1856) first thyroidectomized dogs and a few lived long enough

to develop conditions resembling myxedema in humans. Dott (1923) thyroidectomized dogs, taking precautions to leave the parathyroids intact, and kept the animals alive for long periods of time. They grew subnormally, assumed a dull, lethargic appearance, and skeletal development was markedly reduced as shown by radiograms. Similar results with dogs were reported by Binswanger (1936) from an extensive study of thyroidectomy of young puppies.

Kojimi (1917) thyroidectomized growing rats and observed retarded growth, reduced calcium and nitrogen retention, and decreased feed intake. Hammett (1927a, b) observed like effects and also reported that both relative and absolute weights of the heart, liver, lungs, kidneys, and spleen were below that of normal controls. Salmon (1938) reviewed the literature indicating that thyroidectomy of young animals results in more acute symptoms of hypothyroidism than if the operation is performed on older animals. She thyroidectomized rats at birth and observed early growth stasis with such animals reaching a maximum weight of about 30 grams. Replacement therapy started at time of operation resulted in practically normal growth, whereas therapy begun later was ineffective.

Simpson (1924) thyroidectomized one each of 17 pairs of twin lambs and left mates as controls. When the operation was performed two or three weeks after birth, marked stunting resulted with the controls reaching a weight three times that of the operated animals. The experimental animals showed the usual short legs, short dished face, slipped wool, and "pot belly". If operation was delayed until the animal was three or four months of age, retardation of growth was only slight.

Similar symptoms of hypothyroidism have been recorded for a great number of animals, including the mouse (Davenport and Swingle, 1927), the guinea pig (Silberberg and Silberberg, 1940; Williams et al., 1941), the cat (Dott, 1923), the rabbit (Kunde, 1926; Basinger, 1916), the goat (Simpson, 1924; Reineke and Turner, 1941), the cow (Brody and Frankenbach, 1942), and the monkey (Fleischmann, Schumacker and Straus, 1943).

The above are only a few of the many papers that have appeared on the general effects of thyroidectomy. (For more complete reviews see Kojimi, 1917; Schneider, 1939; Hoskins, 1927; Hammett, 1923; Salmon, 1938; Kendall, 1929; Harington, 1933). Apparently the results of hypothyroidism are analogous in all mammals and in general are more severe the earlier in life the thyroid function is impaired. Hammett (1929) has expressed the belief that thyroidectomy is more severe in older animals, but he stands alone in this view.

The effect of hypothyroidism on the histophysiology of tissues has not been studied extensively. Simpson (1927) studied the skeletal muscle of thyroidectomized sheep and reported that the development of the cytoplasm had been arrested and that the cells

resembled those of much younger sheep. *In vitro* studies of Maeda (1927) indicated that thyroidectomy resulted in a lowered oxygen consumption of all tissue. Hammett (1926) expressed the belief that thyroid deficiency resulted in decreased size of the cells, but had no good evidence in support of his theory. The work of Von Haam and Cappel (1940) with heart tissue *in vitro* showed that the rate of cell division of heart tissue is accelerated by thyroid treatment.

Activity of the growth zones of the bones is clearly depressed by hypothyroidism. Dott (1923), concluded that epiphyseal activity in the thyroidectomized dog was reduced by 81 per cent as judged by radiograms of the ends of the long bones. He reported that there were no degenerative changes which took place and that the result was simply an arrest of cell activity. To all appearances the bones remained in much the same state as those of younger dogs. Boettiger and Osborn (1938) likewise found the epiphyseal picture of 70 day old dwarf mice to be much the same as 25 to 30 day old normals. Thyroid treatment restored the picture to normal. Todd, Wharton and Todd (1938) made an extensive study of skeletal maturation of sheep thyroidectomized at one to five months of age. They found deficient growth and modelling of the epiphysis and when ossification was as completed as would be possible in operated animals, the epiphysis was abnormal in character and was inadequate to cap the growing end of the shaft. The long bones were short due to decreased velocity of growth, and duration of growth was not extended to compensate for this deficiency. The authors stated that the skeletal proportions were similar to those of primitive wild sheep. Laqueur, Dingemans and Freud (1941) observed similar derangements in bones of young rats thyroidectomized at an early age. The detailed histological studies of Silberberg and Silberberg (1940) on guinea pigs and of Becks, Ray, Simpson and Evans (1942) on rats have shown that thyroidectomy results in virtually a cessation of proliferation of the epiphyseal cartilage. Thus the evidence is clear that hypothyroidism suppresses bone growth. It is of interest that König (1937) has reported that thyroid preparations have been used successfully in treatment of slow healing bone fractures.

Effects of Hypothyroidism on Metabolism and Utilization of Nutrients.—It is well known that oxygen uptake is low in hypothyroidism. Blood flow is reduced and anemia is usually present (Kendall, 1929; Abramson and Sidney, 1942).

Feed intake is low in hypothyroid animals including man (Kojimi, 1917; Kunde, 1926; Johnston and Maroney, 1939; Richter, 1933), and as would be expected, nitrogen retention is decreased below that of normal. The efficiency of utilization of nitrogen by hypothyroid animals is not established. Protein requirement is low in

hypothyroidism and nitrogen excretion is usually lower than in normal individuals (Kendall, 1929). Johnston and Maroney (1939), however, have reported a case of cretinism in which nitrogen excretion was further lowered by administration of a small amount of thyroid. Nitrogen retention was increased in others but was accounted for by increased nitrogen ingestion.

The effect on endogenous metabolism of nitrogen has not been studied extensively. Palladin and Savrow (1927) reported that thyroidectomy does not change the excretion of creatinine. Allison and Leonard (1941), working with thyroidectomized rats, observed a decrease in creatine excretion with no change in creatinine. Glaser (1942) thyroidectomized rats with like results and found further that the operated animals had a greater tolerance for injected creatine. Since the injected creatine could not be accounted for by changes in creatinine excretion or by storage in tissues they suggested that the thyroid exerts some unknown influence on creatine metabolism.

Kendall (1929), Harington (1933) and Lerman (1941) have reviewed the literature on the effects of hypothyroidism on carbohydrate metabolism which indicates that the results have been quite variable. There is a tendency for blood sugar to be low (Bodansky, 1942) and liver glycogen is frequently low. Increased sugar tolerance is usually associated with hypothyroidism, but Harington (1933) has suggested this may be due to decreased absorption from the gut. This suggestion is supported by the report of Althausen and Stockholm (1938) that dextrose absorption from the intestines is low after thyroidectomy. They have suggested that the rate of sugar absorption may be used as a criterion of thyroid function.

Fat metabolism is altered also in hypothyroidism. There is a tendency for fat to be stored in the tissues (Harington, 1933) and apparently the fat cannot be oxidized for energy as in normal animals (Kommerell, 1929). Blood lipids are high (Schmidt and Hughes, 1938; Lawson, Fleischmann and Block, 1941a, b; Entenman, Chaikoff and Reichert, 1942) and vitamin A is poorly utilized (Patek and Haid, 1941; Lerman, 1941). The ability to transform carotene into vitamin A is also impaired (Salter, 1940).

Andrews and Bullard (1940) reported rapid fattening and good gains of steers which were thyroidectomized. Zorn and Brüggermann (1939) reported like results following thyroidectomy of 30 to 40 kg. pigs, while older pigs gave no such response. They reported the fattening to occur at the expense of inefficient utilization of feed and probably represented a shift from muscle and skeletal growth to a less efficient fattening process.

The early work of Koeppen (1892) and of Aub and co-workers (1927) with toxic goiter led to the generally accepted belief that hypothyroidism was accompanied by a decreased calcium and phosphorus excretion as opposed to increased excretion in hyperthyroid-

ism (Hunter, 1930, 1934). More recent work, however, has changed this view. As early as 1897 Magnus-Levy noted a decrease in calcium excretion following treatment of myxedematous patients with thyroid. Continued treatment, however, led to increased excretion. Since no intake values were given, this increase may have been due to increased ingestion. Silvestri and Tossati (1907) also reported reduced excretion of calcium following administration of thyroid to patients with low metabolic rates. It is clear that total calcium retention is low in hypothyroidism and the available evidence indicates that utilization of available minerals is lower than normal. Thus, Breitbarth (1940) has reported that calcium excretion of thyroidectomized dogs was lowered by administration of small amounts of thyroid. At the same time calcium intake was increased.

Other Effects of Hypothyroidism.—In addition to subnormal growth, hypothyroidism causes a depression of other processes such as lactation (Graham, 1934a; Preheim, 1940), egg production (Winchester, 1939) and growth of hair (Chang, 1926). Thus, hypothyroidism appears to be unfavorable to all the "anabolic" processes.

Variability in Response to Thyroidectomy.—Although in general the effects of hypothyroidism have been rather uniform, the results of thyroidectomy of rats have been somewhat inconsistent in that some animals, which to all indications have been completely thyroidectomized, continue to grow at a rapid rate while others show severe hypothyroid symptoms. This inconsistency may be explained by the finding of Reinhardt (1942) that certain thyroidectomized rats, which at autopsy show no remnants of thyroid tissue, store radio-active iodine in the cervical region. This would indicate that areas of thyroid tissue too small to be recognized histologically may be present or that tissue other than that having typical thyroid structure may be capable of synthesizing active agents. A small amount of thyroxine is synthesized in thyroidectomized animals by direct combination of iodine with blood proteins (Morton, Chaikoff, Reinhardt and Anderson, 1943). This would not appear, however, to explain the great variability in response to thyroidectomy.

Effects of Hyperthyroidism. Severe Hyperthyroidism.—The effects of severe hyperthyroidism are well known and will be mentioned only briefly here. (For reviews see Schneider, 1939; Cameron and Carmichel, 1920). Thyroid in large amounts is toxic, resulting in loss of nitrogen, extreme emaciation and hyperirritability. Given in large enough quantities, the unusual finding of reduced oxygen consumption and feed intake may be encountered (Kojimi, 1917), followed quickly by death. Usually, however, food intake and gaseous metabolism are increased.

The early work of Koeppen (1892), Magnus-Levy (1897) and Aub and co-workers (1927) led to the generally accepted belief that hyper-

thyroidism was accompanied by loss of calcium leading to osteoporosis and decalcified bone. Some early reports on the effects of hyperthyroidism on experimental animals lent support to this assumption (Parhon, 1912). Parhon, however, cited examples in which this finding has not been reported. Hunter (1934) likewise stressed the point that the finding was not universal in clinical hyperthyroidism. More recent work with experimental animals indicates that uncomplicated hyperthyroidism does not lead to decalcification of bone. Calcium retention, however, is low or nil (Drill, 1941; Smith and McLean, 1938). Since excessive hyperthyroidism is toxic, the reactions of an organism to the conditions are nonspecific and a study of the severe hyperthyroid state actually contributes little to the understanding of normal thyroid physiology. Failure to reckon with this fact coupled with a lack of appreciation of the potency of thyroid materials, especially thyroxine, has led to a great mass of literature which is difficult to evaluate and in many cases is practically meaningless as relates to normal thyroid function.

Most of the experiments that have been reported have dealt with relatively large doses of thyroid material. Parhon (1912) early emphasized the point that dosage largely determines the effect to be expected and suggested that a small amount is "anabolic", whereas a larger amount is "katabolic". Still most workers failed to appreciate thyroid potency and in many cases worked with severe hyperthyroidism. Aub and co-workers (1927) actually criticized early work that had shown small amounts of thyroid to cause an increase in calcium retention (Silvestri and Tossati, 1907) because dosage was "too low."

There are several papers dealing with mild thyroid treatment, however, and these will be reviewed in more detail.

Evidence Indicating that Mild Hyperthyroidism May be Conducive to Rapid Growth. (1) CLINICAL OBSERVATIONS ON HUMANS.—Topper and Cohen (1928) fed thyroid to children who showed no gross signs of hypothyroidism. They observed no increase in metabolic rate but a spurt in growth occurred. Dorff (1935) administered small amounts of thyroid to growing children and obtained results similar to those of Topper and Cohen. To explain the response of children with apparently normal thyroid function, he postulated the condition of "masked hypothyroidism". Molitch and Poliakoff (1938) treated 43 boys of subnormal stature, but with no other signs of hypothyroidism, with thyroid alone or in combination with pituitary extract. One grain of thyroid per day resulted in an average growth of 1.25 inches in six months as compared to 0.75 inches for untreated boys. Variation within the groups cast doubt on the significance of the results, but they were suggestive. Wilkins (1940) has stated in a general discussion of thyroid medication of children that growth is more rapid when hypothyroid cases are made slightly hyperthyroid than when metabolism is merely raised to normal.

The above reports have all come from treating children with some evidence of retarded growth and no results have been reported with strictly normal children. Hertz and Galli-Mainini (1941) and Reilly (1942), however, have presented evidence that a cardinal symptom of hyperthyroidism of children is rapid skeletal growth during childhood and adolescence but that maximum stature is no greater than that of normal individuals. Body weight was not mentioned but would presumably be somewhat below normal due to thin condition. Rapid skeletal growth of dogs fed fresh thyroid has been observed by Moussu (1899).

Johnston and co-workers (Johnston and Maroney, 1939a, b; Johnston, 1941) have studied the effect of mild thyroid treatment on nitrogen and calcium retention of children. In certain cases they have observed greater retention of both elements when small amounts of thyroid were administered to children with normal metabolic rates. Calcium retention was influenced to a greater extent than nitrogen. They have stressed the point, however, that if the metabolic rate is raised much above normal, decreased retention, especially of nitrogen, will occur. Nitrogen excretion was usually increased by thyroid medication but was compensated for by increased food intake.

(2) GROWTH EXPERIMENTS WITH ANIMALS.—Moussu (1899) reasoned that if small amounts of thyroid were essential for normal growth, would not a little extra thyroid result in growth above normal? He used paired littermate dogs and cats and fed small amounts of fresh horse thyroid to one of each pair. He concluded that thyroid in small amounts always resulted in faster growth in young dogs. They became less fat, however, and never reached more than normal stature. He used height as an index of growth and stressed the finding of long limbs. He did not mention the cat in his results. Dott (1923) confirmed Moussu's report of rapid growth of dogs following thyroid medication. He reported that growth stopped prematurely and that the dogs never reached the maximum stature of the controls.

Bircher (1910) fed rats small amounts of thyroid and reported accelerated skeletal growth but body weight was slightly below normal. Schafer (1912) fed small amounts of thyroid to growing rats and observed increased rate of growth and feed intake of females. The males were apparently unaffected. Herring (1917) fed 0.1 to 0.2 grams of fresh sheep thyroid to female rats. On the average, growth rate was not greatly affected. Some animals grew rapidly and to a large size while others were retarded. Hoskins (1916) fed 10 mg. daily of dry thyroid to growing rats and from his observations stated that "the treated animals averaged slightly heavier than controls but the difference is perhaps too slight to be significant. If the loss in fat of the thyroid fed animals be taken into account, an increased weight of the remainder of the body appears." Dulzetto (1928) injected 20 to 25 day old animals with a thyroid extract (not described) and ob-

tained slight, but probably insignificant, gain above controls. Evans, Simpson and Pencharz (1939) injected small quantities of thyroxine into rats and showed growth curves of treated rats that were slightly above those of controls, but the differences were insignificant.

The effect of mild thyroid treatment on growth of mice was studied by Robertson (1928). In a series of trials he found that both males and females responded to feeding of 1.9 mg. fresh thyroid per day by increased rate of growth early in life. The animals reached the same maximum weight as controls but obtained this weight in a shorter period of time. Mean life duration was reduced by about 15 weeks or to about 85 per cent the life span of controls.

No reports have appeared which would indicate that thyroid treatment of guinea pigs or rabbits might lead to 'accelerated growth. Parhon (1912) and Stefanescu (1926) reported that "subtoxic" levels inhibited growth rate, but they still used amounts that markedly increased metabolism. Increased mitotic activity in the epiphyseal cartilages of both rabbits (Coryn, 1939) and guinea pigs (Silberberg and Silberberg, 1940) has been observed following thyroid treatment, but in these cases general body growth was not reported upon.

Since this investigation was started, evidence has been presented that the growth rate of chicks during early life can be increased to a limited extent by feeding small amounts of thyroid while feathering is markedly improved (Parker, 1943; Irwin, Reineke and Turner, 1943).

(3) EFFECT ON FEED INTAKE, AND ABSORPTION.—Zanasi (1934) first reported that mild thyroid treatment stimulated gastric motility in laboratory animals. He also found that several other hormones caused a similar response and attached little significance to this property of thyroid materials. Increased peristalsis of stomach and intestines has been reported also by Rossiiskii (1937), Althausen (1939), Morrison, Samuel and Feldman (1939, 1940) and Castleton and Alvarez (1941).

Eidinova (1936) fed small amounts of thyroid to dogs and by means of Pavlov and Heidenhain pouches determined that the flow of gastric juices was increased above normal and that the "digestive power" of the juices was also increased. Overdoses of thyroid inhibited gastric flow.

Althausen and Stockholm (1938) reported that administration of thyroxine resulted in increased absorption from the intestines of dextrose, xylose, galactose and oleic acid, but not of alanin. They suggested that the increased absorption was due to stimulation of phosphorylation. Fecal excretion of calcium was increased under the same conditions (Althausen, 1939) and was attributed to increased peristalsis. Other drugs which caused increased peristalsis also caused increased calcium excretion while morphine, which inhibits peristalsis, decreased calcium excretion. Glaser (1942) confirmed the work of

Althausen on carbohydrate absorption but observed injury of the liver due to the treatment.

(4) NUTRITIVE BALANCE STUDIES.—Most investigators have reported increased nitrogen excretion upon thyroid administration. This finding has been universal in treatment of myxedema patients and represents nitrogen lost in connection with diuresis. At the same time the fluids in the tissues are reduced and the urine nitrogen is thought to indicate a loss of "deposit protein" (Lerman, 1941; Harington, 1933). This increased excretion of nitrogen disappears upon continued treatment. Similarly, a smaller amount of nitrogen may be lost upon administration of thyroid to normal individuals. Rudinger (1908) stressed the point that increased nitrogen excretion did not occur in states of moderate hyperthyroidism provided sufficient carbohydrate is available to meet the energy requirements of the increased metabolism. Thus, Weymuller, Wyatt and Levine (1932) administered thyroid to well-fed infants and observed no increase in nitrogen excretion and in some cases excretion was actually decreased. Johnston and Maroney (1939) have shown that small amounts of thyroid administered to children lead to increased nitrogen retention so long as metabolism is not raised much above normal. The above reports are not evidence of beneficial effects of mild hyperthyroidism, but do emphasize the fact that ample thyroid activity is essential for optimal nitrogen retention.

Terroine and Babad (1939) reported increased retention of nitrogen by rats even though thyroxine was given in amounts to cause an actual loss in weight. Marx, Magy, Simpson and Evans (1942) have presented evidence that rats receiving "purified thyrotropic" preparations stored more nitrogen than controls but failed to note a similar retention after short periods of thyroxin injection. It is of interest that Zitowskaya (1939) found that livers of thyroid-treated dogs had the ability to synthesize amino acid from pyruvic acid and ammonia *in vitro* at a markedly faster rate than livers of control dogs.

There have been no reports of increased calcium and phosphorus balance due to thyroid treatment of normal humans or experimental animals. However, thyroid medication of hospital patients with apparently normal thyroid function has led to improved calcium balances (Silvestri and Tossati, 1907; Johnston, 1941). Again Johnston has emphasized the point of keeping the metabolic rate near to normal if increased balances are to be expected. The finding of increased skeletal growth of dogs, mice and children in states of mild hyperthyroidism would suggest that the condition is not unfavorable for calcium and phosphorus retention.

(5) OTHER INDICATIONS.—There are several theoretical considerations suggesting that an active thyroid state is associated with rapid growth: (a) The thyrotropic potency of the anterior pituitary is highest during the period of rapid growth of rats (Turner and Cupps,

1940), rabbits (Bergman and Turner, 1941), cattle (Reece and Turner, 1939) and swine (Elijah and Turner, 1942). (b) In rats, a species wherein the male grows more rapidly than the female, the thyrotropic potency of the anterior pituitary is higher in the male than in the female (Turner and Cupps, 1940), whereas the thyrotropic potency of the two sexes is similar in rabbits, a species in which the growth rate of the two sexes is similar (Bergman and Turner, 1941). (c) The pituitaries of slow-growing strains of swine have been reported to be lower in thyrotropic potency than those of faster growing strains (Elijah and Turner, 1942).

It has been well established that treatment of cows or goats with moderate amounts of thyroid will increase milk and fat production for short periods of time (Graham, 1934a, b; Ralston et al., 1940; Reineke and Turner, 1942). No long-term experiments have yet been reported.

Hair and feather growth have also been shown to be affected. Chang (1926) reported that undernourished rats showed a retardation of hair growth. Thyroid fed to such animals kept on a semi-starvation diet improved hair growth in spite of further decreased body weight due to treatment of the originally deficient animals.

Evidence that Mild Thyroid Treatment is Unfavorable to Organisms. The evidence in the preceding section does not suggest that mild thyroid treatment is always favorable to growth but it does emphasize that the reactions of organisms to mild hyperthyroidism have not been appreciated by most investigators. Such factors as genetic constitution, environmental conditions and species differences undoubtedly affect the response to treatment and variation in response of individual animals is to be expected. The reports mentioned suggest that the problem is deserving of further investigation. It appears possible that some species or strains of animals may show a uniformly favorable response to mild thyroid treatment.

In contradiction to the positive evidence of the preceding section, there are a host of reports which have shown "subtoxic" levels of thyroid treatment to be uniformly inhibitory to growth. These publications have been reviewed fully elsewhere (Hoskins, 1916; Cameron and Carmichel, 1920; Kojimi, 1917; Schneider, 1939).

Critical evaluation of these reports, however, indicates that in nearly all cases, even though "subtoxic" levels were used, metabolism was increased to the extent that a state of relatively severe hyperthyroidism was produced. Cameron and Carmichel (1920) seem to be the only investigators to have studied the effects of carefully graded amounts of thyroid material. Working with rats they gave as low as 1 mg. of dried thyroid per 20 grams of body weight. Only a very few animals were given the lower dosages and in this case growth was not appreciably affected. Larger amounts, of course, inhibited growth. Parhon (1912), working with rabbits and Stef-

anescu (1926) with guinea pigs, fed graded amounts of thyroid, but their lowest dosages were in the toxic range.

The work of Herring (1917), Hoskins (1916), Dulzetto (1928), Da Costa and Carlson (1933), Evans, Simpson and Pencharz (1939) and Korenchevsky, Hall and Claphan (1943) with mild treatment of rats, while not showing small amounts of thyroid to be detrimental to animals, failed to show any marked or consistent growth above that of controls.

Variability in Response to Thyroid Treatment.—One common observation of great interest is the variability in tolerance of animals of the same species to thyroid material with environmental and feed factors being constant. Peizer (1906) fed heavy doses of thyroid to rats and observed that in groups where the dosage was usually lethal, some individuals survived the treatment and showed a steady gain in weight. Hoskins (1927) found the response within litters to be fairly uniform but a marked difference occurred between different litters. Work done in the Department of Dairy Husbandry, University of Missouri, bears out these observations on mice, rats, guinea pigs, rabbits and chickens. At present there are no indications as to the cause of this variation in thyroid tolerance. It does offer a possible explanation of the contradictory results that have been reported in some instances. It suggests also that there may be a similar variation in response to milder treatments with thyroid materials.

Thyroid Activity and Vitamin Requirements.—The literature has been reviewed recently citing numerous reports of an "antagonism" between thyroid hormone and vitamins A, C and the B complex (Korenchevsky, Hall and Clapham, 1943). This supposed "antagonism" has been assumed to explain the finding that hyperthyroidism is relieved to a limited extent by vitamin therapy and that thyroid treatment exaggerates vitamin deficiencies. Korenchevsky et al. (1943) interpreted these findings, logically it seems, to indicate that vitamin requirements are increased by thyroid treatment due to increased metabolism. This suggestion is in keeping with the well-established fact that the vitamin B₁ requirement is proportional to the rate of metabolism of the tissues (Drill, 1938).

Korenchevsky, Hall and Clapham (1943) fed rats on a normal ration and found that mild thyroid treatment did not greatly affect the animals while larger doses of thyroid were toxic. Fortifying the ration with cod liver oil, crystalline vitamins C and B and yeast extract relieved the toxic effects. With a vitamin-deficient ration which barely supported control animals, mild thyroid treatment was toxic. Addition of crystalline vitamins relieved the toxic symptoms completely.

Thus, it seems well established that thyroid treatment increases the requirements of most vitamins. It would appear logical to assume that many of the contradictory reports on the effects of small

amounts of thyroid can be explained on the basis of failure to recognize this fact.

Experimental

Four different species of laboratory animals, including mice, rats, guinea pigs and rabbits, were used to determine the effect of mild thyroid treatment on the rate of growth of young animals. Both males and females of the four species were used to determine any sex differences in response.

The general procedure was to treat first a few animals of each species with a wide range in dosage in order to gain some indication as to the tolerance of thyroid material. Most of these exploratory tests have been omitted from the data presented. From the information gained in these preliminary tests, a series of dosages were calculated which at the highest level would be inhibitory to growth. This amount was decreased stepwise, usually by one-half, until a level was reached which apparently did not affect the animals or else to the point where it seemed impractical to reduce the dosage further. If any level appeared to increase rate of growth, the dosage was repeated to obtain sufficient numbers to test significance.

Four different thyroidally active preparations were used in the course of these experiments:

1. Synthetically prepared thyroxine-sodium (British Drug House). This material was used only with mice and was given as a subcutaneous injection of 0.1 cc. of solution at the isoelectric point. The dry powder was dissolved in dilute NaOH, brought to its isoelectric point by addition of HCl, and then made up to volume. The suspension was kept stored in a refrigerator and a new stock was prepared once every 30 days.

The other materials used were three lots of thyroactive iodocasein prepared by the method described by Reineke and Turner (1942). These preparations will be referred to hereafter as thyroactive proteins, or simply as thyroprotein.

2. Thyroactive protein lot I was a highly potent preparation containing 7.14 per cent iodine and having 10.3 per cent the potency of synthetic thyroxine-sodium on a weight basis when injected into tadpoles, or 4.0 per cent the activity of thyroxine-sodium when assayed by oral administration to guinea pigs. This material was used in Experiment 9 with mice and was injected at its isoelectric point.

3. Thyroactive protein lot II was a less potent lot containing 1.8 per cent the activity of thyroxine-sodium when injected into tadpoles. It was used only in Experiment 7 with mice, the material being finely ground and incorporated into the ration.

4. Thyroactive protein lot III contained 7.24 per cent iodine and possessed 9.3 per cent the potency of thyroxine-sodium as assayed by tadpoles, or about 3.0 per cent the activity of thyroxine when given

orally to guinea pigs. This batch of material was used in Experiment 9 with mice and exclusively in all experiments with rats, guinea pigs and rabbits.

Experiments with Mice. Procedure.—The animals used in the mice experiments were all purchased from one commercial breeder and were reported to be descended from a single pair of mice. It is claimed that the strain has been maintained without introduction of outside breeding stock for 37 years. The mice weighed 10 to 14 grams when received and were allowed a few days of recovery from shipment before being placed on experiment. Individual records were kept on all animals and weights were taken one or more times weekly.

The mice were fed a stock diet used in this laboratory supplemented with a commercial dog feed. This stock ration was made up of:

Ground corn	200 parts
Ground oats	150 parts
Wheat shorts	220 parts
Linseed meal	150 parts
Cottonseed meal	50 parts
Soybean meal	50 parts
Skim milk powder	150 parts
Steamed bone meal	20 parts
Salt	10 parts

In Experiments 0, 1, 2, 3, 5 and 6 (Table 1), the stock ration was fed in jars and the dog feed in pellet form. In Experiments 4, 7, 8 and 9, the pellets were ground and added to the stock feed to the amount of 50 per cent of the ration. All feed was given *ad libitum*. The ration has been successful in maintaining good reproduction in mice and rats in this laboratory and does not appear to be deficient in nutritional factors. Records of feed intake by groups were kept in Experiments 4 and 7.

The animals were kept in elevated wire bottom cages and no more than 12 mice were kept in one cage. They were housed in basement laboratories where the temperature was usually maintained between 78° and 85° F.

Ten different trials were conducted involving a total of 493 mice (Table 1). In the first seven experiments, synthetic thyroxine-sodium was used as the test substance and injected subcutaneously in amounts ranging from 0.007 mg. to 0.04 mg. daily, or twice those amounts on alternate days. In the remaining three experiments, three batches of thyroactive iodocasein were used as the thyroidally active agent. Lot I was injected at its isoelectric point while lots II and III were finely ground and mixed with the feed. Male animals were used in Experiment 6 and females in all others.

TABLE 1. GENERAL INFORMATION CONCERNING EXPERIMENTS WITH MICE

Exper. No.	Treatment	Date		No. of Animals	
		Started	Ended	At Start	At End
0	Control females	7/14/41	8/2/41	12	10
	0.01 mg. thyroxine injected daily	7/14/41	8/2/41	12	3
1	Control females	8/5/41	9/9/41	12	9
	0.04 mg. thyroxine injected daily	8/5/41	9/9/41	12	11
	0.02 mg. thyroxine injected daily	8/5/41	9/9/41	12	11
	0.009 mg. thyroxine injected daily	8/5/41	9/9/41	12	11
	0.007 mg. thyroxine injected daily	8/5/41	9/9/41	12	12
2	Control females	9/25/41	1/9/42	30	29
	0.015 mg. thyroxine injected daily	9/25/41	1/9/42	30	28
3	Control females	12/1/41	3/3/42	15	13
	0.03 to 0.06 mg. (1) thyroxine injected alternate days	12/1/41	3/3/42	15	15
4	Control females	1/19/42	2/25/42	10	10
	0.03 to 0.06 mg. (1) thyroxine injected alternate days	1/19/42	2/25/42	10	8
5	Control females	2/6/42	3/13/42	10	9
	0.03 to 0.06 mg. (1) thyroxine injected alternate days	2/6/42	3/13/42	10	9
6	Control males	2/6/42	3/30/42	20	16
	0.06 mg. thyroxine injected alternate days	2/6/42	3/30/42	20	14
7	Control females	2/28/42	4/4/42	11	11
	0.15% lot II thyroprotein in ration	2/28/42	4/4/42	11	11
	0.30% lot II thyroprotein in ration	2/28/42	4/4/42	11	8
	0.60% lot II thyroprotein in ration	2/28/42	4/4/42	11	8
8	Control females	4/3/42	5/8/42	10	7
	0.548 mg. lot I thyroprotein injected alternate days	4/3/42	5/8/42	10	7
	1.096 mg. lot I thyroprotein injected alternate days	4/3/42	5/8/42	10	3
9	Control females	9/10/42	10/15/42	30	22
	0.02% lot III thyroprotein in ration	9/10/42	10/15/42	30	22
	0.04% lot III thyroprotein in ration	9/10/42	10/15/42	30	24
	0.08% lot III thyroprotein in ration	9/10/42	10/15/42	30	22
	0.16% lot III thyroprotein in ration	9/10/42	10/15/42	30	20
	0.32% lot III thyroprotein in ration	9/10/42	10/15/42	15	11

(1) Dosage was increased on 10th day of treatment.

Observations on Growth.—The data obtained on growth are summarized in Table 2 and Figs. 1 and 2. Preliminary trials showed thyroxine in amounts larger than 0.06 mg. daily to be toxic, resulting in a depressed rate of growth and high mortality (Experiment 0). When the dosage was reduced to 0.007 mg. thyroxine daily, growth was not appreciably affected. Dosages ranging from 0.01 to 0.04 mg. daily, however, resulted in a marked increase in growth above that of controls for a period of approximately five weeks (Fig. 1, Experiment 1). Repeated trials with the dosage maintained from 0.015 to 0.03 mg. daily (Experiments 2, 3, 4, 5 and 6) showed a consistent difference in gain in favor of the treated animals. The difference in gain over a five-week period amounted to approximately 28 per cent and was found to be statistically highly significant (Table 3). Male mice responded in a manner similar to females (Experiment 6).

TABLE 2. AVERAGE WEIGHTS OF MICE AT INTERVALS WHILE ON EXPERIMENT

Exper. No.	Treatment		Av. Wt. of Group (gms.)						
0	Control females	7/14 ⁽¹⁾ 15.1 ± 1.01	7/21	7/28	8/2				
	0.01 mg. thyroxine injected daily	15.6 ± 0.89	17.6	18.1	20.1	---	---	---	---
1	Control females	8/5 ⁽¹⁾ 15.9 ± 1.03 ⁽³⁾	8/12	8/19	8/26	9/2	9/9		
	0.04 mg. thyroxine injected daily	16.5 ± 1.24	18.0	18.7	18.9	20.4	20.9 ± 2.14 ⁽³⁾	---	---
	0.02 mg. thyroxine injected daily	17.4 ± 0.75	20.1	20.6	19.6	22.9	24.9 ± 1.58	---	---
	0.009 mg. thyroxine injected daily	17.3 ± 1.27	19.6	21.2	20.7	22.6	24.6 ± 1.10	---	---
	0.007 mg. thyroxine injected daily	16.7 ± 1.18	19.3	20.8	20.9	21.5	22.4 ± 1.87	---	---
	Control females	9/25 ⁽¹⁾ 15.7 ± 1.36	10/4	10/10	10/18	10/24	10/31	1/9	
0.03 mg. thyroxine alternate days	14.9 ± 1.30	17.8	19.0	19.6	20.4	21.4 ± 1.89	23.0	23.1	
3	Control females	12/1 ⁽¹⁾ 15.0 ± 1.18	12/8	12/15	12/22	12/29	1/5	3/3	
	0.03 to 0.06 mg. thyroxine alternate days	14.5 ± 1.54	16.9	17.8	18.6	19.1	20.3 ± 2.41	22.5	23.5
4	Control females	1/19 ⁽¹⁾ 14.2 ± 1.07	1/26	2/3	2/9	2/16	2/25	---	---
	0.03 to 0.06 mg. thyroxine alternate days	13.9 ± 0.91	15.2	16.1	16.9	17.7	17.8 ± 2.13	---	---
5	Control females	2/6 ⁽¹⁾ 14.1 ± 1.34	2/17	2/21	3/3	3/9	3/13	---	---
	0.03 to 0.06 mg. thyroxine alternate days	13.7 ± 0.70	16.9	17.1	18.9	19.6	19.9 ± 1.81	---	---
6	Control males	2/6 ⁽¹⁾ 15.5 ± 1.00	2/17	2/21	3/3	3/9	3/13	---	---
	0.06 mg. thyroxine alternate days	15.5 ± 0.82	18.2	19.5	21.0	21.5	22.1 ± 1.71	---	---
7	Control females	2/28 ⁽¹⁾ 12.1 ± 0.75	3/3	3/14	3/21	3/30	4/4	---	---
	0.15% lot II thyroprotein in ration	11.8 ± 0.81	13.2	17.2	18.1	19.3	19.7 ± 1.86	---	---
	0.30% lot II thyroprotein in ration	12.2 ± 0.63	12.9	16.3	17.7	19.0	19.1 ± 1.93	---	---
	0.60% lot II thyroprotein in ration	12.3 ± 0.76	13.5	18.3	19.5	19.8	20.8 ± 2.08	---	---
	Control females	4/3 ⁽¹⁾ 13.4 ± 1.03	4/11	4/17	4/22	5/2	5/8	---	---
0.548 mg. lot I thyroprotein injected alternate days	13.6 ± 1.23	15.4	16.9	18.2	19.4	19.8 ± 1.72	---	---	
1.096 mg. lot I thyroprotein injected alternate days	13.6 ± 0.79	15.6	17.8	20.2	21.1	21.5 ± 2.08	---	---	
9	Control females	9/10 ⁽¹⁾ 13.1 ± 1.00	9/16	9/23	10/3	10/10	10/15	---	---
	0.02% lot III thyroprotein in ration	13.0 ± 1.16	14.4	15.9	17.9	18.2	19.5 ± 2.16	---	---
	0.04% lot III thyroprotein in ration	12.9 ± 1.27	13.9	16.0	17.7	18.0	19.3 ± 1.73	---	---
	0.08% lot III thyroprotein in ration	12.6 ± 0.86	13.9	16.7	19.5	19.7	20.4 ± 1.81	---	---
	0.16% lot III thyroprotein in ration	13.5 ± 1.13	14.1	16.0	19.4	19.5	20.0 ± 2.30	---	---
	0.32% lot III thyroprotein in ration	13.9 ± 1.38	14.3	16.7	19.0	19.9	21.1 ± 2.84	---	---
			15.1	17.7	20.4	20.7	21.8 ± 2.09	---	---

(1) Date weights were taken.

(2) Dosage was increased on 10th day of treatment.

(3) All such figures are standard deviations.

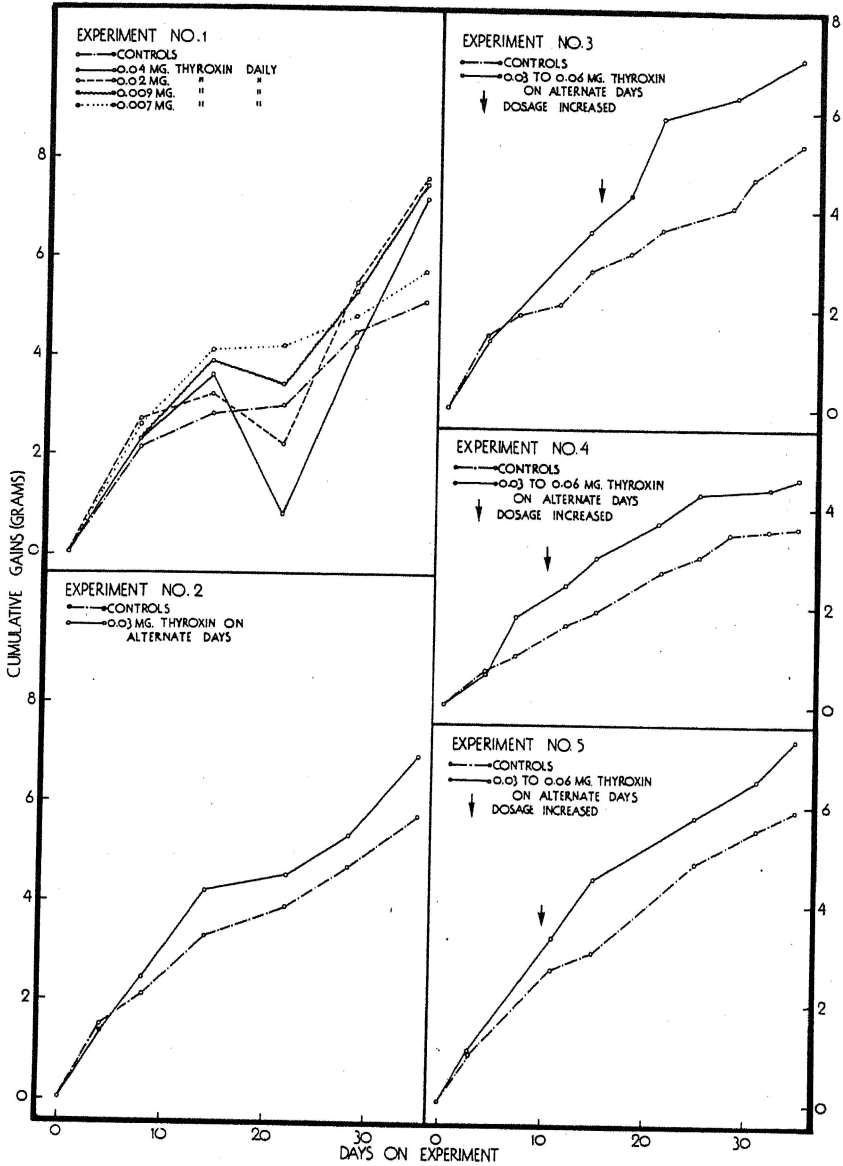


Fig. 1.—Growth curves of mice.

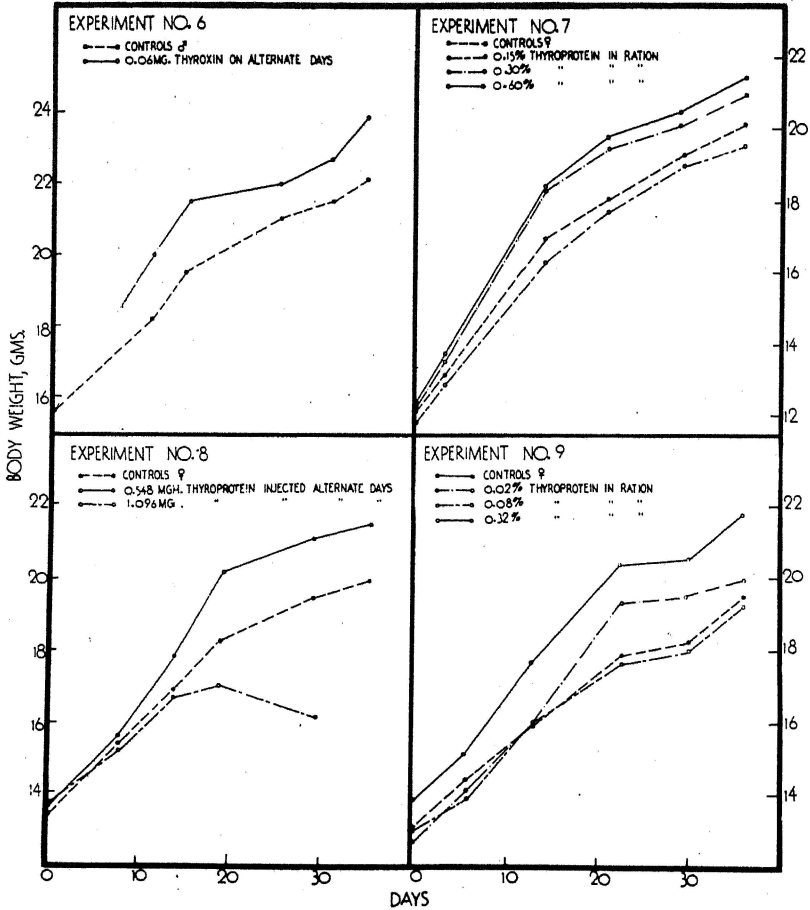


Fig. 2.—Growth curves of mice.

Animals treated for longer than five weeks continued to be heavier than the controls for a period of several weeks, but the difference between control and treated animals gradually became less after the animals reached an average weight of about 20 grams. By the time the treated animals reached a weight of 23 grams, the controls had overtaken them and the maximum size attained was the same in the two groups (Table 2, Experiment 2).

Feeding or injecting thyroactive iodocasein gave results comparable to injection of thyroxine (Experiments 7, 8 and 9). When dosage was reduced to a low level, growth was not appreciably affected although there was a tendency for animals on low dosage to be slightly

TABLE 3.—SUMMARY OF GAIN IN BODY WEIGHT MADE BY CONTROL AND TREATED MICE DURING A FIVE-WEEK PERIOD.¹

	Controls	Treated Animals	Difference in Gain	P Value
Thyroxine treated females				
Gain in weight (gm.)	5.4	6.8	1.8	1
Standard deviation (gm.)	1.94	1.97		
No. of animals	70	105		
Thyroxine treated males				
Gain in weight (gm.)	6.6	8.4	1.8	5
Standard deviation (gm.)	1.95	2.06		
No. of animals	16	14		
Thyroprotein treated females				
Gain in weight (gm.)	6.8	7.8	1.0	1
Standard deviation (gm.)	2.05	2.02		
No. of animals	40	100		

¹Mice in Experiment 0, those receiving 0.15% thyroprotein in Experiment 7, 1.096 mg. thyroprotein in Experiment 8, and 0.02% in Experiment 9, were excluded because the dosages were out of range of response.

lighter than controls (0.15% in Experiment 7, 0.02% in Experiment 9). Heavy dosage resulted in depressed rate of growth and high mortality (1.096 mg. injected in Experiment 8). Amounts between these extremes resulted in a significant increase in gain during a five-week period (Table 3). Skeletal growth, as measured by length of mice, was likewise significantly increased above that of controls (Table 4).

TABLE 4.—SUMMARY OF GAIN IN BODY LENGTH MADE BY CONTROL AND THYROACTIVE PROTEIN-FED MICE DURING A FIVE-WEEK PERIOD.¹

	Controls	Treated Animals	Difference in Gain	P Value
Gain in length (mm.)	19.0	23.0	4.0	1
Standard deviation (mm.)	4.0	4.1		
No. of animals	40	100		

¹Animals receiving 1.096 mg. of thyroprotein in Experiment 8 and those receiving 0.02% in Experiment 9 were excluded because dosages were out of range of response.

Thus, our observations confirm the report of Robertson (1928) that mild thyroid treatment increases the rate of growth of mice early in life but that the maximum size attained is not increased. Robertson reported a decrease of 15 per cent in mean duration of life, but the short time experiments used in these trials showed no appreciable difference in mortality (Table 1).

The question naturally arises whether the average increase in gain of the treated animals could be attributed to stimulation of slow-growing mice, to a general increase in gain of all animals, or to some change in the distribution of gains. Tabulation of the gains made by the treated and control animals showed that the minimum and maximum gains made by the two groups were similar. Extremely small gains were somewhat more frequent in the control animals, however, and the modal gain of the treated animals was

approximately 1 gram heavier than the modal gain of the controls (Fig. 3).

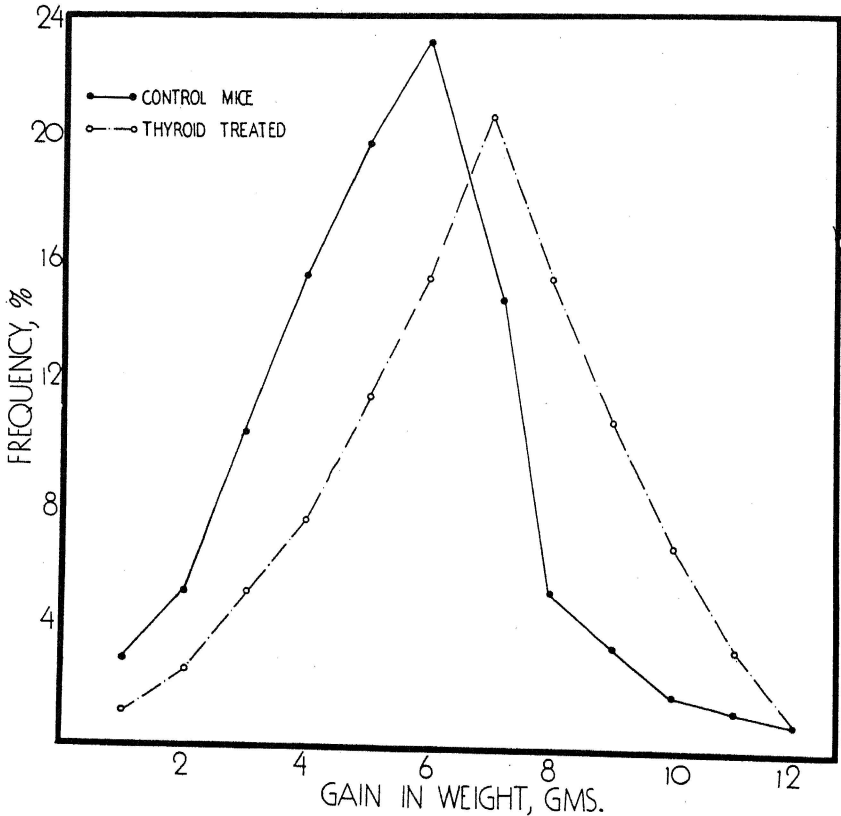


Fig. 3.—Frequency polygons of gains made by control and treated mice.

Effect of Thyroid Treatment on Composition of Carcass.—The composition of whole ground carcasses (with feed content of gut removed) of three different groups of control and treated mice is shown in Table 5. These data have been published in more detail previously (Koger, Hurst and Turner, 1943) and will be summarized here. During the period of accelerated growth of the treated animals the average protein and moisture content of the tissues, on a percentage basis, was increased above that of the controls, whereas the fat and energy content was lower than normal. These differences became less with continued treatment.

One observation of considerable interest is that while the absolute amount of protein and fat contained in the carcasses was different in

TABLE 5. COMPOSITION OF TISSUES OF CONTROL AND TREATED MICE

Treatment	Carcass wt. gm.	Dry Matter %	Wet basis				Calories per 100 gm.
			N. %	Prot. %	Fat %	Ash %	
Controls	15.7	36.3	3.14	19.6	11.0	3.9	224
Thyroxine for 27 days	16.9	34.3	3.27	20.5	9.0	3.5	206
Controls	17.2	37.5	2.86	17.9	11.8	3.4	232
Thyroxine for 43 days	18.0	34.3	2.95	18.4	10.7	3.4	218
Controls	21.0	36.7	2.95	18.4	9.8	3.4	214
Thyroxine for 93 days	22.0	35.4	2.96	18.7	9.4	3.7	206

control and treated mice, the average total energy content of the two groups was found to be almost identical in three different observations (Table 6).

TABLE 6. ABSOLUTE AMOUNTS OF DRY MATTER, PROTEIN, FAT AND ENERGY CONTAINED IN THE CARCASSES OF CONTROL AND TREATED MICE

Treatment	Dry Matter gm.	Protein gm.	Fat gm.	Total Calories
Controls	5.70	3.06	1.73	35.2
Thyroxine for 27 days	5.80	3.46	1.51	34.9
Controls	6.44	3.08	2.10	39.9
Thyroxine for 43 days	6.17	3.31	2.03	39.3
Controls	7.49	3.87	2.05	44.8
Thyroxine for 93 days	7.58	4.10	2.07	45.4

Feed Intake and Utilization.—Feed intake was positively correlated with rate of gain. Dosages of thyroid material which increased the rate of growth caused a corresponding increase in feed intake, whereas a small dosage that resulted in gains slightly lower than normal caused a decrease in feed intake (Table 7). Dosages that accelerated growth rate caused an increase in feed intake from 20 to 30 per cent above that of controls.

TABLE 7.—AVERAGE DAILY FEED INTAKE AND DAILY GAINS MADE BY CONTROL AND TREATED MICE.

Exper. No.	Treatment	Weight Gain	Feed Intake
4	Controls	gm. .10	gm. 3.6
	0.03 mg. thyroxine daily	.13	4.6
7	Controls	.22	3.3
	0.15% thyroprotein in ration	.21	3.0
	0.30% thyroprotein in ration	.25	3.8
	0.60% thyroprotein in ration	.26	4.2

The quantity of protein, fat and energy stored per unit of feed intake was calculated for two different lengths of treatment (Table 8). During the period in which the growth rate of the treated animals was more rapid than the controls, the treated animals stored more protein and gained more in body weight per unit of feed intake than the untreated controls. During this same time the controls stored more fat and total energy per unit of feed intake. Since continued thyroid treatment maintained an increased feed intake and growth was stimulated for only a limited period of time, the efficiency of the treated animals decreased rapidly, and following the period of accelerated growth the treated animals stored less of all nutrients per unit of feed intake than the controls.

TABLE 8.—GAIN IN BODY WEIGHT AND AMOUNTS OF NUTRIENTS STORED PER 100 GRAMS FEED INTAKE BY CONTROL AND TREATED MICE.

	Body Weight	Protein	Fat	Calories
	gm.	gm.	gm.	
Controls	8.8	0.75	0.66	10.9
Thyroxine for 27 days	8.6	0.90	0.21	8.1
Controls	2.7	0.44	0.59	9.3
Thyroxine for 43 days	2.7	0.48	0.49	7.1

Experiments with Rats.—The general information concerning the experiments with rats is shown in Tables 9 and 10. The thyroid preparation used was lot III thyroactive iodocasein and was ordinarily

TABLE 9. GENERAL INFORMATION CONCERNING EXPERIMENTS WITH MALE RATS

Exper. No.	Treatment	Date Started	Date Ended	No. of Animals		Per Cent Survival
				At Start	At End	
1	Controls, Missouri strain	9/9/42	10/17/42	12	11	92
	0.01% thyroprotein (1) in ration	9/9/42	10/17/42	12	12	100
	0.02% thyroprotein in ration	9/9/42	10/17/42	12	11	92
	0.04% thyroprotein in ration	9/9/42	10/17/42	12	10	83
	0.08% thyroprotein in ration	9/9/42	10/17/42	12	10	83
	0.16% thyroprotein in ration	9/9/42	10/17/42	12	11	92
	0.32% thyroprotein in ration	9/9/42	10/17/42	12	11	92
2	Controls, Missouri strain	10/10/42	1/29/43	18	16	89
	0.01% thyroprotein in ration	10/10/42	1/29/43	18	14	78
	0.02% thyroprotein in ration	10/10/42	1/29/43	18	15	83
	0.04% thyroprotein in ration	10/10/42	1/29/43	18	14	78
	0.08% thyroprotein in ration	10/10/42	1/29/43	18	15	83
3	Controls, Missouri strain	11/20/42	3/11/43	20	20	100
	0.005% thyroprotein in ration	11/20/42	3/11/43	20	20	100
4	Controls, Missouri strain	12/1/42	3/21/42	15	14	93
	0.0075% thyroprotein in ration	12/1/42	3/21/42	15	13	87
	0.0100% thyroprotein in ration	12/1/42	3/21/42	15	12	
5	Controls, Sprague-Dawley strain	12/20/42	4/13/43	8	7	88
	0.005% thyroprotein in ration	12/20/42	4/13/43	8	5	63
	0.010% thyroprotein in ration	12/20/42	4/13/43	8	8	100
	0.020% thyroprotein in ration	12/20/42	4/13/43	8	7	88
	0.040% thyroprotein in ration	12/20/42	4/13/43	8	5	63
	4.000 mg. thyroprotein injected daily	12/20/42	4/13/43	8	7	88

(1) Thyroactive Protein lot III

TABLE 10. AVERAGE WEIGHTS OF MALE RATS AT INTERVALS WHILE ON EXPERIMENT

Exper. No.	Treatment	Average weight of group (gms.)									
1	Controls, Missouri strain	9/9	9/17	9/24	10/1	10/10	10/17	---	---	---	---
	0.01% thyroprotein in ration	174 ± 13 ⁽¹⁾	177	189	207	221	230 ± 33	---	---	---	---
	0.02% thyroprotein in ration	180 ± 15	177	193	209	215	228 ± 13	---	---	---	---
	0.04% thyroprotein in ration	181 ± 19	177	182	203	216	223 ± 35	---	---	---	---
	0.08% thyroprotein in ration	174 ± 12	165	181	201	201	220 ± 18	---	---	---	---
	0.16% thyroprotein in ration	174 ± 20	160	175	186	198	195 ± 31	---	---	---	---
	0.32% thyroprotein in ration	183 ± 14	172	183	189	192	191 ± 27	---	---	---	---
		166 ± 16	137	144	152	153	156 ± 25	---	---	---	---
2	Controls, Missouri strain	10/10	10/27	11/1	11/12	11/22	12/1	12/19	1/5	1/17	1/29
	0.01% thyroprotein in ration	83 ± 8	123	155	190	215	233 ± 14	249	257	265	268 ± 24 ⁽¹⁾
	0.02% thyroprotein in ration	73 ± 14	102	123	152	177	201 ± 15	232	236	240	241 ± 27
	0.04% thyroprotein in ration	76 ± 11	109	131	159	179	199 ± 23	219	234	245	260 ± 38
	0.08% thyroprotein in ration	68 ± 14	95	113	141	172	187 ± 16	204	216	215	219 ± 20
		68 ± 12	75	89	109	127	151 ± 19	180	182	180	185 ± 27
3	Controls, Missouri strain	11/20	11/28	12/11	1/1	1/9	1/21	1/30	2/16	2/25	3/11
	0.005% thyroprotein in ration	67 ± 12	101	148	213	233	249 ± 26	262	278	282	289 ± 32
		71 ± 10	105	158	218	231	255 ± 24	268	283	282	291 ± 26
4	Controls, Missouri strain	12/1	12/7	12/22	1/5	1/20	2/1	2/10	---	3/11	3/21
	0.0075% thyroprotein in ration	77 ± 10	107	160	217	229	248 ± 20	255	---	270	276 ± 28
	0.0100% thyroprotein in ration	75 ± 8	108	157	213	230	247 ± 15	257	---	265	267 ± 14
		78 ± 9	107	155	209	220	231 ± 26	242	---	245	244 ± 37
5	Controls, Sprague-Dawley strain	12/20	1/2	1/9	1/16	1/23	2/9	2/16	2/25	3/11	4/13
	0.005% thyroprotein in ration	35 ± 3	94	130	168	199	251 ± 17	269	282	290	304 ± 40
	0.010% thyroprotein in ration	37 ± 2	99	139	175	213	257 ± 8	260	269	275	283 ± 17
	0.020% thyroprotein in ration	36 ± 4	100	139	168	194	220 ± 28	241	260	275	289 ± 35
	0.040% thyroprotein in ration	35 ± 3	99	138	172	204	248 ± 20	257	263	277	286 ± 9
	4.0 mg. thyroprotein injected daily ⁽²⁾	36 ± 3	95	131	152	179	220 ± 21	228	235	242	254 ± 30
		36 ± 2	98	125	156	189	185 ± 32	205	227	232	266 ± 40

(1) All such figures are standard deviations.

(2) Injection stopped 3/10/42.

fed incorporated into the ration. The material was injected into one group each in Experiment 5 with males and Experiments 9 and 10 with females in order to check its effectiveness by injection. In this case it was given as a subcutaneous injection at its isoelectric point.

The ration used was identical with that described in the section devoted to mice and consisted of one-half a standard ration used in this laboratory and one-half ground commercial dog pellets, fortified with cod liver oil.

The animals used came from two different sources. The rats used in Experiments 1 to 5 with males and Experiments 1 to 8 with females were bred at this Station and were of a strain maintained in the Department of Agricultural Chemistry, University of Missouri. They will be referred to hereafter as the Missouri strain. The remaining animals were of the Sprague-Dawley strain purchased from that firm at weaning age.

The rats were housed in well-ventilated basement laboratories (with one exception which will be explained later) where the temperature usually remained between 78° and 85° F. They were caged in elevated wire bottom cages in groups of three to five rats per cage. Individual records were kept on all animals and weights were taken usually at weekly intervals. To simplify presentation, many of the weekly weighings will be omitted.

Both males and females were used and since the response of the two sexes was different, the work with each will be described separately.

Experiments with Males.—Five separate experiments involving 307 animals were conducted with male rats (Tables 9 and 10, Figs. 4 and 5). Experiment 1 was exploratory in nature, and almost mature rats were treated with a wide range of dosages to determine the tolerance to the thyroid material. It was evident that amounts larger than 0.08 per cent in the ration were toxic (Table 10), with no significant changes appearing with smaller amounts.

In Experiments 2, 3 and 4, young rats weighing 65 to 85 grams were used and dosages ranged from 0.005 per cent to 0.08 per cent of the ration. At the levels of 0.005 and 0.0075 per cent, body weight was not affected (Figs. 4 and 5) but amounts in excess of 0.0075 per cent caused a progressive depression of growth rate. Skeletal development, as reflected in nose-anus length, was also depressed but not to as great an extent as body weight (Table 22).

Young rats of the Sprague-Dawley strain weighing from 30 to 35 grams were placed on 0.005, 0.01, 0.02 and 0.04 per cent thyroprotein in the ration (Experiment 5). In this instance the responses were somewhat different from those observed in the Missouri strain. Practically no effect was observable until the animals reached a weight of approximately 150 grams. At this point the growth curves separated rapidly (Fig. 5) and the controls continued to grow more rapidly than animals on any treatment. Feeding at the level of 0.005 per

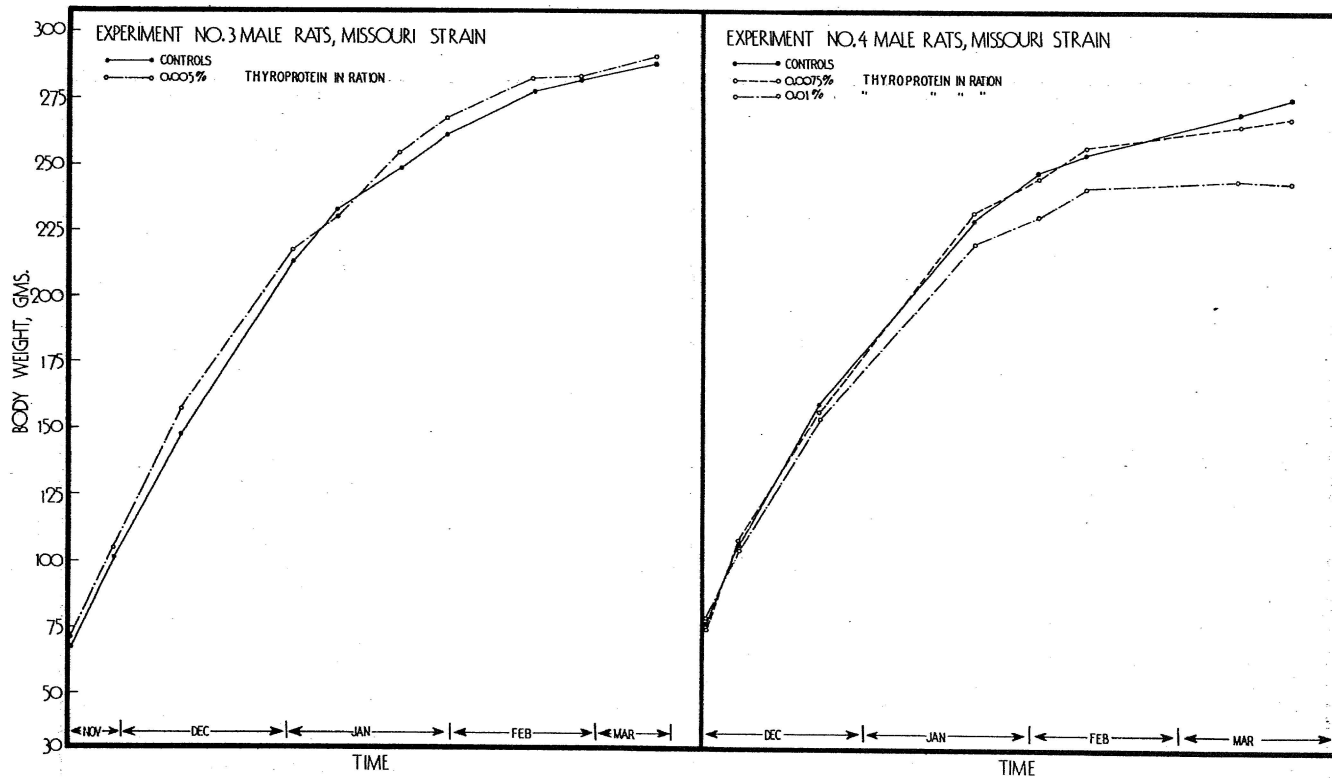


Fig. 4.—Growth curves of male rats.

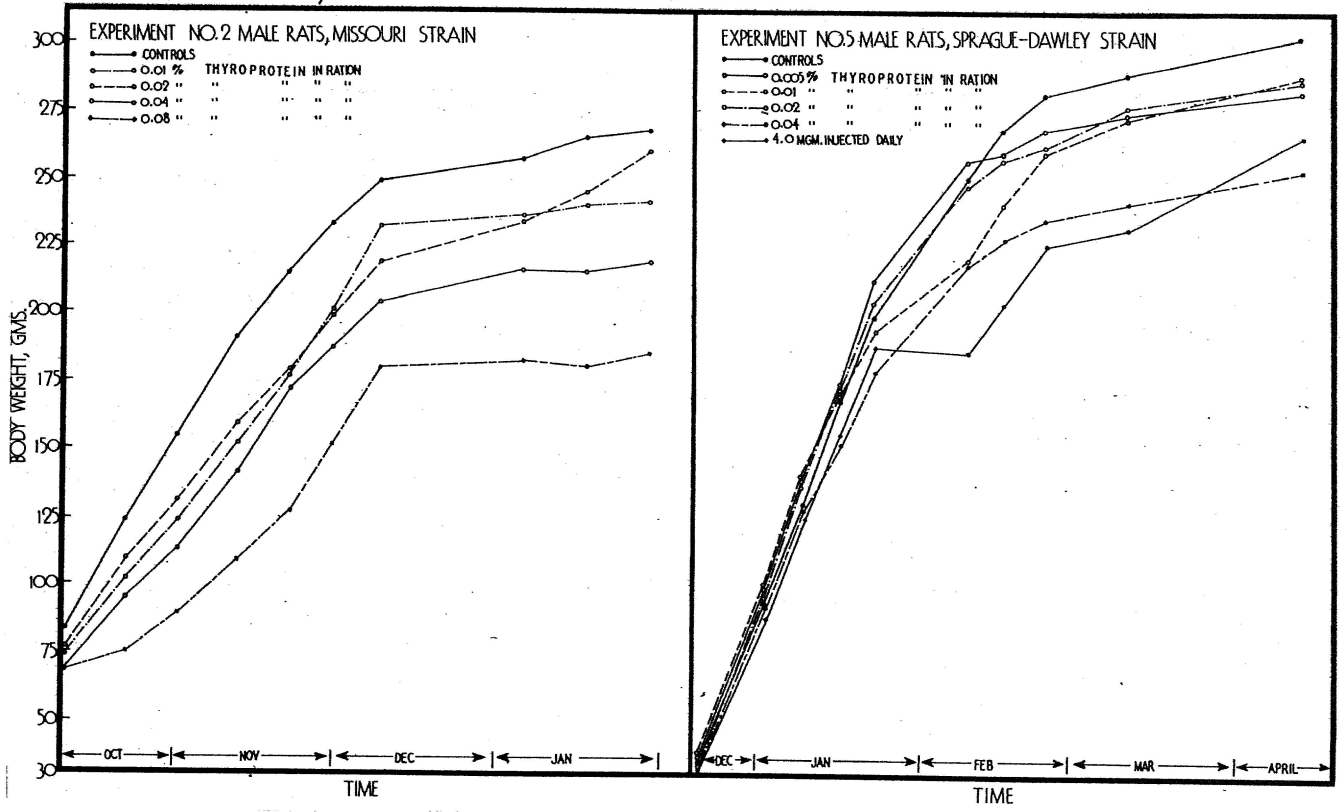


Fig. 5.—Growth curves of male rats.

cent caused a depression in growth in this strain which was not observed in the Missouri strain (Experiment 3). In addition to the feeding levels mentioned above, one group of the Sprague-Dawley rats were injected with 4.0 mg. per rat daily for three months. This produced about the same inhibition of growth as 0.04 per cent in the ration. When the injections were terminated, growth rate increased (Fig. 5).

The effects of thyroprotein on the skeletal development of the Sprague-Dawley rats were different from those observed in the Missouri strain. At the termination of the experiment, the average nose-anus length of controls was 23.5 cm. while treated animals averaged 23.5, 24.0, 25.0 and 25.1 cm. for the respective levels of 0.005, 0.01, 0.02 and 0.04 per cent thyroprotein in the ration (Table 23). Statistical treatment by analysis of variance showed a probability of 5 per cent that the observed differences were due to chance variation. Such a difference would ordinarily be considered probably significant. Thus, while weight was significantly depressed by all levels of thyroprotein fed to Sprague-Dawley male rats, the skeletal development was somewhat greater on the higher levels of feeding than that of the controls. While the difference in length in favor of the treated animals was not highly significant for the number of animals involved, it appears that the difference was not due to chance variation.

It is interesting to note that while the Sprague-Dawley rats were more sensitive to thyroid treatment as regards depression of weight, they showed an accelerated skeletal development which was not observed in the Missouri strain.

Experiments with Females. Ten different trials involving a total of 486 animals were conducted with female rats (Table 11). As with the males, both Missouri and Sprague-Dawley strains were used. Treatment, feed, etc., were identical with that of the males except that dosage levels were varied.

Experiment 1 (Table 12) was a preliminary trial to determine the tolerance to thyroid treatment. From this trial it was evident that 0.32 per cent in the ration was toxic, with some indication that 0.04 and 0.08 per cent caused a slight increase in gain above the controls.

In Experiments 2, 3 and 4, groups of 10 animals each were used, and dosage varied from 0.01 to 0.08 per cent of the ration. In all three trials the growth curves of the animals on 0.04 and 0.08 per cent were somewhat above the controls (Figs. 6 and 7). The gains made by rats in these experiments over a period of two and one-half months were treated statistically by analysis of variance to test the significance of the difference between control and treated animals (Table 13). The probability value was slightly less than 5 per cent, being suggestive, but not conclusive, of a significant difference in favor of the treated animals.

TABLE 11. GENERAL INFORMATION CONCERNING EXPERIMENTS WITH FEMALE RATS

Exper. No.	Treatment	Date		No. Animals		Per cent Survival
		Started	Ended	At start	At end	
1	Controls, Missouri strain	9/12/42	11/20/42	12	12	100
	0.01% thyroprotein (1) in ration	9/12/42	11/20/42	12	12	100
	0.02% thyroprotein in ration	9/12/42	11/20/42	12	11	92
	0.04% thyroprotein in ration	9/12/42	11/20/42	12	11	92
	0.08% thyroprotein in ration	9/12/42	11/20/42	12	7	58
	0.16% thyroprotein in ration	9/12/42	11/20/42	12	7	58
	0.32% thyroprotein in ration	9/12/42	11/20/42	12	6	50
2	Controls, Missouri strain	10/24/42	2/13/43	10	10	100
	0.01% thyroprotein in ration	10/24/42	2/13/43	10	10	100
	0.04% thyroprotein in ration	10/24/42	2/13/43	10	7	70
	0.08% thyroprotein in ration	10/24/42	2/13/43	10	6	60
3	Controls, Missouri strain	11/7/42	3/15/43	10	8	80
	0.04% thyroprotein in ration	11/7/42	3/15/43	10	6	60
	0.08% thyroprotein in ration	11/7/42	3/15/43	10	7	70
4	Controls, Missouri strain	11/7/42	3/15/43	10	8	80
	0.04% thyroprotein in ration	11/7/42	3/15/43	10	6	60
	0.08% thyroprotein in ration	11/7/42	3/15/43	10	6	60
5	Controls, Missouri strain	11/20/42	3/16/43	30	28	93
	0.08% thyroprotein in ration	11/20/42	3/16/43	30	27	90
6	Controls, Missouri strain	11/20/42	3/16/43	30	27	90
	0.04% thyroprotein in ration	11/20/42	3/16/43	30	27	90
7	Controls, Missouri strain	11/20/42	3/16/43	25	23	92
	0.02% thyroprotein in ration	11/20/42	3/16/43	25	23	92
8	Controls, Missouri strain	12/10/42	3/6/43	12	10	83
	0.16% thyroprotein in ration	12/10/42	3/6/43	12	7	58
	0.32% thyroprotein in ration	12/10/42	3/6/43	12	5	42
9	Controls, Sprague-Dawley strain	12/20/42	4/20/43	8	7	88
	0.04% thyroprotein in ration	12/20/42	4/20/43	8	5	63
	0.08% thyroprotein in ration	12/20/42	4/20/43	8	4	50
	0.16% thyroprotein in ration	12/20/42	4/20/43	8	3	38
	0.32% thyroprotein in ration	12/20/42	4/20/43	8	2	25
	4.0 mg. thyroprotein injected daily (2)	12/20/42	4/20/43	8	6	75
10	Controls, Sprague-Dawley strain	1/12/43	5/13/43	8	7	88
	0.04% thyroprotein in ration	1/12/43	5/13/43	8	6	75
	0.08% thyroprotein in ration	1/12/43	5/13/43	8	3	38
	0.16% thyroprotein in ration	1/12/43	5/13/43	8	3	38
	0.32% thyroprotein in ration	1/12/43	5/13/43	8	2	25
	4.0 mg. thyroprotein injected daily (2)	1/12/43	5/13/43	8	7	88

(1) Thyroactive iodocasein lot III

(2) Injection ceased on 3/10/43.

TABLE 12. AVERAGE WEIGHTS OF FEMALE RATS AT INTERVALS WHILE ON EXPERIMENT.

Exper. No.	Treatment	Average weight of group (gms.)										
1	Controls, Missouri strain	9/12 ⁽¹⁾	9/21	9/26	10/8	10/19	10/24	11/6	-----	-----	-----	11/20 ⁽²⁾
	0.01% thyroprotein in ration	142 ± 16 ⁽²⁾	146	153	162	167	172 ± 12	177	-----	-----	-----	186 ± 16
	0.02% thyroprotein in ration	146 ± 8	153	161	172	177	179 ± 8	185	-----	-----	-----	194 ± 11
	0.04% thyroprotein in ration	139 ± 18	138	150	161	167	169 ± 20	175	-----	-----	-----	181 ± 16
	0.08% thyroprotein in ration	139 ± 20	141	153	155	171	176 ± 21	178	-----	-----	-----	189 ± 11
	0.16% thyroprotein in ration	138 ± 18	128	146	159	168	170 ± 16	190	-----	-----	-----	198 ± 18
0.32% thyroprotein in ration	149 ± 17	141	151	165	179	181 ± 15	187	-----	-----	-----	188 ± 26	
		141 ± 12	134	140	150	160	130 ± 17	154	-----	-----	-----	154 ± 19
2	Controls, Missouri strain	10/24 ⁽¹⁾	11/6	11/19	11/28	12/5	1/1	1/9	1/21	1/30	-----	2/13
	0.01% thyroprotein in ration	112 ± 8	144	160	165	172	189 ± 10	193	199	203	205	205 ± 22
	0.04% thyroprotein in ration	111 ± 11	146	162	169	174	195 ± 9	202	203	208	215	215 ± 10
	0.08% thyroprotein in ration	108 ± 6	133	150	163	176	194 ± 13	200	206	210	211	211 ± 18
		113 ± 7	145	163	179	186	195 ± 11	193	208	209	220	220 ± 15
3	Controls, Missouri strain	11/7 ⁽¹⁾	11/14	11/29	12/5	1/1	1/9	1/21	1/30	2/25	-----	3/15
	0.04% thyroprotein in ration	112 ± 6	130	154	162	178	184 ± 12	191	196	198	197	197 ± 15
	0.08% thyroprotein in ration	110 ± 4	134	161	170	180	187 ± 8	193	197	201	201	201 ± 12
		113 ± 7	121	142	163	185	191 ± 9	197	208	214	217	217 ± 10
4	Controls, Missouri strain	62 ± 4	85	126	138	164	172 ± 11	176	177	192	195	195 ± 14
	0.04% thyroprotein in ration	60 ± 3	76	117	139	175	189 ± 7	185	193	193	191	191 ± 10
	0.08% thyroprotein in ration	67 ± 3	94	135	152	180	184 ± 9	190	198	203	200	200 ± 11
5	Controls, Missouri strain	11/20 ⁽¹⁾	11/28	12/5	12/11	1/1	1/9	1/21	1/30	1/25	-----	3/16
	0.08% thyroprotein in ration	67 ± 3	101	125	135	168	175 ± 9	190	198	208	210	210 ± 17
		68 ± 3	91	116	125	160	167 ± 11	178	185	188	195	195 ± 16
6	Controls, Missouri strain	34 ± 1	57	85	97	146	158 ± 13	163	172	183	183	183 ± 15
	0.04% thyroprotein in ration	33 ± 2	51	73	89	131	143 ± 12	163	175	186	187	187 ± 17
7	Controls, Missouri strain	51 ± 4	79	102	117	148	156 ± 10	168	178	186	189	189 ± 13
	0.02% thyroprotein in ration	50 ± 1	74	100	111	149	153 ± 7	165	175	181	183	183 ± 11
8	Controls, Missouri strain	12/10 ⁽¹⁾	12/31	1/9	1/16	1/23	1/30	2/14	2/20	-----	-----	3/6
	0.16% thyroprotein in ration	48 ± 3	101	111	123	132	143 ± 10	151	155	-----	-----	158 ± 14
	0.32% thyroprotein in ration	48 ± 2	88	100	105	114	124 ± 17	130	132	-----	-----	145 ± 20
		49 ± 4	84	92	98	107	123 ± 14	136	141	-----	-----	150 ± 24
9 and 10	Controls, Sprague-Dawley strain	1 ⁽³⁾	13	20	27	34	51	67	81	108	-----	121
	0.04% thyroprotein in ration	35 ± 2	94	124	144	158	184 ± 11	202	211	223	226	226 ± 15
	0.08% thyroprotein in ration	34 ± 2	87	109	127	142	164 ± 10	176	184	190	197	197 ± 14
	0.16% thyroprotein in ration	33 ± 3	77	97	105	115	130 ± 13	133	137	163	155	155 ± 16
	0.32% thyroprotein in ration	35 ± 2	81	96	111	126	152 ± 16	152	146	147	147	147 ± 21
	4.0 mg. thyroprotein injected daily	34 ± 3	74	91	97	109	123 ± 20	127	143	145	187	187 ± 23
		34 ± 2	81	97	120	133	156 ± 21	158	155	187	200	200 ± 16

(1) Date weights were taken.

(2) Standard deviation.

(3) Days on experiment when weighed.

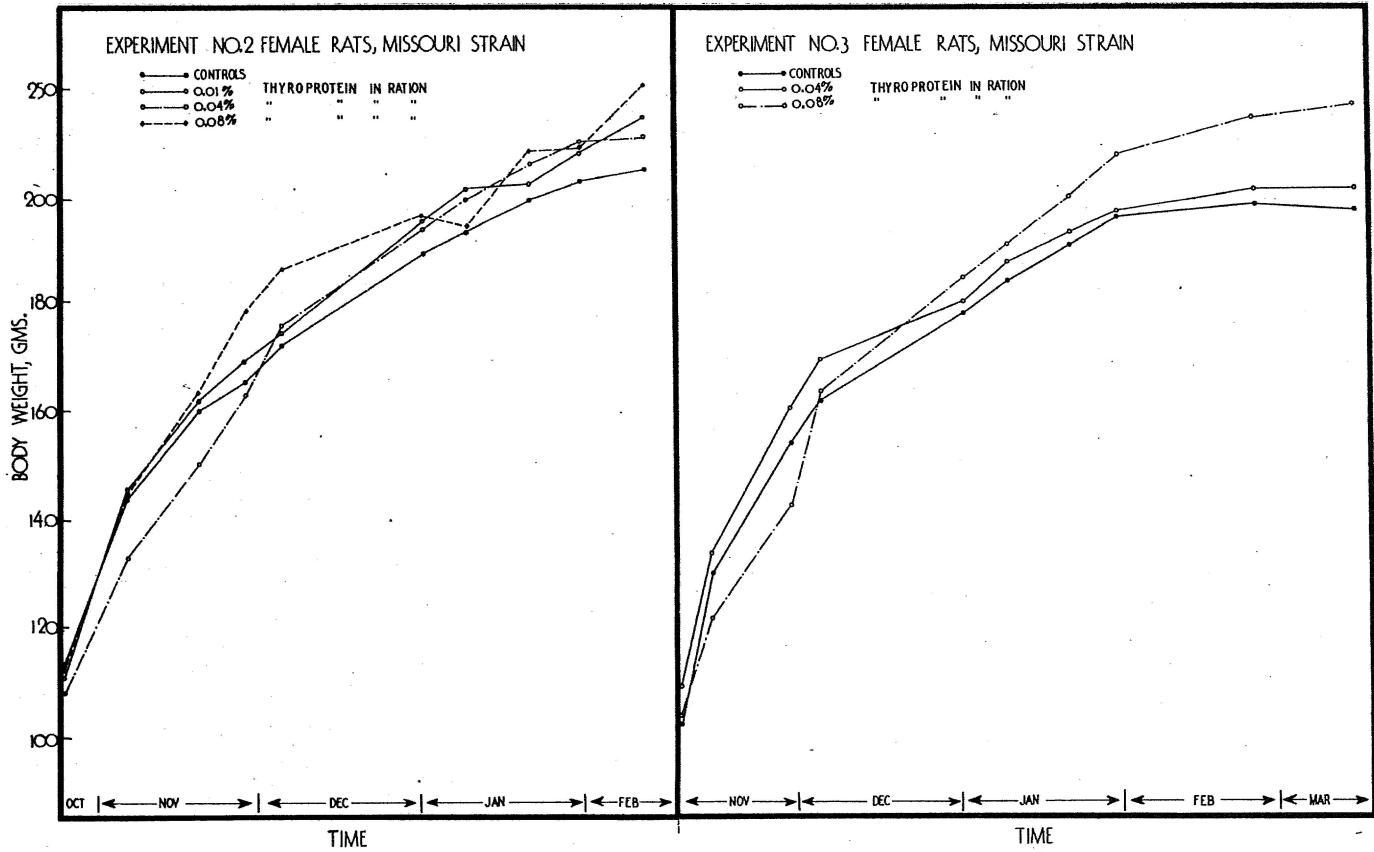


Fig. 6.—Growth curves of female rats.

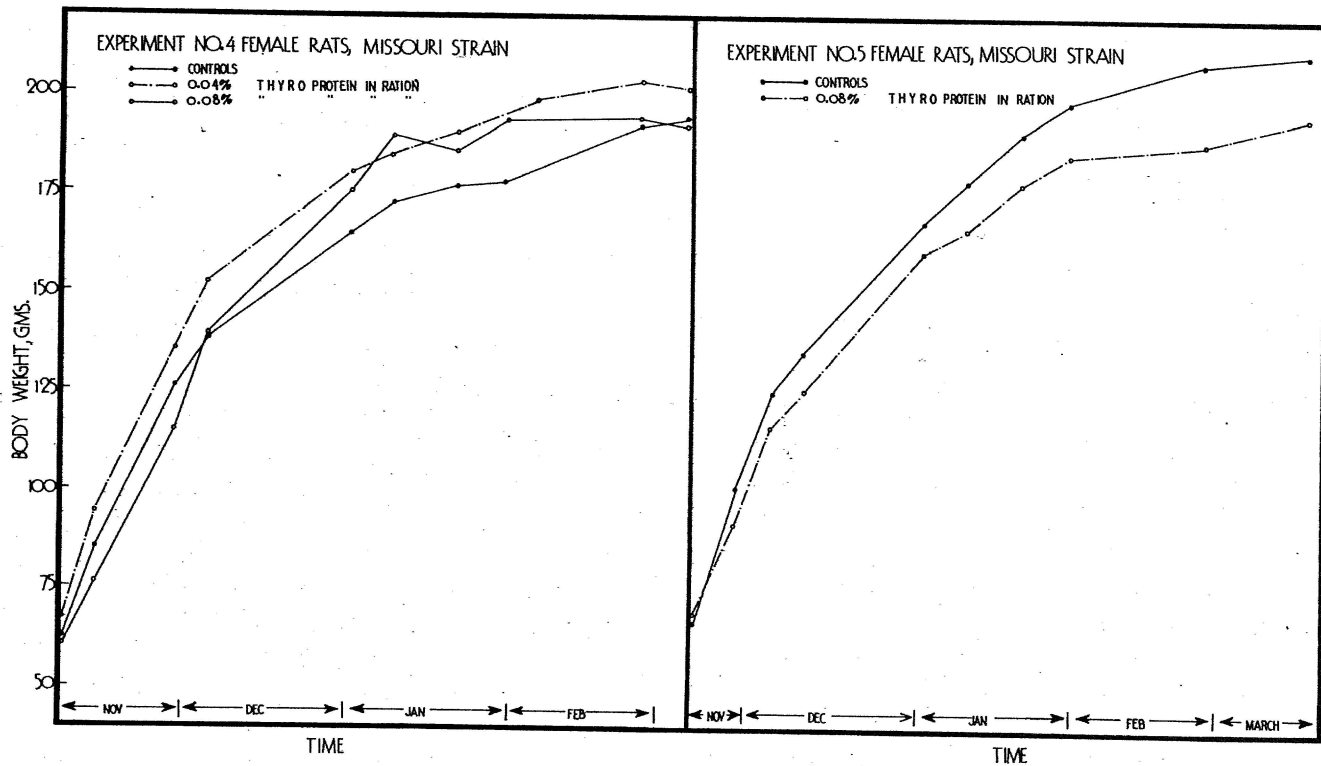


Fig. 7.—Growth curves of female rats.

TABLE 13.—SUMMARY AND VARIANCE TABLE OF GAINS MADE BY FEMALE RATS IN EXPERIMENTS 2, 3 AND 4 DURING TWO AND ONE-HALF MONTHS.

Exper. No	Average Gains (gm.)		
	Controls	0.04% in Ration	0.08% in Ration
2	77	86	82
3	72	77	78
4	92	129	117
Total	244	292	277
Average	80	97	92

Source	Variance Table		
	Degrees Freedom	Sx ₂	Variance
Controls—treated	1	420	420 ¹
Error	65	...	95 ²

¹F = 4.42, P = less than 5 per cent.

²Error variance was calculated from the individual gains as described by Snedecor (1939), pp. 235 to 238.

Larger numbers of more uniform animals were available for Experiments 5 and 6. In these experiments, 0.08 per cent in the ration resulted in a depressed rate of growth as compared to controls (Fig. 8), while 0.04 and 0.02 per cent caused no appreciable change in growth rate. Casual examination would indicate that these experiments are in direct contradiction to trials 2, 3 and 4. A more detailed study of mortality in the different trials, however, shows that in Experiments 2, 3 and 4 the mortality rate of the treated animals was greater than that of controls while in the latter the mortality rate was similar to that of the untreated animals (Table 11). Since the animals that died on experiment were, on the average, lighter in weight than the remainder of the group (Table 14), it appears that the positive results in Experiments 2, 3 and 4 may have been spurious due to a greater number of light animals dying among the treated animals than among the controls. In Experiments 5 and 6 the mortality

TABLE 14.—MORTALITY RATE AND RELATIVE WEIGHT OF FEMALE RATS DYING ON EXPERIMENTS 2 TO 6.

Exper. No.	Treatment	Mortality	Average Relative Weight
		%	%*
3	0.04%	30	84
	0.08%	40	80
	Control	20	83
4	0.04%	40	82
	0.08%	30	79
	Control	20	79
5	0.04%	40	81
	0.08%	40	80
	Control	10	81
6	0.08%	13	82
	Control	10	85
	0.04%	10	82

*Average of the ratios:

weight of dead animal

average weight of group

Animals dying first two weeks of experiment were not included.

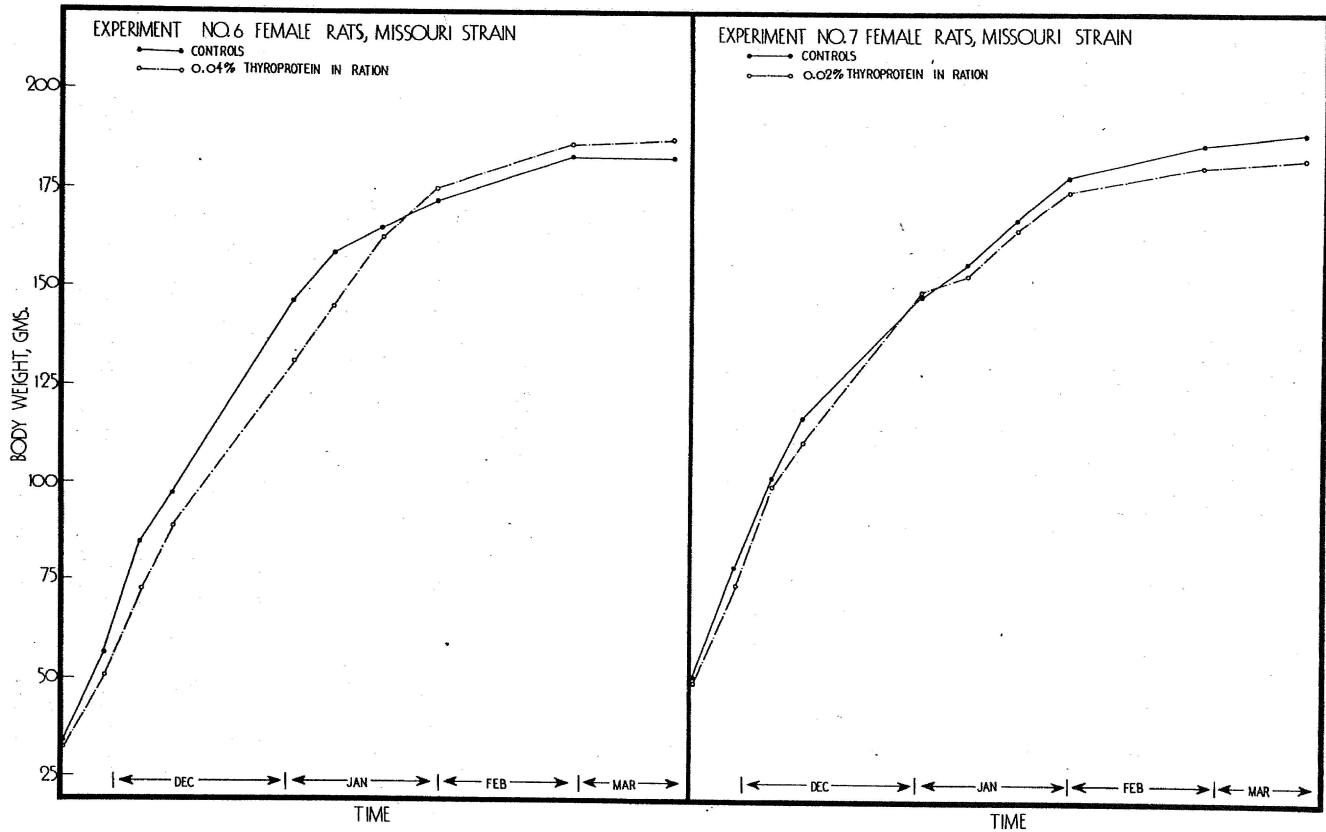


Fig. 8.—Growth curves of female rats.

rate was similar among the different groups, and it would appear that results obtained in the latter more nearly reflect the average effect of thyroid treatment than the results in the former trials.

In Experiment 8 the animals were housed in different quarters from those in all the other experiments. Temperature was variable with weather conditions and time of day. The building was not usually heated and therefore the mean temperature was much lower than in the laboratory where the other experiments were conducted. It was thought that the increased metabolism due to thyroid treatment might be beneficial under such environment. The growth of the controls was subnormal while 0.16 and 0.32 per cent thyroprotein in the ration further depressed the rate of gain (Fig. 9).

Young animals of the Sprague-Dawley strain were used in Experiments 9 and 10. Rats weighing from 30 to 40 grams were placed on amounts ranging from 0.04 to 0.32 per cent of the ration and in addition one group in each trial received injections of 4.0 mg. thyroactive iodocasein until March 10, 1943. In each case the controls grew at a significantly faster rate than animals on any thyroid treatment. The severity of growth depression paralleled roughly the level of thyroprotein in the ration. Injection of 4.0 mg. of the material caused about the same depression of growth as 0.04 per cent in the ration.

The skeletal development of female rats and the various levels of thyroprotein treatment followed the same trend as body weight (Tables 22 and 24).

Thus the results with female rats leave several questions concerning the response to mild thyroid treatment unanswered. The Sprague-Dawley rats were clearly more sensitive to thyroid treatment than the Missouri strain, and it seems likely, in view of their sensitivity, that smaller amounts than those employed would not have accelerated growth rate. On the other hand, the results with the Missouri strain are not so clear. Even though the high mortality rate in the treated animals may possibly explain the positive results obtained in Experiments 2, 3 and 4, the fact remains that the results are suggestive of certain animals responding to mild thyroid treatment by limited acceleration of growth.

The Effect of Thyroprotein Feeding on Metabolism of Rats. The carbon dioxide production of four animals on each level of thyroid treatment in Experiment 5 with male rats and Experiment 12 with females was determined by means of a modified Haldane metabolism unit. Carbon dioxide production by each animal was measured on three consecutive days and rats were fasted five to eight hours before determinations were made. The temperature in the metabolism unit was maintained at 29° C.

The total energy metabolism was not calculated for the reason that carbon dioxide production appeared to be a valid index for group

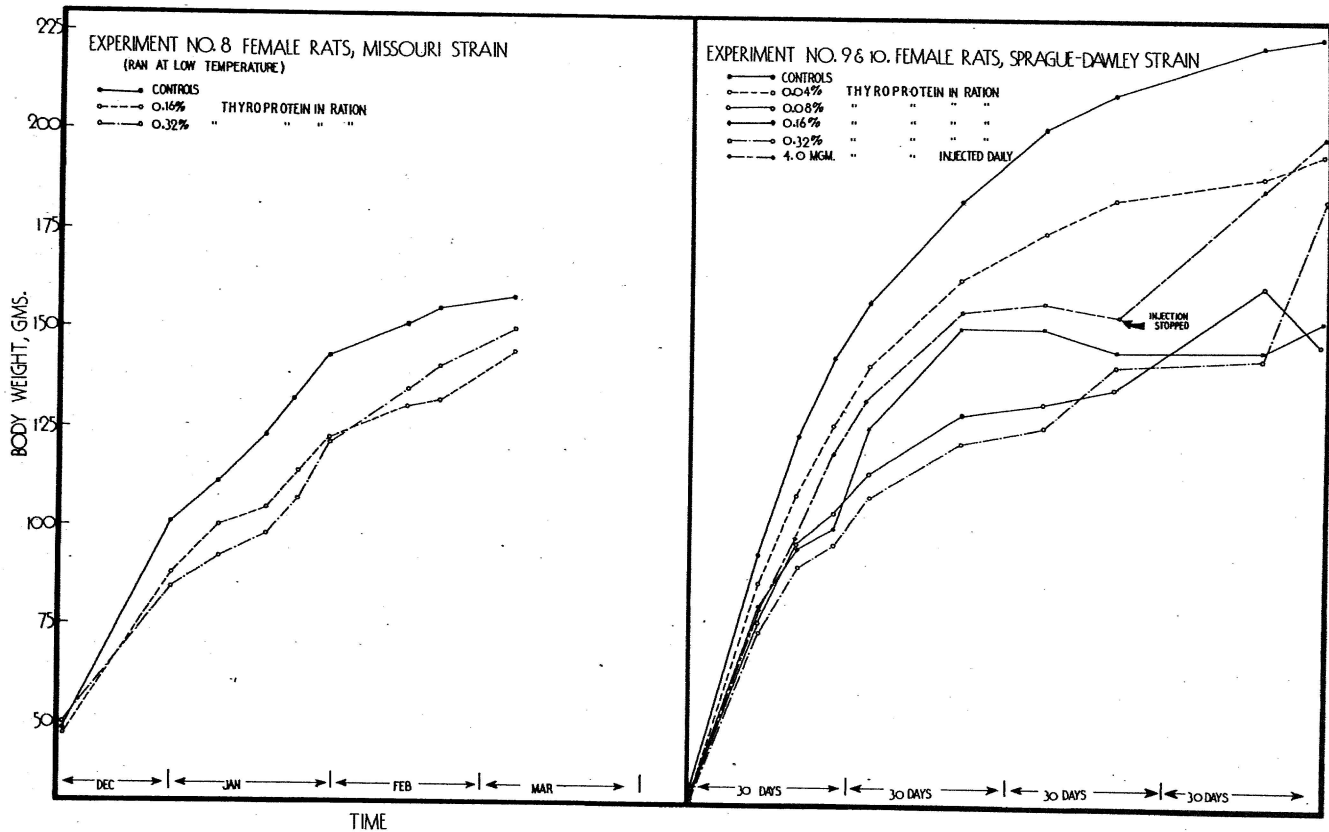


Fig. 9.—Growth curves of female rats.

comparisons under the conditions used, and avoided the errors incurred in indirect calculation of energy metabolism. Also, the carbon dioxide output was not determined under basal conditions but each group was given identical treatment, other than thyroid treatment, to insure comparable results. Resting metabolism is of more interest than basal rate in these experiments.

The results have been summarized in Tables 15 and 16. Feeding thyroprotein at the levels of 0.005, 0.01 and 0.02 per cent in the ration to males did not apparently affect carbon dioxide production. Feeding 0.04 per cent in the ration caused a significant rise in carbon dioxide production while injection of 4.0 mg. caused an even greater increase. It is of interest to compare the effects of growth rate with that on metabolism. Thyroprotein feeding at the levels of 0.005, 0.01 and 0.02 per cent in the ration caused a significant depression in rate of body weight gain, whereas the carbon dioxide production, at least of the animals tested, was not apparently affected on these levels.

TABLE 15.—CARBON DIOXIDE PRODUCTION OF FEMALE RATS.

Treatment	Average Weight of Animals gm.	Mg. CO ₂ /hr./100 gm. Body Weight				Average**
		1	Animal No.* 2	3	4	
Controls	209	209	248	232	201	218
Thyroprotein-fed						
0.04% in ration	180	348	305	315	312	320
0.08% in ration	171	439	406	402	395	411
0.16% in ration	121	489	464	456	456	466
0.32% in ration	129	689	484	724	635	638
4.0 mg. injected	137	458	451	583	484	494

*Figure for each animal represents an average of determinations on 3 consecutive days.

**The difference between means necessary for a probability of 5 per cent is 96 mg., for 1 per cent 132 mg.

TABLE 16.—CARBON DIOXIDE PRODUCTION OF MALE RATS.

Treatment	Average Weight of Animals gm.	Mg. CO ₂ /hr./100 gm. Body Weight				Average**
		1	Animal No. 2	3	4	
Controls	284	213	208	184	201	202
Thyroprotein-fed						
0.005% in ration	245	197	173	174	223	192
0.01% in ration	256	166	189	245	149	187
0.02% in ration	267	179	196	195	207	194
0.04% in ration	211	305	294	300	274	293
4.0 mg. injected	203	539	514	505	506	516

*Figure for each animal represents an average of determinations on 3 consecutive days.

**The difference between means necessary for a probability of 5 per cent is 63 mg., for 1 per cent, 86 mg.

The carbon dioxide production by females was increased by all levels of thyroprotein given in Experiment 12 and roughly paralleled the dosage employed (Table 17). Injection of 4.0 mg. thyroprotein and feeding 0.04 per cent in the ration caused an increase in carbon dioxide production of females comparable to the increase in males caused by the same treatment.

TABLE 17. GENERAL INFORMATION CONCERNING EXPERIMENTS WITH GUINEA PIGS

Exper. No.	Sex and Treatment	Date		At Start	At End	Per Cent Survival
		Started	Ended			
1	Control males	11/21/42	5/27/43	8	6	75
	0.0025% thyroprotein in ration	11/21/42	5/27/43	8	8	100
	Control females	11/21/42	5/27/43	8	8	100
	0.0025% thyroprotein in ration	11/21/42	5/27/43	8	8	100
2	Control males	11/28/42	5/27/43	8	8	100
	0.005% thyroprotein in ration	11/28/42	5/27/43	8	6	75
	Control females	11/28/42	5/27/43	8	8	100
	0.005% thyroprotein in ration	11/28/42	5/27/43	8	4	50
3	Control males	12/5/42	5/27/43	8	8	100
	0.01% thyroprotein in ration	12/5/42	5/27/43	8	8	100
	Control females	12/5/42	5/27/43	8	8	100
	0.01% thyroprotein in ration	12/5/42	5/27/43	8	4	50
4	Control males	12/13/42	5/27/43	8	7	88
	0.02% thyroprotein in ration	12/13/42	5/27/43	8	4	50
	Control females	12/13/42	3/16/42	8	8	100
	0.02% thyroprotein in ration	12/13/42	3/16/42	8	0	0

Experiments with Guinea Pigs.—Preliminary experiments (not reported in this paper) showed that guinea pigs were more sensitive to thyroid materials than mice or rats and that about 0.02 per cent thyroactive casein (lot III) in the ration was all that guinea pigs would tolerate. In addition it was found necessary to take precautions against animals selecting feed.

Using this information as a guide, four experiments were set up with dosages ranging from 0.0025 per cent to 0.02 per cent thyroprotein in the ration (Table 17). Technical difficulties encountered in mixing small amounts of thyroprotein into the feed made it seem impractical to attempt further reduction of dosage. The thyroactive iodocasein was incorporated into a ration made by mixing 75 parts of a finely ground standard rabbit concentrate feed with 25 parts of alfalfa leaf meal. This feed was not as palatable as might be desired and growth rate was not rapid. The pigs remained in a thrifty, vigorous condition, however. Fresh cabbage was fed daily to supply vitamin C.

The animals were kept in a building which was usually unheated and the temperature varied with weather conditions. In extremely cold weather the building was heated to prevent bursting of water pipes and the mean temperature was usually above freezing during the winter months.

The animals used were purchased from a commercial breeder as soon as they were old enough to withstand shipment. The ancestry of the guinea pigs is not known, but they were judged as a heterogeneous lot as to color and uniformity of conformation. Individual records were kept and the animals were weighed weekly.

The responses of the two sexes to thyroid treatment were different and will therefore be discussed separately.

Results with Males.—In the first three experiments, the growth curves of the treated animals on 0.0025, 0.005 and 0.01 per cent thyroprotein were consistently slightly above the curves of their respective controls until about March 15, 1943 (Table XVIII, Figs. 10 and 11). At this time the growth rate of the treated animals became depressed and by May 1, 1943, the treated animals were losing weight while the controls continued to grow. It appears likely that the increase in temperature with the approach of spring was at least partly responsible for the depression of growth during the latter part of the experiments. It is possible that the factor of age also affected the response.

TABLE 18. AVERAGE WEIGHTS OF GUINEA PIGS AT INTERVALS

Exper. No.	Sex and Treatment	Av. Wt. of Group (gms.)											
1	Control males	11/21	12/5	1/4	1/25	2/16	3/9	3/30	4/20	5/4	5/27		
	0.0025% in ration	163 ± 15	247	370	438	515	575 ± 7	645	700	757	787 ± 12		
	Control females	165 ± 8	244	357	420	483	548 ± 28	620	670	704	721 ± 31		
	0.0025% in ration	168 ± 3	227	342	402	463	526 ± 65	571	587	598	556 ± 27		
2	Control males	11/28	12/29	1/18	2/9	2/23	3/9	3/30	4/20	5/4	5/27		
	0.005% in ration	154 ± 5	282	330	432	490	498 ± 40	587	626	670	683 ± 61		
	Control females	160 ± 9	270	336	420	460	481 ± 70	531	593	613	635 ± 72		
	0.005% in ration	165 ± 10	286	340	415	467	475 ± 33	489	511	503	467 ± 53		
3	Control males	12/5	12/29	1/18	2/9	2/23	3/9	3/30	4/20	5/4	5/27		
	0.01% in ration	161 ± 9	276	316	406	421	453 ± 50	548	573	614	620 ± 69		
	Control females	166 ± 8	278	332	398	432	468 ± 34	538	579	608	613 ± 13		
	0.01% in ration	167 ± 9	281	319	405	452	467 ± 13	541	520	550	469 ± 20		
4	Control males	12/13	1/4	1/25	2/9	2/23	3/16	4/6	4/20	5/4	5/27		
	0.02% in ration	149 ± 4	261	357	388	421	471 ± 63	550	622	687	694 ± 19		
	Control females	133 ± 17	229	284	340	389	446 ± 88	---	---	---	---		
	0.02% in ration	138 ± 9	201	240	324	345	---	---	---	---	---		

The fact that slight acceleration of growth during the first part of the experiment occurred in all three trials would indicate that the response was due to thyroid treatment and not to variation. Statistical treatment of gains made during the first 45 days on Experiments 1, 2 and 3 showed the difference in favor of the treated animals to be significant (Table 19).

Thus, under the conditions used in these experiments, it appears that 0.0025, 0.005 and 0.01 per cent thyroprotein in the ration caused a slight but probably significant acceleration of growth for a short period of time. Continued treatment at the same levels caused a depression of growth and an actual loss of weight with the advent of warmer weather. It would be of interest to know whether this de-

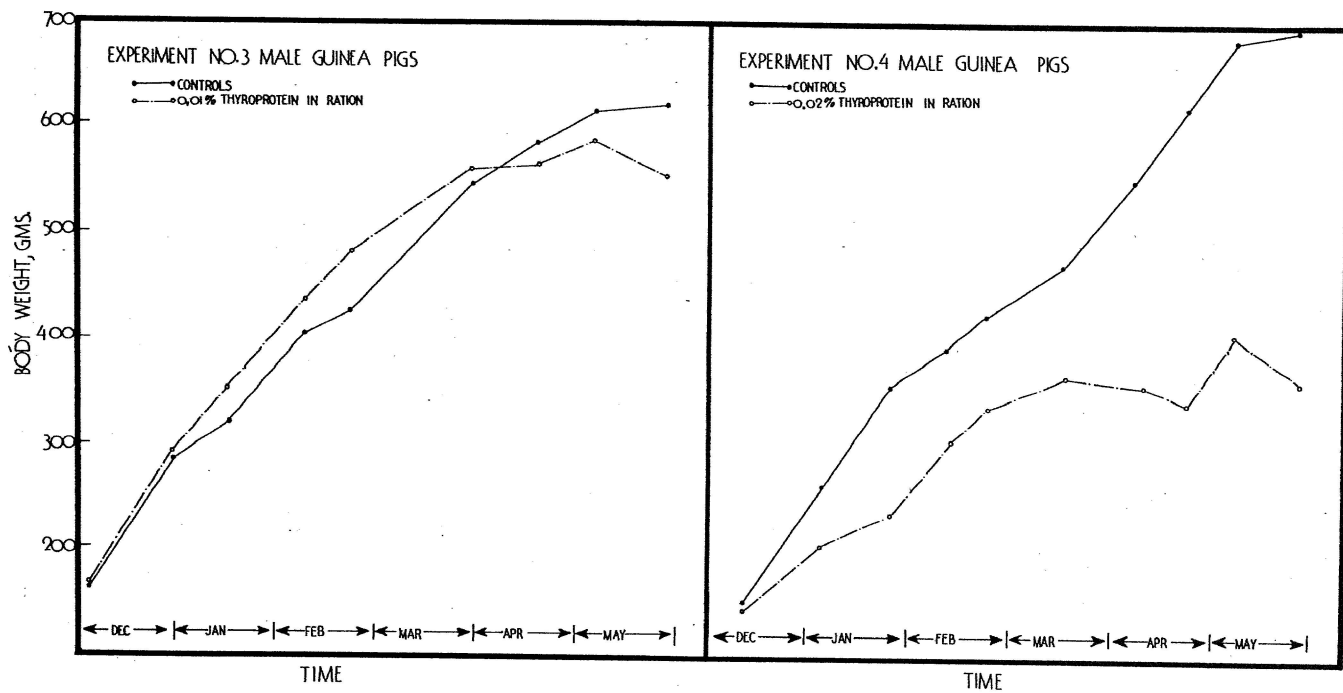


Fig. 10.—Growth curves of male guinea pigs.

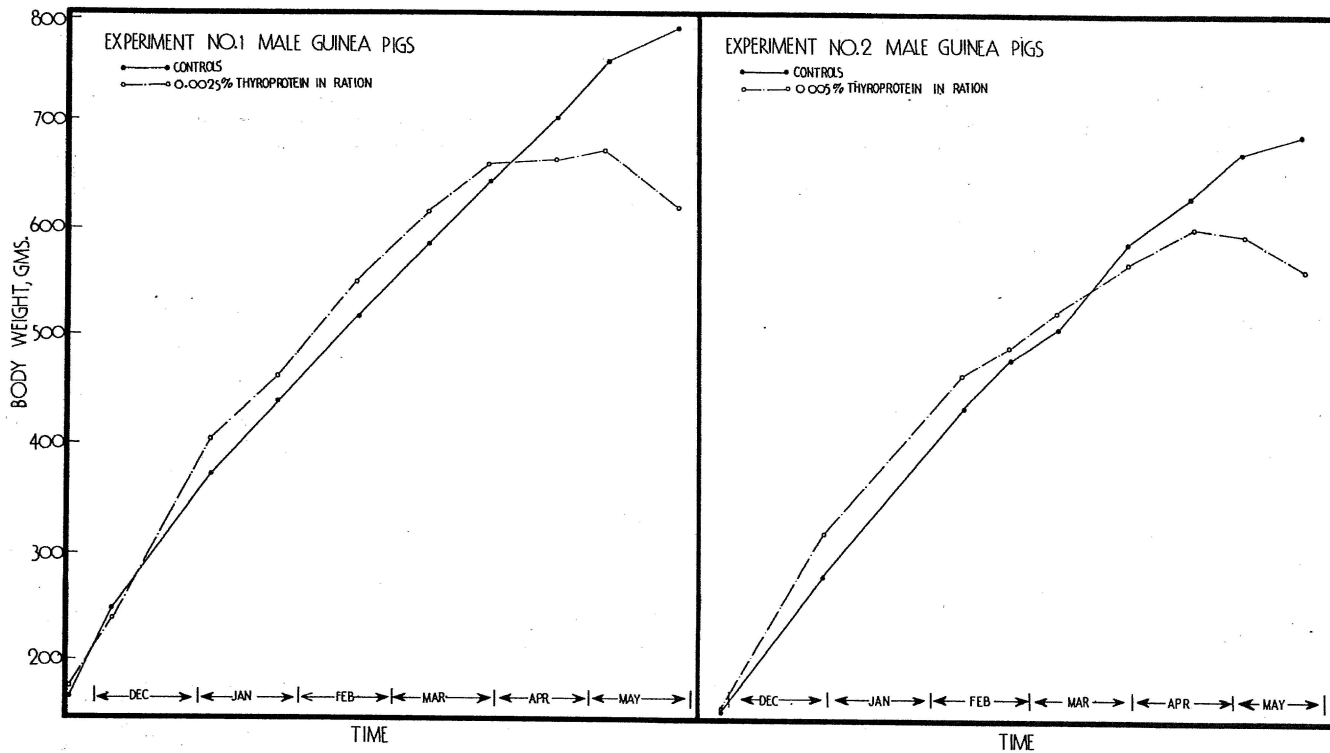


Fig. 11.—Growth curves of male guinea pigs.

TABLE 19.—SUMMARY AND VARIANCE TABLE OF GAINS MADE BY MALE GUINEA PIGS DURING THE FIRST 45 DAYS ON EXPERIMENT.

Exper. No.	Average Gain in Weight (gm.)		Treated
	Controls		
1	207		236
2	176		221
3	155		190
Total	538		647
Average	179		216
Source	Variance Table		
Controls—treated	Degrees Freedom	Sx ²	Variance
Error	1	1980	1980*
	38	...	350**

*F = 5.66, P = less than 5 per cent.

**Error variance was calculated from individual gains as described by Snedecor (1939).

pression would have occurred if temperature had remained constant, but conditions used in these experiments left this question unanswered. Schmidt and Genther (1938) have reported that guinea pigs are more sensitive to thyroid treatment in a warm environment. Thyroid material at the level of 0.02 per cent of the ration depressed growth at all times (Fig. 11).

Results with Females.—No evidence of accelerated growth rate was noted in females on any level of thyroprotein feeding. Feeding 0.0025, 0.005 and 0.01 per cent in the ration did not affect noticeably the rate of growth during the first period of the experiments, but by March 15, growth of the treated animals began to be depressed (Figs. 12 and 13). About May 1 the females began losing weight in a manner similar to males.

Feeding at the level of 0.02 per cent of the ration resulted in death of all treated females in approximately 45 days.

A marked difference in tolerance of thyroid material of the two sexes as observed in rats was not evidenced in guinea pigs. Judging from effects on growth curves, there was no great difference in the tolerance of male and female guinea pigs to thyroid treatment. The mortality rate of treated animals was greater in the females, however, than in the males.

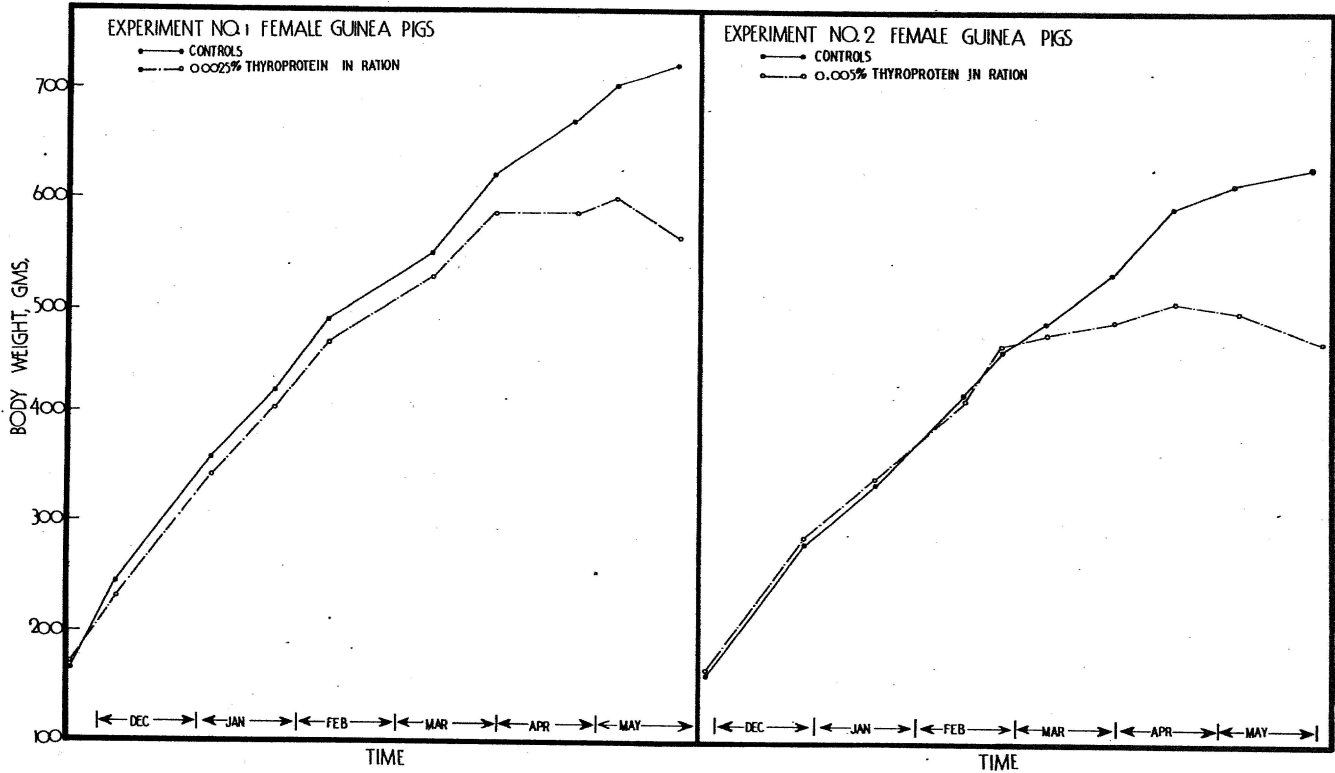


Fig. 12.—Growth curves of female guinea pigs.

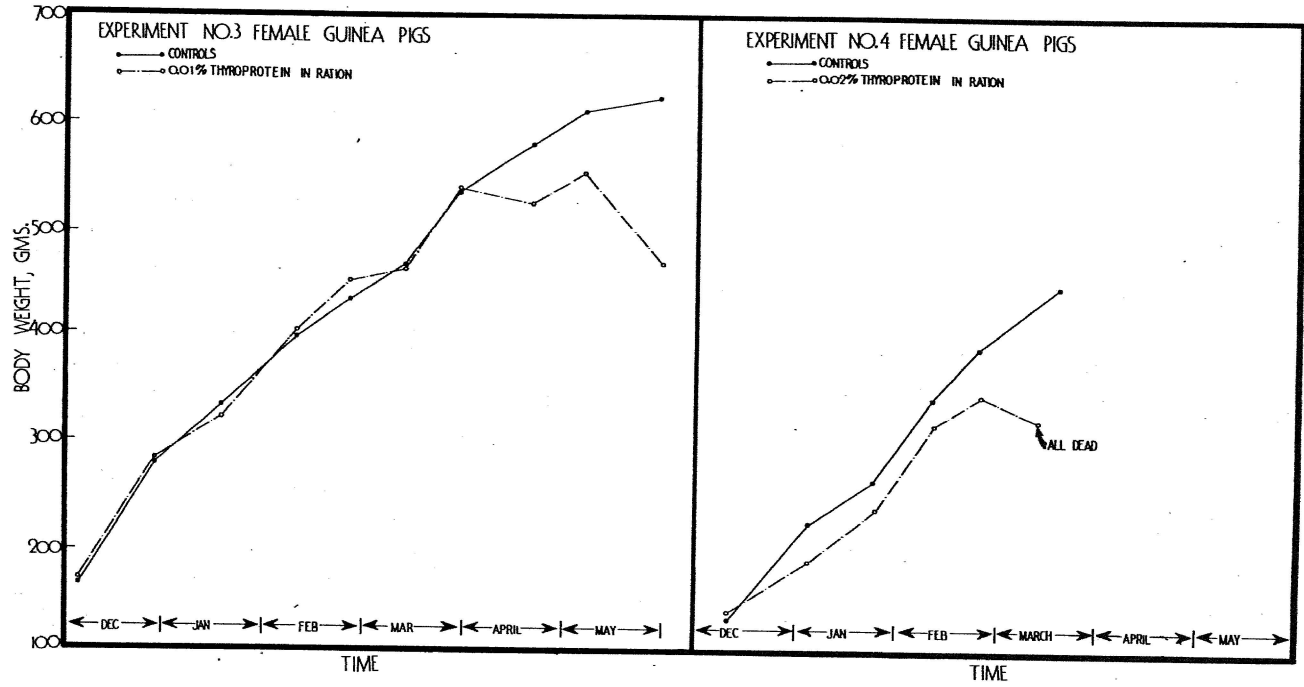


Fig. 13.—Growth curves of female guinea pigs.

Experiments with Rabbits.—Three different experiments were conducted with young rabbits with the dosage of thyroactive iodocasein (lot III) ranging from 0.0025 to 0.08 per cent of the ration (Tables 20 and 21). New Zeland White rabbits weighing from 600 to 1200 grams were purchased for experimental animals, both male and female animals being used.

The animals were maintained in individual cages in a building which was heated only enough to prevent freezing of water pipes, and the temperature varied according to the weather conditions and time of year. The ration consisted solely of a commercial complete rabbit feed. The thyroprotein was passed through a number 100 screen, mixed with a small amount of dry, finely ground grain and then incorporated into the complete ration. The feed was given *ad libitum* to all animals.

TABLE 20. GENERAL INFORMATION CONCERNING EXPERIMENTS WITH RABBITS

Exper. No.	Sex and Treatment	Date Started	Date Ended	No. of Animals		Per Cent Survival	
				At Start	At End		
1	Control males	9/19/42	12/5/42	4	4	100	
	0.0025% thyroprotein in ration	9/19/42	12/5/42	4	3	75	
	0.0075% thyroprotein in ration	9/19/42	12/5/42	4	4	100	
	0.020% thyroprotein in ration	9/19/42	12/5/42	4	3	75	
	0.040% thyroprotein in ration	9/19/42	12/5/42	4	4	100	
	Control females	9/19/42	12/5/42	4	4	100	
	0.0025% thyroprotein in ration	9/19/42	12/5/42	4	4	100	
	0.0075% thyroprotein in ration	9/19/42	12/5/42	4	4	100	
	0.020% thyroprotein in ration	9/19/42	12/5/42	4	4	75	
	0.040% thyroprotein in ration	9/19/42	12/5/42	4	4	100	
	0.080% thyroprotein in ration	9/19/42	12/5/42	4	0	0	
	2	Control males	10/20/42	3/6/42	4	3	75
		0.005% thyroprotein in ration	10/20/42	3/6/42	4	4	100
		0.010% thyroprotein in ration	10/20/42	3/6/42	4	4	100
Control females		10/20/42	3/6/42	4	4	100	
0.005% thyroprotein in ration		10/20/42	3/6/42	4	4	100	
0.010% thyroprotein in ration		10/20/42	3/6/42	4	4	100	
3	Control females	12/13/42	5/1/43	8	8	100	
	0.005% thyroprotein in ration	12/13/42	5/1/43	8	8	100	
	0.010% thyroprotein in ration	12/13/42	5/1/43	6	5	83	

Experiment 1 with rabbits was started in September 1942. Animals of both sexes were placed on rations containing 0.0025, 0.0075, 0.02, 0.04 and 0.08 per cent thyroactive iodocasein, and on control feed containing no thyroactive protein.

There was no apparent difference in the response of males and females to thyroid treatment so the weights of the two sexes were averaged for plotting growth curves (Fig. 14). Feeding thyroprotein at the levels of 0.0025 and 0.075 per cent of the ration did not greatly affect the growth rate of the rabbits while 0.02 per cent and larger amounts caused a progressive depression of growth (Fig. 14). The

TABLE 21. AVERAGE WEIGHTS OF RABBITS AT INTERVALS

Exper. No.	Treatment and Sex										
1	Controls		9/19	10/7	10/14	10/19	10/30	11/17	11/30	12/16	1/5
	Males		921 ± 96	1454	1619	1716	2090 ± 70	2553	2777	3192	3491 ± 102
	Females		1137 ± 50	1514	1711	1871	2113 ± 60	2574	2791	3204	3498 ± 40
	Both sexes		1029	1484	1665	1794	2102	2564	2784	3198	3490
	0.0025% thyroprotein in ration										
	Males		1017 ± 93	1562	1781	1896	2359 ± 120	2833	2944	3365	3619 ± 111
	Females		1001 ± 74	1441	1531	1594	1942 ± 116	2524	2716	3175	3477 ± 150
	Both sexes		1009	1502	1656	1745	2151	2678	2830	3270	3548
	0.0075% thyroprotein in ration										
	Males		1028 ± 79	1487	1672	1766	2107 ± 150	2535	2810	3272	3538 ± 127
	Females		991 ± 34	1365	1555	1685	2025 ± 80	2506	2780	3290	3526 ± 131
	Both sexes		1010	1426	1614	1726	2066	2521	2795	3281	3532
	0.0020% thyroprotein in ration										
	Males		927 ± 96	1170	1420	1500	1783 ± 206	2305	2438	2709	2953 ± 250
	Females		1070 ± 83	1356	1518	1653	1849 ± 315	2153	2568	2853	3061 ± 195
	Both sexes		999	1263	1469	1577	1816	2229	2503	2781	3007
	0.0040% thyroprotein in ration										
	Males		1061 ± 126	1225	1458	1579	1717 ± 204	1903	2037	2453	2559 ± 271
Females		1061 ± 133	1221	1618	1700	1844 ± 237	2277	2179	2663	2581 ± 300	
Both sexes		1061	1223	1538	1640	1776	2040	2108	2558	2570	
0.008% thyroprotein in ration											
Females		989 ± 69	1173	1217	1119	1230 ± 63	1124	1120	1132	----	
2	Controls		10/20	11/6	11/17	11/29	12/14	1/9	2/6	2/20	3/6
	Males		915 ± 124	1248	1664	2071	2515 ± 100	2966	3343	3335	3377 ± 306
	Females		819 ± 42	1108	1314	1678	2255 ± 38	2697	3155	3198	3419 ± 97
	Both sexes		867	1179	1489	1875	2385	2832	3249	3267	3398
	0.005% thyroprotein in ration										
	Males		717 ± 31	1248	1671	2134	2549 ± 63	3076	3461	3349	3549 ± 123
	Females		723 ± 15	1168	1480	1765	2339 ± 98	2785	3210	3340	3503 ± 116
	Both sexes		720	1208	1576	1950	2444	2931	3336	3354	3526
	0.01% thyroprotein in ration										
	Males		951 ± 76	1503	1759	2123	2652 ± 119	2893	2953	3024	3063 ± 84
	Females		943 ± 48	1327	1722	2040	2535 ± 205	2962	3343	3415	3423 ± 132
	Both sexes		947	1415	1741	2082	2594	2928	3148	3220	3243
3	Control females		12/13	12/29	1/9	1/23	2/6	3/6	4/3	4/17	5/1
	0.05% thyroprotein in ration		753 ± 73	1367	1676	2037	2446 ± 201	2921	3502	3749	3823 ± 298
	0.01% thyroprotein in ration		766 ± 64	1414	1611	1956	2295 ± 266	3019	3469	3710	3718 ± 178
	0.01% thyroprotein in ration		764 ± 39	1408	1700	2015	2449 ± 165	3069	3434	3625	3635 ± 216

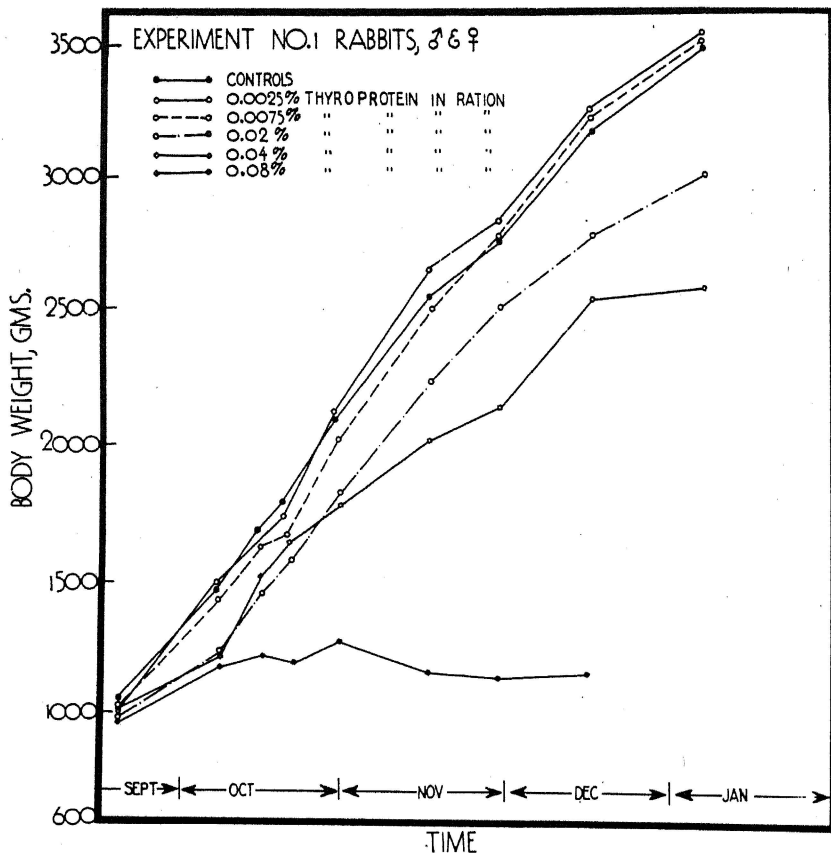


Fig. 14.—Growth curves of rabbits.

level of 0.08 per cent was extremely toxic and resulted in death of all animals within three months.

From the beginning of this experiment, it was evident that 0.02 per cent thyroprotein in the ration was slightly inhibitory to growth, but the animals on 0.0025 and 0.0075 per cent grew slightly faster than controls. This difference could easily be accounted for by chance variation, but it seemed desirable to test more animals on dosages that were not inhibitory to growth. It appeared impractical to reduce the dosage below 0.0025 per cent due to difficulties in securing a homogeneous mixture with such small amounts of thyroprotein.

Accordingly, Experiments 2 and 3 were instituted with thyroprotein being fed at the levels of 0.005 and 0.01 per cent of the ration.

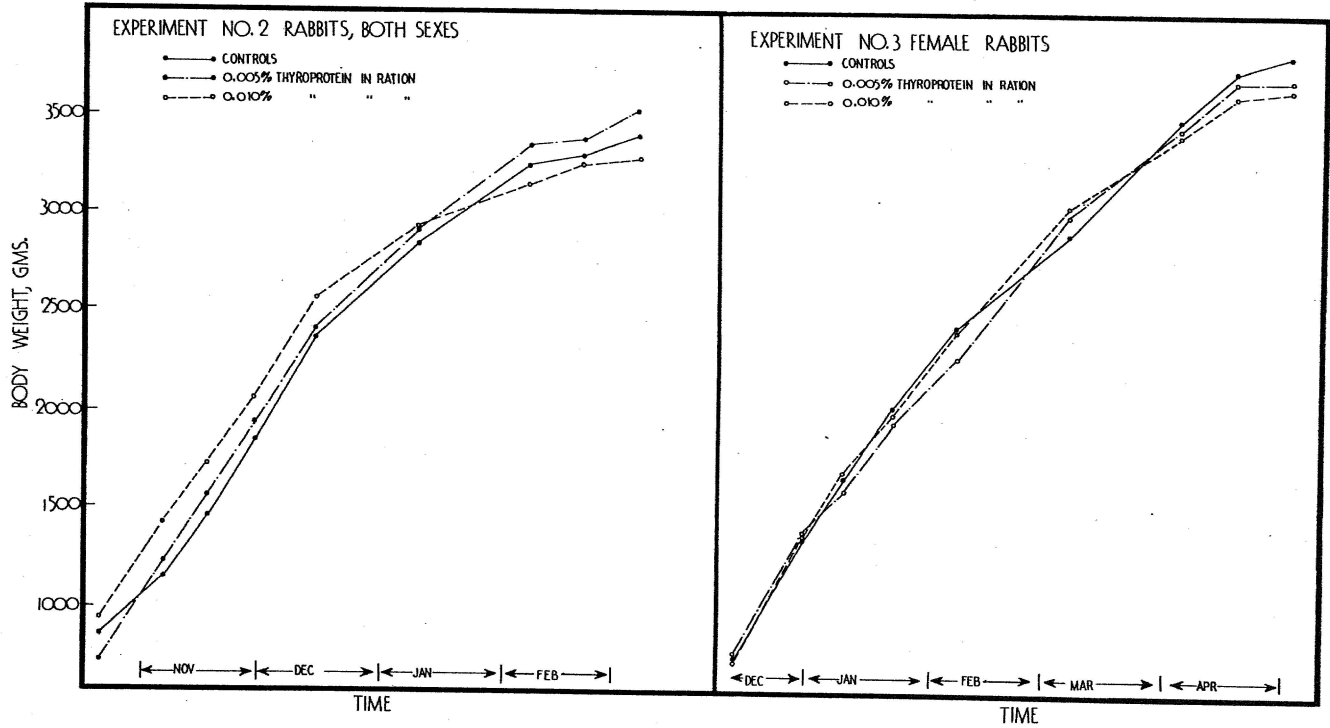


Fig. 15.—Growth curves of rabbits.

At these levels of feeding thyroprotein, growth was almost identical with that of controls (Fig. 15) with any differences easily explained by chance variation.

The results of the rabbit experiments indicate that feeding small amounts of thyroprotein did not affect the growth rate of animals used in these experiments, while feeding at the level of 0.02 per cent of ration and larger amounts progressively depressed growth.

Briefly, the results obtained from the growth experiments indicate that the growth rate of male and female mice was consistently and significantly increased by administration of a rather wide range in dosage of either synthetic thyroxine-sodium or of thyroactive iodocasein. These levels of thyroid treatment also caused an increase in feed intake. The maximum size finally attained by control and treated mice was similar.

The effects of feeding thyroprotein to rats were variable with different strains and sexes. In the Missouri strain, small amounts of thyroactive protein caused no noticeable effects on rate of growth, on the average, but there was some evidence of slight acceleration of growth of a few females. The rate of gain in body weight was depressed in both sexes of the Sprague-Dawley strain by all levels of thyroprotein given. The nose-anus length of males, however, was greater in the treated animals than in the controls.

The growth rate of male guinea pigs was slightly accelerated by low levels of thyroprotein during the early periods of the experiments. The same dosages were toxic when the weather became warm. Growth of female guinea pigs was depressed by all levels of treatment.

Small amounts of thyroprotein did not affect the rate of growth of rabbits, while larger amounts caused progressive depression of growth.

EFFECT OF THYROPROTEIN FEEDING ON ORGAN WEIGHTS

The effects on weight of most organs due to feeding relatively large amounts of thyroid material have been fairly well established. The effects of mild thyroid treatment, however, have not been investigated extensively. The internal organs were removed from many animals on various levels of treatment at the termination of the growth experiments described in the preceding section and the data obtained from weighing these organs will be presented.

Review of Literature

Hypertrophy of the heart, liver, spleen, kidneys and adrenals following administration of thyroid to experimental animals is well known (Hoskins, 1916; Herring, 1917; Hewitt, 1920; Cameron and Carmichael, 1920; Addis, Karnofsky, Lew and Poo, 1938; Sternheimer, 1939; Korenchevsky, Hall and Clapham, 1943). This finding has been almost universal among experimenters and apparently, large amounts of thyroid material are not required to elicit this response. The in-

crease in weight of these organs appears to be due to true growth since it is accompanied by increased nitrogen content of the tissues and increased mitotic figures (Addis et al., 1938; Sternheimer, 1939).

The pancreas has been reported also to undergo hypertrophy following thyroid treatment. Kojimi (1917) reported that the islet tissue of the pancreas underwent hypertrophy as judged by increased numbers of mitotic figures in pancreas from animals which had undergone thyroid treatment. Florentin and Wolff (1940) confirmed the report of Kojimi. Cameron and Carmichel (1920) reported that the weight of rabbit pancreas was increased by thyroid feeding and cited several earlier reports confirming this response. Fraenkel-Conrat et al. (1942) reported an increased insulin content of normal rat pancreas following thyroxine injection. The insulin content of pancreas of hypophysectomized rats was decreased by the same treatment.

The available evidence indicates that the weight, and to a greater extent, the activity, of the thyroid gland is depressed by thyroid administration (Cameron and Carmichael, 1920; Hewitt, 1920; Kamiovski, 1938; Azerad et al., 1939; Irwin, Reineke and Turner, 1943; Korenchevsky, Hall and Clapham, 1943).

The few reports concerning the effects of thyroid feeding on the pituitary are not in complete agreement. It seems well established that the basophilic cells are increased while the relative number of acidophils are reduced (Severinghaus et al., 1934; Campbell and Wolfe, 1934). These observations were made following large doses of thyroid.

Most reports indicate that the weight of the pituitary is decreased by thyroid feeding (Herring, 1917; Campbell and Wolfe, 1934; Evans and Simpson, 1930). However, Hoskins (1916); Herring (1917) and Cohen (1935), working with small numbers of animals, reported increased weight of pituitaries of male rats following thyroid feeding. Irwin, Reineke and Turner (1943) observed a slight reduction in pituitary weight of male chicks due to feeding small amounts of thyroid while the effect in females was inconclusive.

The effects of feeding thyroid on the thymus, testes and ovaries are not well known and reports are conflicting in most instances, the matter of dosage likely being the confusing issue.

Reinhardt and Wainman (1942) reported that small amounts of thyroxine caused an increase in size of the thymus in rats. Their data showed that the effect was unmistakable in castrate animals, but the effect on normal unoperated rats was inconclusive. The average weight in the controls was 222 mg. as compared to 235 mg. in thyroid treated animals, a difference easily within limits of chance variation. Korenchevsky, Hall and Clapham (1943) noticed no consistent effect of small amounts of thyroid on the thymus of male or female rats. Toxic dosages resulted in a decrease in relative and absolute size of thymus. Calvin (1936) fed thyroid to pregnant rats and reported a

subnormal size in thymus of the offspring. Irwin, Reineke and Turner (1943) fed nontoxic amounts of thyroid to growing chicks and observed a slight decrease in size of the thymus in males, while in females the effects were variable and inconclusive.

Apparently the effect of thyroid on the testes is variable with dosage and species. Hoskins (1916) and Da Costa and Carlson (1933) reported a depression in both absolute and relative weight of rat testes following moderately large doses of thyroid. Cameron and Carmichael (1920) and Azerad et al. (1939), feeding smaller amounts of thyroid to rats, reported an increase in the size of the testes. Smelser (1939) gave varying amounts of thyroxine or thyroid to rats and observed decreased activity of the testes and a slight reduction in size in most cases. Irwin, Reineke and Turner (1943) have observed a depression in weight of testes of growing chicks fed small amounts of thyroactive protein.

Ovarian function is abnormal in both hypo- and severe hyperthyroidism, but the effects of mild hyperthyroidism on the ovary has not been studied to any extent. Kraatz (1939) reported that brief treatment of virgin female rats with thyroid previous to mating, resulted in increased litter size if thyroid treatment was carried out in a cool environment. Treatment during hot weather reduced the litter size. Herring (1917) and Korenchevsky, Hall and Clapham (1943) reported hypertrophy of the ovaries of female rats due to thyroid treatment. Luteinization was pronounced in most cases. Weichert and Boyd (1933) have reported that typical pseudo-pregnancy was produced in rats by feeding dried thyroid. Kamiovsky (1938) fed large amounts of thyroid to rats and reported a depression of ovarian weight.

Experimental

The data on organ weights reported herein were obtained from rats used in the previously described growth experiments. The animals were killed at the end of the growth experiments or else continued on the experimental rations until such time as the animals could be killed.

The animals were killed with ether, their organs quickly removed, trimmed and weighed. The small glands were weighed immediately upon removal from the animals. The larger glands and organs were maintained in a saturated atmosphere in *petri* dishes to prevent drying until weighing. All organs were weighed within two or three hours after removal from the animal.

The data obtained by weighing the glands and organs are summarized in Tables 22 to 25, inclusive.

Comparison of the effects produced by various levels of thyroprotein treatment presents a difficult problem due to the fact that the body weights of the animals in the different groups varied. Since the normal relationship of body weight to organ weight was known

TABLE 22. ORGAN WEIGHTS OF MALE RATS OF MISSOURI STRAIN

	Controls	Treatment						
		0.005	0.01	0.02	0.04	0.08	0.16	0.32
Number of animals	49	20	25	26	22	23	11	11
Weight of animals, gm.	255	277	234	231	225	199	200	156
S.D. (1)	42	26	27	38	27	35	27	25
Length of animals, cm.	22.8	23.3	22.7	22.5	22.7	21.8	21.7	21.4
S.D.	1.6	1.0	1.7	2.0	1.8	1.9	1.3	1.2
Weight of organs								
Pituitary, mg.	7.6	8.4	7.5	7.1	7.8	7.5	7.9	6.9
S.D.	1.2	1.5	1.6	1.3	0.9	1.3	1.4	.51
Thyroid, mg.	18.5	18.2	19.6	17.6	16.7	16.2	16.0	16.6
S.D.	4.1	2.9	3.6	4.5	3.6	4.4	4.1	2.7
Adrenal, mg.	27.5	27.0	30.2	30.7	32.1	35.3	39.9	38.0
S.D.	10.3	3.9	8.6	7.1	6.9	6.3	7.2	9.4
Thymus, mg.	44.5	37.5	44.0	33.5	41.6	33.5	---	---
S.D.	4.6	6.1	3.1	6.0	7.7	8.6	---	---
Testes, gm.	3.10	3.0	3.2	3.1	3.2	2.9	2.9	2.3
S.D.	.42	.48	.35	.39	.27	.56	.39	.41
Weight of organ per 10 cm. of length.								
Pituitary, mg.	3.3	3.6	3.3	3.2	3.4	3.4	3.6	3.2
Thyroid, mg.	8.1	7.8	8.6	7.8	7.4	7.4	7.4	7.8
Adrenal, mg.	12.1	11.6	13.3	13.6	14.1	16.2	18.4	17.7
Thymus, mg.	19.5	33.7	19.4	14.9	18.3	15.4	---	---
Testes, gm.	1.4	1.3	1.4	1.4	1.4	1.3	1.3	1.1

(1) Standard deviation

only for the Sprague-Dawley females (Lauson, Golden and Sevringhaus, 1942) and for the pituitary in males of the Missouri strain (Mixner and Turner, 1942) comparison to normal values was not possible in most cases. Furthermore, the lowered weight in the treated animals was due largely to an absence of fat and comparison to a normal relationship would not be valid. Comparison based on organ weight in relationship to body length would appear to be more logical. Consequently, the organ weights were divided by body length to correct for differences in size of animals. This procedure is subject to criticism due to the fact that organ size does not vary directly with body size, but the range in body length was small and the error introduced by this factor was negligible. This correction was not necessary in Tables 23 and 24 since body length varied only slightly. Statistical comparison of such data is of doubtful value; therefore, conclusions reached regarding the effect of various levels of treatment are based on knowledge of the variability within groups of similarly treated animals and general trends produced by increasing amounts of thyroprotein.

TABLE 23. ORGAN WEIGHTS OF SPRAGUE-DAWLEY MALE RATS

	Controls	Treatment			
		Thyroprotein fed in ration, %			
		0.005	0.01	0.02	0.04
Number of animals	7	4	7	7	5
Weight of animals, gm.	330	293	285	283	271
S.D. (1)	40	17	35	9	30
Length of animals, cm.	23.5	23.5	24.0	25.0	25.1
S.D.	1.0	0.4	0.6	0.6	0.6
Organ weights					
Pituitary, mg.	9.3	9.5	9.7	8.3	8.8
S.D.	1.5	0.5	1.2	0.7	1.0
Thyroid, mg.	16.6	14.1	14.5	17.1	16.9
S.D.	2.5	2.3	2.0	2.1	1.4
Adrenal, mg.	50.0	45.0	32.8	24.0	48.0
S.D.	9.8	6.3	13.0	4.6	
Thymus, mg.	348	325	312	352	325
S.D.	50	37	44	40	53
Testes, gm.	3.08	3.17	3.10	3.12	3.10
S.D.	.06	.28	.31	.21	.14
Heart, gm.	1.06	1.04	1.09	1.07	1.28
S.D.	.14	.06	.11	0.04	0.07
Liver, gm.	11.2	11.0	11.3	11.9	13.1
S.D.	2.0	0.3	1.4	0.5	2.7
Kidney, gm.	2.58	2.54	2.47	2.49	2.88
S.D.	0.22	0.40	0.35	0.13	0.24

(1) Standard deviation

TABLE 24. ORGAN WEIGHTS OF FEMALE RATS OF THE MISSOURI STRAIN

	Controls	0.01	Treatment				
			Thyroprotein fed in ration, %				
			0.02	0.04	0.08	0.16	0.32
Number of animals	125	20	32	46	46	6	5
Weight of animals, gm.	197	202	181	194	200	187	154
S.D. (1)	22	22	16	18	18	26	19
Length of animals, cm.	20.7	20.8	20.6	20.8	20.9	20.9	19.9
S.D.	1.5	1.6	1.3	1.4	1.4	1.7	1.6
Organ weights							
Pituitary, mg.	11.4	10.3	10.3	10.1	10.8	11.2	8.9
S.D.	2.3	2.1	1.5	2.3	1.6	2.1	1.2
Thyroid, mg.	19.3	16.4	20.1	19.2	21.5	25.1	20.9
S.D.	4.9	4.3	5.1	5.4	4.3	4.0	5.2
Adrenal, mg.	38.2	37.5	49.9	46.0	52.7	63.7	48.7
S.D.	10.3	11.0	10.9	8.5	7.1	10.6	7.2
Thymus, mg.	213	227	---	216	240	---	---
S.D.	87	41	---	57	61	---	---
Ovary, mg.	67.2	64.6	64.8	76.7	83.9	122.6	48.0
S.D.	15.6	14.1	16.0	14.6	15.0	21.6	12.5

(1) Standard Deviation

TABLE 25. ORGAN WEIGHTS OF SPRAGUE-DAWLEY FEMALE RATS

	Controls	Treatment			
		Thyroprotein fed in ration, %			
		0.04	0.08	0.16	0.32
Number of animals	11	8	6	6	4
Weight of animals, gm.	229	179	168	162	119
S.D. (1)	15	14	16	21	19
Length of animals, cm.	22.9	22.1	21.5	21.2	21.1
S.D.	0.9	0.7	1.0	0.8	1.1
Organ weights					
Pituitary, mg.	13.1	8.0	6.8	6.7	7.7
S.D.	1.1	0.6	0.6	0.9	0.8
Thyroid, mg.	14.1	11.1	12.2	10.4	12.2
S.D.	2.0	3.2	3.4	1.1	2.8
Adrenal, mg.	51.8	63.0	74.0	59.4	57.4
S.D.	4.2	10.0	14.0	6.7	5.0
Thymus, mg.	165	264	272	235	218
S.D.	79	90	71	92	35
Ovary, mg.	73.6	63.9	70.6	54.1	49.8
S.D.	10.8	12.8	20.0	20.0	5.7
Heart, mg.	764	943	1000	935	995
S.D.	43	64	90	70	100
Liver, gm.	9.6	10.7	11.7	12.1	11.3
S.D.	0.6	1.3	0.8	1.5	1.8
Kidney, gm.	1.86	2.38	2.57	2.59	2.57
S.D.	0.24	0.34	0.32	0.36	0.41
Organ weight per 10 cm. of length.					
Pituitary, mg.	5.7	3.6	3.2	3.2	3.7
Thyroid, mg.	6.2	5.0	5.7	4.9	5.7
Adrenal, mg.	22.6	28.5	34.4	28.0	27.2
Thymus, mg.	72	119	127	111	103
Ovary, mg.	32.1	28.9	32.8	25.5	23.6
Heart, mg.	334	427	465	441	472
Liver, gm.	4.2	4.8	5.4	5.7	5.4
Kidney, gm.	.81	1.07	1.20	1.22	1.22

(1) Standard deviation

Taking into consideration the limitations of the data mentioned above, the effects of feeding thyroprotein on the various glands and organs were as follows:

Effect on Pituitary.—The size of the pituitaries of male rats was not affected by the levels of thyroprotein used in these trials. There was a slight indication of depression of the size of the pituitary in females of the Missouri strain and a marked depression in females of the Sprague-Dawley strain due to thyroprotein feeding.

Effect on Thyroid.—Any differences in size of the thyroids of the different groups of animals were within limits of experimental error due to the great variability of size of the gland within groups of similar treatment. However, there was a tendency for the thyroids of treated animals to be slightly lighter than those of controls, especially in females of the Sprague-Dawley strain. Histological examination of the glands showed the thyroids of all treated animals to be inactive and filled with colloid.

Effect on Adrenals.—Marked hypertrophy of the adrenals occurred in both sexes of the Missouri strain and in females of the Sprague-Dawley strain due to thyroid treatment. The extent of hypertrophy paralleled roughly the level of thyroprotein fed. This response confirms numerous reports in the literature. Surprisingly, however, the adrenals of males of the Sprague-Dawley strain were not enlarged by any dosage employed. In fact, although showing no general trend due to dosage, the adrenals of some groups of the treated animals were statistically significantly lighter than those of the controls. This point should be investigated further, because if this finding should be substantiated, it will be of great interest due to the unusual response.

Effect on Thymus.—Thymus weights of treated animals in males of either strain or of females of the Missouri strain did not vary significantly from the controls, nor was any general trend due to treatment evident. In females of the Sprague-Dawley strain, the thymus of treated animals was consistently and significantly greater than that of the controls. The average thymus weight of the controls agreed closely with the value reported by Lauson, Golden and Sevringhaus (1942) for normal females of the same strain. Their data showed that maximum thymus size was reached when the animals weighed approximately 120 grams and thereafter declined. It would appear, therefore, that the enlarged thymus observed in treated females in these trials was due in part to the smaller size of the animals and also to failure of the thymus to regress as in normal controls.

Effect on the Ovary.—Feeding thyroprotein at the levels of 0.01, 0.02 and 0.04 per cent of the ration to females of the Missouri strain did not apparently affect the weight of the ovaries. Feeding at the levels of 0.08 and 0.16 caused an increased weight of the ovaries with histological evidence of intense luteinization. This observation agrees with certain reports in the literature. Feeding thyroprotein at the level of 0.32 per cent of the ration, an extremely toxic dosage, resulted in ovaries somewhat lighter in weight than in the controls. The ovaries of the Sprague-Dawley strain were depressed on all levels of treatment. Sexual immaturity may possibly account for this result since the treated animals were smaller in size.

Effect on Testes.—Moderate amounts of thyroprotein did not result in any apparent change in the size of the testes. Feeding

thyroprotein at the level of 0.32 per cent in the ration, an extremely toxic dosage, to males of the Missouri strain resulted in a relatively light testis. Whether this was due to an active depression on the testes or simply a non-specific result of depressed growth is not known.

Effect on Heart, Liver and Kidneys.—Both absolute and relative weight of these organs was increased due to thyroprotein feeding of male and female Sprague-Dawley rats. The degree of enlargement paralleled the amount of thyroprotein fed. With the smallest dosages in males, no noticeable effect occurred.

It should be pointed out that conclusions based on a study of this sort should be accepted with a great deal of reservation. In order to get a clear picture of the changes in endocrine glands due to thyroid treatment, it would be necessary to follow the changes from early life to maturity to determine any shift in sequence of changes. Furthermore, statements that thyroid treatment produces certain changes should be qualified by levels of treatment with which these changes occur. For example, it is well known that thyroid produces enlargement of the heart, liver and kidneys, and the data from these experiments bear out the fact. On the other hand, the lowest dosages given to male rats did not bring about this response (Table 23).

The lowest levels of thyroprotein treatment given male rats apparently did not affect the weight of any of the glands or organs weighed. Larger amounts in either sex resulted in hypertrophy of the heart, liver and kidneys. The adrenals were enlarged in most cases, but males of the Sprague-Dawley strain failed to show this response. The pituitary weight of females was depressed by thyroprotein treatment, while in males, pituitary size was unaffected. The weight of the thyroid was not significantly altered, but there was a tendency for thyroids of treated animals to be reduced in size and they showed histological evidence of inactivity. The ovaries were enlarged by moderate levels of thyroprotein in the Missouri strain while larger dosages caused a depression. Ovaries of the Sprague-Dawley strain were depressed by all levels of treatment. Moderate amounts of thyroprotein did not affect testes weight, while large amounts caused a reduced weight. The thymus was enlarged in thyroprotein-fed females of the Sprague-Dawley strain, but was unaffected in the other animals.

Thus, the effects of thyroprotein feeding on organ weights was variable with dosage, sex and strain of animals.

EFFECT OF THYROPROTEIN FEEDING ON HYPOPHYSEAL THYROTROPIN AND GONADOTROPIN

A knowledge of the effects of thyroid activity on the hormones of the pituitary will be necessary for a better understanding of thyroid physiology. Direct experimental evidence on this point is meager at the present time.

When the animals were killed at the end of the experiments described in the preceding sections, their pituitaries were removed, weighed and kept frozen until enough pituitary substance for assay purposes became available. The results of the assays made on these pituitaries will be reported.

Review of Literature

Although there is good indirect evidence that thyroid activity influences the pituitary hormones, very few assays of pituitaries from animals with varying levels of thyroid activity have been reported.

Reineke, Bergman and Turner (1941) and Stein and Lisle (1942) have reviewed the available evidence concerning the effects of thyroidectomy on the pituitary hormones. The reports concerning thyrotropin and lactogen are inconclusive and no very marked departure from normal concentrations has been reported. Reports agree that gonadotropin is reduced in hypothyroidism. Reineke et al. (1941) reported that the pituitaries from thyroidectomized goats showed a marked decrease in the factor which elevates blood sugar and in gonadotropin, while lactogen and thyrotropin were unchanged.

It would be expected that the thyrotropic potency of the pituitary would be reduced following thyroid treatment and Kuchinsky (1933) and Hohlweg and Junkmann (1933) have reported this to be the case. There is indirect evidence also that hyperthyroidism results in decreased thyrotropin secretion. The stimulation of thyroid glands of test animals by injection of thyrotropic hormone is reduced by simultaneous injection of thyroxine or feeding of thyroid (Aron et al., 1931; Loeser and Thompson, 1941). Loeb and Seibert (1930) fed thyroid to partially thyroidectomized guinea pigs and reported that the thyroid remnant failed to hypertrophy and that the acinar epithelium was low and the acini filled with hard colloid. A decrease in thyrotropic hormone content of blood and urine has been reported in thyrotoxicosis (Cope, 1938), although the evidence is not conclusive. Reforzo-Membrives (1943) has reported a thyroid-inhibiting principle in pituitaries from animals receiving thyroid treatment. He injected normal and hypophysectomized guinea pigs with pituitaries from thyroid-fed rats and reported that the weight of the thyroid glands and metabolism of these guinea pigs were reduced below that of control animals.

Increased gonadotropic potency of the pituitary due to thyroid feeding has been reported by Evans and Simpson (1930), Van Horn (1933) and Cohen (1935). No other reports on the effect of thyroid feeding on pituitary gonadotropin were encountered. Smelser (1939), however, reported that experimental hyperthyroidism did not affect the gonadotropic hormone in the blood of castrate rats.

Only one report concerning the effect of thyroid feeding on pituitary lactogenic hormone is available. Reece and Turner (1937)

found that injection of 0.01 mg. thyroxine daily into rats produced no significant change in pituitary lactogen, while larger doses which were toxic caused a marked depression. Hurst et al. (1941) injected thyroxine into goats in amounts which increased milk flow, but observed no change in the lactogenic hormone excreted in the urine.

Experimental

The pituitaries were removed from the animals at the end of the growth experiments previously described and kept frozen until sufficient quantity of substance became available for biological assay. It was planned originally to assay the pituitaries for growth hormone potency but no suitable assay method was developed. A part of the pituitaries was lost accidentally and as a result only a limited amount of material was available for assay. This was assayed for thyrotropic and gonadotropic potency by the method of Bergman and Turner (1939) using day old male White Leghorn chicks as the assay animals. The pituitary material was ground in a smaller mortar and suspended in distilled water before injection into chicks.

Pituitaries from control animals and animals receiving various levels of thyroprotein in the ration were available from male and female rats of the Missouri strain and female rabbits. The assay data are summarized in Table 26.

TABLE 26. DATA OBTAINED FROM PITUITARY ASSAYS

Treatment Given Chicks	No. of Chicks	Mgm. Pit. Injected	Av. Wt. of Chick Thyroids	Av. Wt. of Chick Gonads
Control chicks	15	8	2.88	10.3
Injected with pit. from male rats				
Control rats	14	8	6.26	17.9
0.005% thyroprotein in ration	10	8	4.12	15.3
0.020% thyroprotein in ration	11	8	3.48	14.2
0.040% thyroprotein in ration	14	8	2.58	15.8
0.080% thyroprotein in ration	15	8	2.70	15.7
Control chicks	14	10	3.01	8.7
Injected with pit. from female rats				
Control rats	13	10	5.44	8.6
0.01 and 0.02% thyroprotein in ration	12	10	4.51	12.8
0.08% thyroprotein in ration	14	10	3.97	8.8
0.16 and 0.32% thyroprotein in ration	13	10	3.43	13.1
Control chicks	15	15	3.48	12.6
Injected with pit. from female rabbits				
Control rabbits	14	15	4.38	21.8
0.005% thyroprotein in ration	13	15	4.47	20.5
0.010% thyroprotein in ration	12	15	4.08	18.4

Pituitary material from each type of animal was injected at a constant level so that the thyroid weight of chicks receiving pituitary from control animals could be compared directly with the weights of those receiving pituitary from the thyroprotein-fed animals. Units of

thyrotropic and gonadotropic hormone contained in the various groups of pituitary were not calculated for the reason that the level of stimulation was out of the range of sensitive response in many cases. Direct comparison of the weight of chick glands appears to be suitable for comparative purposes.

The effect of feeding thyroprotein on the thyrotropic potency of rat pituitaries was unmistakable, resulting in a marked depression in thyrotropic potency in every instance, with the extent of depression being positively correlated with dosage of thyroprotein employed. Apparently, the thyrotropic potency of male rat pituitaries was depressed more by a given level of thyroprotein than the potency of the female pituitaries. The higher levels of thyroprotein treatment resulted in pituitaries which, at the levels given, caused no stimulation whatever of the chick thyroids. There was no significant difference in stimulation due to the various batches of rabbit pituitary. The amount of pituitary substance given was too low for a sensitive assay, however, so the results were inconclusive.

The injection of pituitary substance from control and thyroprotein-fed animals resulted in chick gonads of comparable weight in all three assays. Thus, there was no indication of a change in gonadotropic potency of the pituitaries due to the thyroprotein feeding of the animals used in these trials. This observation agrees with the report of Smelser (1939), of unchanged gonadotropic potency of the blood of castrate rats following thyroid treatment. It fails to confirm the several early reports of increased pituitary gonadotropin following thyroid treatment, and it is suggested that the age and sexual activity of the animals involved may have influenced the results. Gonadotropin secretion in animals at the height of sexual activity, as in these experiments, would not be expected to be increased easily.

These assays have demonstrated clearly that treatment with thyroactive preparations result in decreased thyrotropin content of the pituitary. Gonadotropic potency of pituitaries was not affected by thyroprotein feeding in these trials.

DISCUSSION

Probably the most striking feature arising from these investigations has been the marked variability in response of different animals to thyroid treatment. Differences in species, strains and sexes were very evident. Furthermore, there was a marked variability in the response of similar animals within a subgroup. This was noted especially in groups of animals treated with rather large amounts of thyroactive material which were toxic in effect. In such groups, the growth of the majority of the animals was markedly depressed and most of the individuals assumed an unthrifty appearance. There were occasional animals, however, which survived such treatment apparently unharmed, grew normally, or nearly so, and had a thrifty appearance.

The marked variability in response undoubtedly accounts for much of the confusion existing in the literature concerning the effects of thyroid treatment. In fact, many of the conflicting reports have been confirmed in the course of this investigation. This variability in response emphasizes the danger in generalization of results and extrapolation from one type of animal to another. Determination of the underlying causes of these variations in response will add much to the knowledge of animal physiology and is a fertile field for future investigation.

Mice used in these experiments responded uniformly over a rather wide range in dosage with an accelerated rate of growth. This response confirms the report of Robertson (1928). It is significant, however, that Dr. J. A. Cameron of the Medical School University of Missouri (unpublished data) has fed 0.08 per cent thyroactive casein in the ration to a cancer strain of mice (Strong A) and observed inhibition of growth. The same amount induced accelerated growth in mice used in these experiments (Experiment 9).

The only other indications of accelerated growth in body weight occurred in male guinea pigs and inconsistently in female rats of the Missouri strain. During the first few weeks of the guinea pig experiments, the growth curves of treated males on low levels of treatment were slightly above those of controls. This difference was eventually reversed, however, and the treated animals actually lost weight with the advent of warm weather. The growth rate of control guinea pigs was slow throughout the experiments due to a relatively unpalatable ration, and it is doubtful if the same response to thyroprotein feeding would have occurred on a better ration.

Results were inconclusive with female rats of the Missouri strain, but the earlier experiments indicated a slight acceleration of growth of certain females. Experiments repeated later did not confirm these indications, however. Thyroprotein feeding at any of the levels employed was inhibitory to body weight gain in both sexes of Sprague-Dawley rats. At the end of the experiments, however, male rats of the Sprague-Dawley strain had a greater nose-anus length than controls. Bircher (1910) has likewise observed increased skeletal growth with reduced body weight of male rats treated with thyroid tissue.

The response to thyroid treatment is undoubtedly influenced by environmental conditions, and it is emphasized that the results obtained in these trials might have been different had environment (temperature and humidity) been changed. Another factor that should be considered is the increased sensitivity of organisms to thyroid materials with increased age. Treatment during these trials was held relatively constant so that the degree of hyperthyroidism increased with age. This change in sensitivity should be considered when thyroid materials are given over a period of time.

Apparently, the homeostatic mechanism of the animals was impaired somewhat by treatment with thyroactive preparations, and the treated animals were more sensitive to environmental changes than controls. The indication was present even with dosages which did not inhibit growth and is illustrated well in Experiment 1 with mice (Fig. 1). There was a tendency for the growth curves of treated animals to be more uneven throughout than those of controls. It would appear that small amounts of thyroid material which would no more than replace the thyroxine produced by the animal's own thyroid, would not produce unfavorable effects so long as environmental conditions are favorable since it seems well established that excessive thyroid activity results in depression of the animal's own thyroid. However, this adjustment removes one of the factors which contributes to the steady state of the animal, and unfavorable environmental conditions cannot be met by such animals as effectively as by normal animals. Thus, animals receiving nontoxic amounts of thyroactive materials may be at some disadvantage in adjusting themselves to high environmental temperature.

Mice and female rats were relatively tolerant of thyroactive preparations while male rats and to a greater degree guinea pigs and rabbits were relatively intolerant of such materials. A comparison of the sensitivity of mice and male rats is interesting since *a priori* considerations might suggest the two species would react similarly. Mice receiving 0.32 per cent thyroprotein in the ration (Experiment 9) consumed an average of approximately 12 mg. of thyroprotein daily and grew at a markedly accelerated rate. Male rats receiving 0.04 per cent in the ration consumed not more than 10.0 mg. thyroprotein daily and their growth was greatly depressed by this amount. Thus, mice, about one-tenth the size of male rats, not only tolerated amounts of thyroprotein which were toxic to rats, but actually thrived upon it. The tolerance of mice for thyroactive materials is therefore remarkable, and it is suggested that this response may be influenced by the ability to dissipate heat.

Another interesting observation was the failure of low levels of thyroprotein to increase the metabolism of Sprague-Dawley male rats. Since the thyrotropic hormone of the pituitary and thyroid activity were decreased by thyroprotein feeding, it might be suggested that the small amount of thyroid activity contained in the thyroprotein consumed merely replaced the reduced output of the thyroid, and that net thyroid activity remained unchanged. This assumption would not explain the reduced rate of gain in body weight, however.

The results of this investigation indicate that there are certain strains of some species of animals that will respond to mild thyroid treatment by acceleration of growth rate. This was illustrated well in the experiments with mice. On the other hand, the response of rats, rabbits and guinea pigs to treatment would indicate that the

welfare of most growing animals cannot be improved by administration of thyroactive materials and that in certain cases, treatment at any level may be detrimental. Thus, treatment of male Sprague-Dawley rats with extremely low dosages caused a depression of body weight gains and unthrifty appearance. This indicates that in many animals, their own thyroid produces all the thyroxine that is compatible with the well-being of the individual during growth. Certain individuals, and even strains of animals that have been developed under artificial conditions, are probably hypothyroid and small amounts of thyroid may be beneficial.

SUMMARY

Four different species of animals, including mice, rats, guinea pigs and rabbits, have been treated with graded amounts of thyroactive preparations to determine the effects of mild hyperthyroidism on growing animals. The dosage for each species extended over a range which at the lowest level did not apparently affect the growth of the animals, and at the highest level, inhibited growth.

It has been demonstrated that feeding or injecting thyroactively active iodocasein produces effects comparable to synthetic thyroxine.

The rate of growth of young mice was consistently accelerated by injecting 0.01 to 0.03 mg. crystalline thyroxine-sodium daily or by feeding 0.04 to 0.32 per cent thyroactive iodocasein in the ration. The period of accelerated growth extended for a period of approximately five weeks, after which time the controls gradually overtook the treated animals and the maximum size attained by the two groups was similar. Larger amounts of thyroprotein or thyroxine were toxic and inhibited growth. Smaller amounts did not affect growth greatly, although there was a tendency for the treated animals to grow somewhat slower than normal. Feed intake was increased by treatment.

The percentage of water and protein in the tissues of treated mice was increased, and fat content was decreased as compared to the composition of the tissues of untreated mice. The average total energy contained in the carcass was similar in the control and treated animals.

Thyroactive iodocasein fed up to the level of 0.04 per cent of the ration apparently did not affect the growth rate of female rats of the Missouri strain. Fed at the levels of 0.04 and 0.08 per cent of the ration, it caused indications of a limited acceleration of growth in some females of the Missouri strain, but for the most part their growth rate was not affected or else was depressed. Larger amounts resulted in marked inhibition of growth. Growth rate of males of the Missouri strain was not affected by the lowest levels of thyroprotein and was depressed by larger amounts. The rate of gain in both sexes of Sprague-Dawley rats was inhibited by all levels of thyroprotein fed. The nose-anus length of the males of this strain, however, was slightly increased in the thyroprotein-fed animals.

The growth rate of male guinea pigs was slightly accelerated during the early periods of the experiments by small amounts of thyroactive iodocasein. The same dosages became toxic with the arrival of warm weather. Growth of female guinea pigs was depressed on all levels of thyroprotein given.

The growth rate of male or female rabbits was unaffected by extremely small amounts of thyroprotein, while larger amounts caused progressive depression of growth.

The different animals ranked in order of their ability to tolerate thyroid treatment are as follows: mice, female rats, male rats, rabbits and guinea pigs.

The lowest levels of thyroactive protein fed to male rats apparently did not affect the weight of any of the glands or organs weighed. Larger amounts fed to either sex resulted in hypertrophy of the heart, liver and kidneys. The adrenals were enlarged due to treatment in most cases, but males of the Sprague-Dawley strain failed to show this response. The pituitaries of females were depressed by thyroprotein feeding, while male pituitaries were unaffected. The weight of the thyroid was not significantly altered, but there was a tendency for thyroids of treated animals to be reduced in size. The ovaries were enlarged by moderate levels of thyroprotein in the Missouri strain, while larger amounts caused a depression. The ovaries of Sprague-Dawley animals were depressed by all levels of thyroprotein given. Small or moderate amounts of thyroprotein did not affect the weight of the testes, while larger amounts resulted in a reduced weight. The thymus was enlarged in treated females of the Sprague-Dawley strain, but was unaffected in the other animals.

The thyrotropic potency of rat pituitaries was reduced by thyroprotein feeding, while gonadotropic potency apparently was not affected by any level of treatment.

BIBLIOGRAPHY

- Abramson, D. L., and Sidney, M. F. 1942. Resting peripheral blood flow in the hyperthyroid state. *Arch. Int. Med.* 69:409.
- Addis, T., Karnofsky, D., Lew, W., and Poo, J. L. 1938. The protein content of the organs and tissues of the body after administration of thyroxine and dinitrophenol and after thyroidectomy. *J. Biol. Chem.* 124:33.
- Allison, J. B., Glaser, C., and Leonard, S. L. 1939. The relation of thyroid to creatine and creatinine excretion in the rat. *Proc. Soc. Exper. Biol. and Med.* 42:491.
- Allison, J. B., and Leonard, S. L. 1941. The effects of estrogen and thyroidectomy in female rats on the excretion of creatine and creatinine. *Am. J. Physiol.* 132:185.
- Althausen, T. L. 1939. A study of the influence of the thyroid gland on the digestive tract. *Tr. Am. Soc. Study of Goiter*, p. 37.
- Althausen, T. L., and Stockholm, M. 1938. Influence of the thyroid gland on absorption in the digestive tract. *Am. J. Physiol.* 123:577.
- Andrews, F. N., and Bullard, J. F. 1940. The effect of partial thyroidectomy on the fattening of steers. *Proc. Am. Soc. Animal Prod.*, p. 112.
- Aron, M., Van Caulaert, C., and Stahl, J. 1931. L'équilibre entre l'hormone pré hypophysaire et l'hormone thyroïdienne dans le milieu intérieur, à l'état normal et à l'état pathologique. *Compt. rend. Soc. de biol.* 107:64.
- Aub, J. C., Bauer, W., Heath, C., and Ropes, M. 1927. Studies of calcium and phosphorus metabolism. III. The effects of the thyroid hormone and thyroid disease. *J. Clin. Investigation* 7:97.
- Azérad, E., Simonnett, H. et Wolfshaut, C. 1939. Etude expérimentale des effets de la thyroxine et des hormones sexuelles féminines (folliculine, progesterone) sur le rat male adulte. *Rev. Franc. Endocrinol.* 17:86.
- Basinger, H. R. 1916. The control of experimental cretinism. *Arch. Int. Med.* 17:260.
- Baumann, E. 1896. Über das Normale Vorkommen von Jod im Thierkörper. *Z. Physiol. Chem.* 21:319.
- Becks, H., Ray, R. D., Simpson, M. E., and Evans, H. M. 1942. Effect of thyroxin and the anterior pituitary growth hormone on endochondral ossification. *Arch. Path.* 34:334.
- Bergman, A. J., and Turner, C. W. 1939. A comparison of the guinea pig and chick thyroid in the assay of thyrotropic hormone. *Endocrinology* 24:656.
- Bergman, A. J., and Turner, C. W. 1941. Thyrotropic hormone content of rabbit pituitary during growth. *Endocrinology* 29:313.
- Bernard, Claude, 1855. *Lecons de physiologie experimentale.* Les Ouvres, T 1 et 2, p. 96. Paris.
- Bernard, Claude. 1859. *Lecons sur les propriétés physiologiques des liquides de l'organisme.* Les Ouvres, T 6 et 7, p. 411.
- Binswanger, F. 1936. *Studien zur Physiologie der Schilddrüse.* III. Schilddrüse und Wachstum (Studien am Hund). *Endokrinologie* 17:150.
- Bircher, E. 1910. Zur Wirkung der Thyreodintabletten auf das Knochenwachstum. *Arch. f. Klin. Chir.* 91:554.
- Bodansky, A. 1924. Effect of thyroxin upon the blood sugar of normal and thyroidectomized sheep. *Am. J. Physiol.* 69:518.
- Boettiger, E., and Osborn, C. M. 1938. A study of natural growth and ossification in hereditary dwarf mice. *Endocrinology* 22:447.
- Breitbarth, B. 1940. Study on the phosphate metabolism in congenital athyroidism. *Ztschr. f. Kinder.* 62:52.
- Brody, S., and Frankenbach, R. F. 1942. Growth and development. LIV. Age changes in size, energy metabolism and cardio-respiratory activities of thyroidectomized cattle. *Mo. Agri. Exper. Sta. Res. Bul.* 349.
- Calvin, D. B. 1936. Effect of thyroid feeding and thyroidectomy on thymus size in new-born rat. *Proc. Soc. Exper. Biol. and Med.* 34:724.

- Cameron, A. T., and Carmichael, J. 1920. The comparative effects of thyroid and iodine feeding on growth of rabbits and white rats. *J. Biol. Chem.* 45:69.
- Cameron, A. T., and Carmichael, J. 1921. The effect of thyroxine on growth in white rats and rabbits. *J. Biol. Chem.* 46:35.
- Campbell, Mary, and Wolfe, J. M. 1934. Effect of feeding thyroid on anterior hypophysis of the female albino rat. *Proc. Soc. Exper. Biol. and Med.* 32:205.
- Canzanelli, R. G., and Rapport, D. 1939. Effect of thyroglobulin and thyroxine on oxygen uptake of tissue *in vitro*. *Endocrinology* 25:707.
- Castleton, K. B., and Alvarez, W. C. 1941. The rate of rhythmic contraction of the small bowel of rabbits as influenced by experimentally produced hyperthyroidism. *Am. J. Dig. Dis.* 8:473.
- Chang, H. C. 1926. The specific influence of the thyroid gland on hair growth. *Am. J. Physiol.* 77:562.
- Chapman, A. 1941. Extrathyroidal iodine metabolism. *Endocrinology* 29:686.
- Cohen, R. 1935. Effect of experimentally produced hyperthyroidism upon the reproductive and associated organs of the male rat. *Am. J. Anat.* 56:143.
- Cope, C. L. 1938. The anterior lobe in Graves' disease and in myxedema. *Quart. J. Med.* 7:151.
- Coryn, G. 1939. Recherche experimentale sur l'influence des glandes endocrines sur l'histologie du cartilage de conjugaison. *Ann. d'Anat. path.* 16:27.
- Curling, T. B. 1850. Two cases of absence of the thyroid body and symmetric swellings of fat tissue at sides of the neck, connected with defective cerebral development. *Med. Chir. Trans.* 33:303.
- Da Costa, E., and Carlson, A. J. 1933. The effect of feeding desiccated thyroid upon the sexual maturation of the albino rat. *Am. J. Physiol.* 104:247.
- Davenport, C. B., and Swingle, W. W. 1927. Effects of operations upon the thyroid glands of female mice on the growth of their offspring. *J. Exper. Zool.* 48:395.
- Dorff, G. B. 1935. Masked hypothyroidism. *J. Pediat.* 6:788.
- Dott, N. M. 1923. An investigation into the functions of the pituitary and thyroid glands. I. Technique of their experimental surgery and a summary of their results. *Quart. J. Exper. Physiol.* 13:241.
- Drill, V. A. 1938. The effect of experimental hyperthyroidism on the vitamin B content of some rat tissues. *Am. J. Physiol.* 122:486.
- Drill, V. A. 1941. Bone calcium during hyperthyroidism. *Proc. Soc. Exper. Biol. and Med.* 48:448.
- Dulzetto, F. 1928. The action of thyroid extracts on somatic growth of the albino rat. *Boll. d. Soc. ital. d. biol. sper.* 3:945. *Chem. Abs.* 23:2748.
- Durrant, E. P. 1928. Effect of desiccated thyroid feeding on growth of the guinea pig. *Am. J. Physiol.* 85:364.
- Eidinova, M. 1936. The action of hormones upon the excitability of the digestive glands. II. Actions of thyroid preparations upon gastric secretion as determined by the functional state of the glandular apparatus. *Bull. biol. Med. exptl. U. S. S. R.* 1:316.
- Elijah, H. D., and Turner, C. W. 1942. The weight and thyrotropic hormone content of anterior pituitary of swine. *Mo. Agr. Exp. Sta. Res. Bul.* 357.
- Entenman, C., Chaikoff, I. L., and Reichert, F. L. 1942. Role of nutrition in response of blood lipids to thyroidectomy. *Endocrinology* 30:794.
- Evans, H. M., and Simpson, M. E. 1930. Some effects on the hypophysis of hyper- and hypothyroidism. *Anat. Rec.* 45:215.
- Evans, H. M., Simpson, M. E., and Pencharz, R. I. 1939. Relation between the growth promoting effects of the pituitary and the thyroid hormone. *Endocrinology* 25:175.

- Farr, L. E., and Alpert, L. K. 1940. The effect of endocrine extracts on the amino acids in the blood with incidental findings on blood sugar and urea. *Am. J. Physiol.* 128:772.
- Fleischmann, W., Schumacker, H. B., and Straus, W. L. 1943. Influence of age on the effect of thyroidectomy in the Rhesus monkey. *Endocrinology* 32:238.
- Florentin, P., and Wolff, R. 1940. Effet de l'hormone thyrotrope anté-hypophysaire sur le pancréas endocrine. *Essai d'interprétation. Compt. rend Soc. biol.* 133:138.
- Fox, E. L. 1892. A case of myxoedema treated by taking extracts of thyroid by mouth. *Brit. Med. J.* vol. 2 for 1892, p. 941.
- Fraenkel-Conrat, H., Herring, V. V., Simpson, M. E., and Evans, H. M. 1942. Effect of thyroxin on the insulin content of the rat pancreas. *Endocrinology* 30:485.
- Garrison, F. H. 1917. An introduction to the history of medicine. 2nd ed. W. B. Saunders Co., Philadelphia and London.
- Glaser, Charles. 1942. Effect of thyroidectomy on the excretion and retention of creatine and creatinine in the male rat. *Endocrinology* 30:564.
- Graham, W. R. 1934a. The effect of thyroidectomy and thyroid feeding on milk secretion and fat production of cows. *J. Nutrition* 7:407.
- Graham, W. R. 1934b. The action of thyroxine on milk and milk-fat production of cows. *Biochem. J.* 28:1368.
- Graver, Robert C., Starkey, W. F. and Saier, E. 1942. The influence of stilbestrol and thyroxine on galactose absorption and liver function. *Endocrinology* 30:474.
- Hammett, F. S. 1923. Studies on the thyroid apparatus. IX. The effects of the loss of the thyroid and parathyroid glands at 100 days of age on the growth of body length, body weight and tail length of male and female albino rats. *Am. J. Physiol.* 63:218.
- Hammett, F. S. 1926. Studies on the thyroid apparatus. XXIX. The role of the thyroid apparatus in growth. *Am. J. Physiol.* 76:69.
- Hammett, F. S. 1927a. Studies on the thyroid apparatus. XXXIX. The role of the thyroid and parathyroid glands in growth of the heart and lungs. *Am. J. Anat.* 39:219.
- Hammett, F. S. 1927b. Studies of the thyroid apparatus. XL. The role of the thyroid apparatus in growth of the liver, kidneys and spleen. *Am. J. Anat.* 39:239.
- Hammett, F. S. 1929. Thyroid and growth. *Quart. Rev. Biol.* 4:353.
- Harrington, C. R. 1933. The thyroid gland—its chemistry and physiology. Oxford University Press. London.
- Herman, H. A., Graham, W. R., and Turner, C. W. 1938. The effect of thyroid and thyroxine on milk secretion in dairy cattle. *Mo. Agr. Exper. Sta. Res.* 275.
- Herring, P. T. 1917. The action of thyroid upon growth of the body organs of the rat. *Quart. J. Physiol.* 11:231.
- Hertz, S., and Galli-Mainini, C. 1941. Effect of thyroid hormone on growth in thyrotoxic and myxedematous children and adolescents. *J. Clin. Endocrinology* 1:518.
- Hewitt, James Arthur. 1920. The effect of administration of small amounts of thyroid gland on the size and weight of certain organs in the male white rat. *Quart. J. Exper. Physiol.* 12:347.
- Hohlweg, W., and Junkmann, K. 1933. Über die Beziehungen Zwischen Hypophysen-vorderlappen und Schilddrüsen. *Arch. f. d. ges. Physiol.* 232:148.
- Hoskins, E. R. 1916. The growth of the body and organs of the albino rat as affected by feeding various ductless glands. *J. Exper. Zool.* 21:295.
- Hoskins, M. M. 1927. The effects of acetyl-thyroxin on the new-born rat. *J. Exper. Zool.* 48:373.
- Horsley, Victor A. 1884. Quoted by Paget (1919).
- Hunter, Donald. 1930. Calcium and phosphorus metabolism. *Lancet* 1:947.

- Hunter, D. 1934. Studies in calcium and phosphorus metabolism in generalized diseases of the bones. *Proc. Roy. Soc. Med.* 28:1619.
- Hurst, V., Meites, J., and Turner, C. W. 1941. The effect of thyroxine on lactogenic hormone in urine of dairy goats. *J. Dairy Sci.* 24:499.
- Johnston, Joseph A. 1941. Factors influencing retention of nitrogen and calcium in period of growth. V. Further evidence of the anabolic effect of thyroid on calcium metabolism. *Am. J. Dis. Child.* 62:1172.
- Johnston, J. A., and Maroney, J. W. 1939a. Factors affecting retention of nitrogen and calcium in period of growth. I. Effect of thyroid on nitrogen retention. *Am. J. Dis. Child.* 58:965.
- Johnston, J. A., and Maroney, J. W. 1939b. Factors affecting retention of nitrogen and calcium in growth. II. Effect of thyroid on calcium retention. *Am. J. Dis. Child.* 58:1187.
- Irwin, M. R., Reineke, E. P., and Turner, C. W. 1943. Effect of feeding thyroactive iodocasein on growth, feathering, and weights of glands of young chicks. *Poultry Sci.* (in press).
- Kamiovski, N. O. 1938. Investigation of the interaction between thyroid gland and ovaries. I. The effect of hyperthyroidism on the ovaries of rats. *Problemy Endocrinol. (U. S. S. R.)* 3:8.
- Kendall, Edward C. 1929. Thyroxine. Chemical Catalog Company. New York.
- Kocher, T. 1878. *Cor.-Bl. f. Sweiz Aerzte Basel.* 8:702. Quoted by Garrison (1917).
- Kocher, T. 1883. Über Kropfextirpation und ihre Folgen. *Arch. f. klin. Chir.* 29:254.
- Koepfen, H. 1892. Über Knochenerkrankungen bei Morbus Basedowii. *Neurologisches Centralblatt.* 11:219.
- Koger, M., Hurst, V., and Turner, C. W. 1942. Relation of thyroid to growth. I. Effects of crystalline thyroxine upon rate of growth, food intake and body composition of female albino mice. *Endocrinology* 31:237.
- Kojimi, M. 1917. Effect upon metabolism of castration, of thyroidectomy, of parathyroidectomy and of thyroid and parathyroid feeding. *Quart. J. Exper. Physiol.* 11:351.
- Kommerell, B. 1929. Über den Einfluss von Schilddrüsendarreichung auf den Eiweiß- und Fettstoffwechsel. *Biochem. Z.* 208:112.
- König, W. 1937. The treatment of slow-healing bone fractures. *Therapie Gegenwart.* 78:17. *Chem. Abs.* 31:4722.
- Korenchevsky, V., Hall, K. and Clapham, B. 1943. Effects of vitamins on experimental hyperthyroidism. *Brit. Med. J.*, vol. 1 for 1943, p. 245.
- Kraatz, C. P. 1939. Effect of brief experimental hyperthyroidism on reproduction in the rat. *Proc. Soc. Exper. Biol. and Med.* 40:499.
- Kuchinsky, G. 1933. Über die Bedingungen der Sekretion des thyreo-tropen Hormons der Hypophyse. *Arch. f. exper. Path. u. Pharmakol.* 170:510.
- Kunde, Margarete M. 1926. Studies on experimental cretinism. III. Nutritional disturbances, pellegra and xerophthalmia. *Proc. Soc. Exper. Biol. and Med.* 23:812.
- Laqueur, E., Dingemans, E., and Freud, J. 1941. The influence of the hypophysis and thyroid on the growth of rats. *Acta. brevia Neerl.* 11:46.
- Lauson, H. D., Golden, J. B., and Sevringhaus, E. L. 1942. Normal endocrine gland weights of female rats of the Sprague-Dawley strain throughout the growth period and adult life. *Endocrinology* 31:46.
- Lawson, W., Fleischmann, W., and Block, W. 1941a. Hypothyroidism in childhood. I. The basal metabolic rate, serum cholesterol and urinary creatine before treatment. *J. Clin. Endocrinology* 1:3.
- Lawson, W., Fleischmann, W., and Block, W. 1941b. Hypothyroidism in childhood. II. Sensitivity to thyroid medication as measured by serum cholesterol and creatine excretion. *J. Clin. Endocrinology* 1:14.
- Lerman, Jacob. 1941. Physiology of the thyroid gland. *J. A. M. A.* 117:349.

- Loeb, Leo, and Siebert, W. J. 1930. Oral administration of anterior pituitary tablets and our laboratory preparations on compensatory hypertrophy of thyroid gland. *Proc. Eoc. Exper. Biol. and Med.* 27:495.
- Loeser, A., and Thompson, K. W. 1934. Hypophysenvorderlappen, Jod, und Schilddrüse. *Der Mechanismus der Schilddrüsenwirkung des Jods.* *Endokrinologie* 14:144.
- Lombard, H. C. 1883. Sur les fonctions du corps thyroïde d'Après des documents d'Après des documents recents. *Rev. Mé. de la Suisse Rom.* 3:593.
- Maeda, M. 1927. Thyroid and tissue respiration. *Folia Pharmacol. Japan* 3:796.
- Magnus-Levy, Adolf. 1897. Gas—und Stoffwechseluntersuchungen bei Schilddrüsenfütterung, Myxodema, Morbus Basedowii und Fettleibigkeit. *Ztschr. f. klin. Med.* 33:269.
- Marx, W., Magy, D., Simpson, M. E., and Evans, H. M. 1942. Effect of purified pituitary preparations on urine nitrogen in the rat. *Am. J. Physiol.* 137:544.
- Mixner, J. P., and Turner, C. W. 1942. Pituitary weight of growing male albino rat related to body weight. *Endocrinology* 31:261.
- Molitch, M., and Poliakoff, S. 1938. Clinical results of anterior pituitary therapy in children. A comparison of the value of oral and hypodermic preparations. *Endocrinology* 22:422.
- Morrison, Samuel, and Feldman, Maurice. 1939. The effect of the thyroid on the motility of the gastro-intestinal tract. *Am. J. Digest. Dis.* 6:549.
- Morrison, Samuel, and Feldman, Maurice. 1940a. An experimental study of the effects of the pituitary and thyroid glands on carbohydrate metabolism. *Am. J. Digest. Dis.* 7:453.
- Morrison, S., and Feldman, M. 1940b. Effect of atropine on the gastro-intestinal tract following thyroid medication. *Endocrinology* 27:500.
- Morton, M. E., Chaikoff, D. L., Reinhardt, W. O., and Anderson, E. 1943. Radioactive iodine as an indicator of the metabolism of iodine. VI. The formation of thyroxine and diiodotyrosine by the completely thyroidectomized animal. *J. Biol. Chem.* 147:757.
- Moussu, M. G. 1899. Influence de l'alimentation thyroïdienne sur la croissance reguliere. *Compt. rend Soc. biol.* 51:241.
- Murray, G. R. 1891. Note on the treatment of myxedema by hypodermic injections of an extract of the thyroid gland of a sheep. *Brit. Med. J.*, vol. 2 for 1891, p. 796.
- Oppenheimer, M. J., and Glyer, N. M. 1941. The effect of elevated metabolism on rate of intestinal contractions. *Am. J. Dig. Dis.* 8:471.
- Paget, Stephen. 1919. Sir Victor Horsley. A study of his life and work. London. pp. 54-67.
- Palladin, A., and Savron, E. 1927. Studies on creatinuria in the young and its relation to the thyroid. *Biochem. Ztschr.* 191:1.
- Parhon, Marie, 1912. L'influence de la thyroïde sur le metabolisme du calcium. *Mem. Soc. de Biol.* 72:620.
- Parker, J. E. 1943. Influence of thyroactive iodocasein on growth of chicks. *Proc. Soc. Biol. and Med.* 52:234.
- Patek, A. J., and Haig, Charles. 1941. Effect of administration of thyroid extract and of dinitrophenol upon dark adaptation. *Proc. Soc. Exper. Biol. and Med.* 46:180.
- Peiser, Jul. 1906. Über die Beinflussung der Schilddrüsensubstanz. *Ztschr. f. exper. Path.* 3:513.
- Preheim, Delbert V. 1940. Studies on thyroidectomized rats with special reference to lactation and growth. *Endocrinology* 27:494.
- Ralston, N. P., Cowsert, W. C., Ragsdale, A. C., Herman, H. A., and Turner, C. W. 1940. The yield and composition of the milk of dairy cows as influenced by thyroxine. *Mo. Agr. Exper. Sta. Res. Bul.* 317.
- Reece, R. P., and Turner, C. W. 1937. The lactogenic and thyrotropic hormone content of the anterior lobe of the pituitary gland. *Mo. Agr. Exper. Sta. Res. Bul.* 266.

- Refarzo-Membrives, Juan. 1943. Thyroid-inhibiting action of the hypophysis of rats fed with thyroid. *Endocrinology* 32:263.
- Reilly, W. A. 1942. Thyrotoxicosis in children. *Am. J. Dis. Child.* 63:996.
- Reineke, E. P., Bergman, A. J., and Turner, C. W. 1941. Effect of thyroidectomy of young male goats upon certain A P hormones. *Endocrinology* 29:306.
- Reineke, E. P., and Turner, C. W. 1941. Growth response of thyroidectomized goats to artificially formed thyroprotein. *Endocrinology* 29:667.
- Reineke, E. P., and Turner, C. W. 1942a. Increased milk and milk fat production following the feeding of artificially formed thyroprotein (thyrolactin). *J. Dairy Sci.* 25:393.
- Reineke, E. P., and Turner, C. W. 1942b. Formation *in vitro* of highly active thyroproteins, their biologic assay and practical use. *Mo. Agr. Exper. Sta. Res. Bul.* 355.
- Reinhardt, W. O. 1942. Method for determining completeness of thyroidectomy using radioactive iodine. *Proc. Soc. Exper. Biol. and Med.* 50:81.
- Reinhardt, W. O., and Wainmann, P. 1942. Effect of thyroidectomy, castration, and replacement therapy on thymus, lymph nodes, and spleen in male rats. *Proc. Soc. Exper. Biol. and Med.* 49:257.
- Reverdin, J. L., and Reverdin, A. 1883. Note sur vingtdeux opérations de goitre. *Rev. Méd. de la Suisse Rom.* 3:309.
- Richter, C. P. 1933. The role played by the thyroid gland in the production of gross body activity. *Endocrinology* 17:73.
- Robertson, T. B. 1928. The influence of thyroid alone and of thyroid administered together with nucleic acids upon growth and longevity of the white mouse. *Austral. J. Exper. Biol. and Med.* 5:69.
- Rossiiskii, D. 1937. *Rev. franc. endocrinol.* 15:384. *Chem. Abs.* 32:2595.
- Rudinger, Karl. 1908. Über den Eiweissumsatz bei Morbus Basedowii. *Wein. klin. Wochenschr.* 31:1581.
- Salmon, T. D. 1938. The effect on the growth rate of thyroparathyroidectomy in new-born rats and of the subsequent administration of thyroid, parathyroid and anterior hypophysis. *Endocrinology* 23:446.
- Salter, William Thomas. 1940. The endocrine function of iodine. Harvard University Press. Cambridge, Mass.
- Schäfer, E. A. 1912. The effects upon growth and metabolism of the addition of small amounts of ovarian tissue, pituitary, and thyroid to the normal dietary of white rats. *Quart. J. Exper. Physiol.* 5:203.
- Schiff, M. 1856. Untersuchen über die Zuckerbildung in der Leber. Würzburg. Quoted by Schneider (1939).
- Schiff, M. 1884. Résumé d'une sene d'periences sur les effets de l'ablation des corps thyroïdes. *Rev. Med. de la Suisse Rom.* 4:65.
- Schmidt, L. H., and Genther, D. 1938. Relation of environmental temperature to action of thyroxine. *Endocrinology* 23:553.
- Schmidt, L. H., and Hughes, H. B. 1938. The free and whole cholesterol content of whole blood and plasma related to experimental variations in thyroid activity. *Endocrinology* 22:474.
- Schneider, B. A. 1939. Effects of feeding thyroid substance. *Quart. Rev. Biol.* 14:289.
- Sevringhaus, A. E., Smelser, G. K., and Clark, H. M. 1934. Anterior pituitary changes in adult male rats following thyroxine injections or thyroid feeding. *Proc. Soc. Exper. Biol. and Med.* 31:1125.
- Silberberg, Martin, and Silberberg, Ruth. 1940. The effects of thyroidectomy nad administration of anterior pituitary extract of cattle on the growth of cartilage of immature guinea pigs. *Am. J. Path.* 16:505.
- Silberberg, Martin, and Silberberg, Ruth. 1941. Effects of hormones on the skeleton of mice, guinea pigs, and rats. *Endocrinology* 29:475.
- Silvestri, T., and Tossati, C. 1907. Di una fusione della glanduala tiroide non ancora ben studiota. *Gaz. degli. Ospedali* 28:1067.

- Simpson, Ethel D. 1927. Changes in the growth of skeletal muscle following thyroidectomy in sheep. Proc. Soc. Exper. Biol. and Med. 24:289.
- Simpson, S. 1924. The effect of thyroidectomy on growth in the sheep and goat as indicated by body weight. Quart. J. Exper. Physiol. 14:161.
- Smelser, G. K. 1939. Testicular function and the action of gonadotropic and male hormones in hyperthyroid male rats. Anat. Rec. 73:273.
- Smith, Evelyn E., and McLean, Franklin C. 1938. Effect of hyperthyroidism upon growth and chemical composition of bone. Endocrinology 23:546.
- Smith, P. E. 1933. Increased skeletal effects in A P growth hormone injections by administration of thyroid in hypophysectomized thyroparathyroidectomized rats. Proc. Soc. Exper. Biol. and Med. 30:1253.
- Snedecor, G. W. 1940. Statistical methods. 3rd ed. Iowa State College Press, Ames, Iowa.
- Stefanescu, Maria. 1926. Les Modifications des Cartilages de Conjugaison chez les animaux hyperthyroïdés. Compt. rend. Soc. biol. 95:1570.
- Stein, K. F., and Lisle, M. 1942. The gonad stimulating potency of the pituitary of hypothyroid young male rats. Endocrinology 30:16.
- Sternheimer, Richard, 1939. The effect of a single injection of thyroxine on carbohydrates, protein and growth in the rat liver. Endocrinology 25:899.
- Terroine, Emile, and Babad, Perl, 1939. Role de la thyroxine dans de Metabolism azoté de Croissance. Arch. Internatde Physiol. 48:441.
- Todd, T. W., Wharton, R. E., and Todd, A. W. 1938. The effect of thyroid deficiency upon bodily growth and skeletal maturation in the sheep. Am. J. Anat. 63:37.
- Topper, A., and Cohen, P. 1928. The effect of thyroid therapy in children. Am. J. Dis. Child. 35:205.
- Turner, C. W., and Cupps, P. T. 1940. The effects of certain experimental conditions upon the thyrotropic hormone content of the albino rat. Endocrinology 26:1042.
- Van Haam, E., and Cappel, L., 1940. Effects of hormones upon cell growth *in vitro*. II. The effect of the hormones from the thyroid, pancreas and adrenal gland. Am. J. Cancer 39:354.
- Van Horn, W. M. 1933. The relation of the thyroid to the hypophysis and ovary. Endocrinology 17:152.
- Weichert, C. K., and Boyd, R. W. 1933. Induction of typical pseudopregnancy in the albino rat by means of experimental hyperthyroidism. Anat. Rec. 58:55.
- Weymuller, L. E., Wyatt, T. C., and Levine, S. Z. 1932. The respiratory metabolism in infancy and childhood. XIV. The effect of thyroid therapy on the metabolism of protein in normal infants. Am. J. Dis. Child. 43:1544.
- Wharton, Thomas. 1656. Adenographia. De Glandulis Thyreoidis. Chapt. 18. London.
- Wilkins, Lawson. 1940. Thyroid medication during childhood. J. A. M. A. 114:2382.
- Williams, C., Phelps, D., and Burch, J. C. 1941. Observation on the effect of hypothyroidism on ovarian function in the guinea pig. Endocrinology 29:373.
- Winchester, C. F. 1939. Influence of thyroid on egg production. Endocrinology 24:697.
- Zarrazi, V. 1934. Hormones and gastric motility. Giorm. Clin. Med. 15:1659. Chem. Abs. 31:6711.
- Zitowskaya, I. 1939. Aminosäurensynthese in den Gemeben. II. Einfluss von thyroxine auf die Aminosäurensynthese in Leber und Nieren. Bull. biol. et Med. exper. U. S. S. R. 7:114.
- Zorn, W., and Brügermann, H. 1939. Thyroidectomy in swine for purpose of increasing fat production. Züchtungskunde 14:376. Chem. Abs. 34:1358.