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# NUTRITIONAL REQUIREMENTS IN HYPERTHYROIDISM OF GROWING CHICKS

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# TABLE OF CONTENTS

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# Nutritional Requirements in Hyperthyroidism of Growing Chicks

M. H. MAGHRABI and C. W. TURNER

## INTRODUCTION

The thyroid gland, as a regulator of body metabolism, has been a center of attraction for research workers. A cure for hypothyroidism was achieved by the use of thyroidal substances. However, in hyperthyroidism the answers to the many problems associated with that condition were not easy to approach. Partial surgical ablation of the thyroid gland and the use of antithyroid substances have been advocated.

Through the recent advances in the knowledge of inter-relationships between thyroid function and metabolism of the vitamins, the correction of the abnormal condition through better nutrition was visualized. In hyperthyroidism the amounts of several of the known vitamins, in body tissues, appear to be subnormal. Furthermore, the group of B-vitamins and other vitamins are constituents of enzymes involved in the fundamental physiological processes especially those concerned with energy transformations. As a result of hyperthyroidism, the enzymatic activity is decreased below normal. Addition of several of the known vitamins has been claimed to alleviate the symptoms associated with hyperthyroidism and to reestablish the activity of the enzymes. Consequently, the increase in the metabolic rate may be eliminated and the amount of weight lost may be restored.

Some substances have been reported to possess growth stimulating factor(s), such as liver, yeast, animal protein factor, and a number of antibiotics. Most of these materials were found to be of value in experimental hyperthyroidism.

The induction of hyperthyroidism, by the feeding of thyroactive materials (e. g. thyroprotein), has been used in animal experimentation. Some reports have shown that feeding thyroprotein will stimulate the processes of growth, milk and fat production, and egg production. Evidence for the need of additional nutrients in this condition has been accumulating in the literature.

The present work was undertaken to investigate the effect of thyroprotein feeding on growing chicks and the possibility of meeting the nutritional requirements of the hyperthyroid animal by supplementation of the feed with additional vitamins or growth-promoting factors.

## REVIEW OF THE LITERATURE

### The Thyroid Gland: Historical

Since the time of Hippocrates and Plato the thyroid gland has been described and various functions have been assigned to it (Schneider, 1939). The gland was an object of both speculation and controversy until 1656 when Wharton in his book "Adenographia," listed the functions of the thyroid gland 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.

Claude Bernard in 1855 was the first to differentiate between glands of external secretion and those of internal secretion. Later, Bernard (1859) described the thyroid as one of the glands of internal secretion.

The importance of the thyroid gland was shown by Schiff (1856) when the gland was surgically removed from dogs. Some of the animals survived the operation for several weeks, but most of them soon went into tetany and died.

Kocher (1878) was the first to practice successful removal of the thyroid for treatment of goiter. Later, Kocher (1883) called the pathological condition arising a few months after the removal of the gland, "cachexia strumiprivia" and described a condition similar to myxedema. Reverdin and Reverdin (1883) described the condition as "operative myxedema." Horsley (1884) reported that thyroidectomy of monkeys led to myxedema, the condition being similar to that noted by Schiff on dogs, and to the "operative myxedema" of Reverdin and Reverdin. Lombard (1883) reviewed the previous work on the physiology of the thyroid and stated that the gland was essential for normal development of body and intelligence in children, and for maintenance of normal mentality in the adults. The observation that death from tetany resulted from complete extirpation of the thyroid in animals, led Schiff (1884) to the conclusion that the thyroid had an important role in the nutrition of the central nervous system.

Schiff also found that some of the consequences of thyroidectomy could be alleviated by transplanting a gland from another animal of the same species. He also suggested that the same effect could be achieved by injection of an emulsion or an extract of the thyroid. In man, myxedema was relieved by an injection of an extract of sheep thyroid (Murray, 1891). Fox (1892) was able to relieve myxedema by the oral administration of thyroid. Thus, an approach to the study of the physiology of the gland was accomplished by the extirpation of the thyroid gland followed by replacement therapy.

The association between thyroid activity and metabolic rate was first recorded by Magnus-Levy in 1895. He showed that the rate of metabolism was increased in Graves' disease (where the thyroid is hyperactive) and decreases in myxedema.

Moussu (1899) tested his hypothesis that if a small amount of thyroid was necessary for growth, a little extra might result in more rapid growth, found that feeding fresh thyroid to dogs caused rapid skeletal growth with early maturity.

Certain substances that now are known to contain iodine (e. g. sponge ash) were used by the Chinese fifteen centuries before Christ as a cure for goiter (Schneider, 1939). The isolation of iodine by Courtais (1812) led to its use for the treatment of goiter. Baumann (1896) was the first to show that iodine, in a firm organic combination, is a normal constituent of the thyroid gland. Hutchison (1896) and Oswald (1899) made the discovery that the iodine of the thyroid is bound to a globulin (thyroprotein) which has the same physiological activity as the whole gland. It was not until 1915 that Kendal isolated the hormone of the thyroid gland in a crystalline form and later (1919) named the hormone thyroxine. In 1927 Harrington and Bayer synthesized thyroxine and proved experimentally that the synthetic product had the same effects as thyroxine extracted from the gland.

The wide use of thyroxine in therapy and feeding experiments stimulated research on the production of iodinated proteins similar to the natural thyroglobulin of the thyroid gland. In 1941 Reineke and Turner developed a method for the relatively cheap production of iodinated casein which evoked the same thyroidal effects when given orally to either normal or thyroidectomized animals.

## Functions of the Thyroid Gland

The thyroid gland occupies a central position among the endocrine glands for it regulates many of the physiological processes in the body. Rate of metabolism, volume and composition of the blood, and growth are directly dependent upon the action of the thyroid.

**Metabolic Rate.** The early observations of Magnus-Levy (1897) established the effect of the thyroid on oxygen metabolism. The metabolic rate is low in hypothyroidism and is increased in hyperthyroid individuals or in normal animals fed thyroid substances. That thyroxine is a catalytic agent accelerating the metabolic processes of all cells in the body, was proposed by Plummer in 1921. Dresel (1928) found that excised liver and kidney, from rats rendered hyperthyroid by thyroxine injection, showed increased rate of metabolism. Similar observations were reported by McEachern (1935) and Davis and Hastings (1935). The latter authors also reported that thyroidectomy preceding excision caused reduction in muscle metabolism. Gerard and McIntyre (1932) fed young dogs with 0.6 gr. of desiccated thyroid per kilo per day for a period of 3 to 6 weeks and found that isolated liver, heart, and vagus nerve tissues had an average increase of 25 per cent in oxygen consumption above normal.

Sykes et al. (1948) reported that the heart rate of steers, which had been made hyperthyroid by feeding thyroprotein, could be materially altered by varying feed intake. Also, increases or decreases in energy did not change the essential activity of thyroprotein in the organism or the heart rate, but rather that the effects of thyroprotein and energy seemed to be addative. Singh and Shaffner (1950) determined the rate of oxygen consumption at two-week intervals from hatching to 8 weeks of age for New Hampshire chicks fed thyroprotein at the levels of 5, 10, and 15 gms. per 100 pounds of feed, and for chicks fed high and low caloric diets with and without thyroprotein (7 gm. per 100 pounds of feed). All levels of treatment caused an increase in the rate of oxygen consumption and the response was proportional to the dosage level. Increasing the caloric value of the ration appeared to raise the metabolic rate somewhat, and increased the metabolic response to thyroprotein.

Rabbits exhibited a rapid rise in oxygen consumption with a secondary fall followed by a second rise after a continually increasing dosage of thyroid (initial dose 0.1 gm. daily 6 times per week with dosage increments of 0.1 gm. each week until the 24th week, when the dose became constant.) All rabbits gained weight continuously until they became severely hyperthyroid.

**Carbohydrate Metabolism.** Barnes (1934) advanced the "working hypothesis" that the thyroid may exert an influence upon carbohydrate metabolism through the pituitary, which in turn may influence the adrenals. Barnes showed that thyroid feeding to dogs with pituitary and pancreas removed did not intensify their mild glycosuria. However, the injection of pituitary extract produced severe glycosuria. The effect of the thyroid gland upon the metabolism of carbohydrates, proteins and fat has been reviewed by Astwood (1944). Thyroid feeding to animals had been shown by Coggeshall and Greene (1933) to cause a diminution in liver glycogen, although the animals were kept on a high carbohydrate diet. On the other hand, thyroidectomy leads to increased storage of liver glycogen. Hyperthyroidism accelerates intestinal absorption of sugars and starch, and increases oxidation of glucose in tissue, which leads to depletion of glycogen stores (Althausen, 1940).

**Lipoid Metabolism.** Experimental evidence indicates that the thyroid is concerned with the metabolism of lipoids. Nichols and Perlzweig (1928) reported that in hyperthyroidism there was a decrease in saturated fatty acids of the plasma. Similar observations had been reported by Gilligan et al. (1934) and Hurxthal and Hunt (1935). A rise in the blood lipids of the adult dog after thyroidectomy has been reported (Chaikoff et al., 1941). Duerst (1941) found that thyroidectomy in pigs enhances fat deposition. He had advocated thyroidectomy in pigs as a means of producing a greater amount of lard. Blivaiss (1947) found that thyroidectomized birds accumulated large amounts of fat in different parts of the body.

Feeding excess thyroid hormone to rats resulted in a marked decrease of body fat, the loss being pronounced in the skin, but less so in muscles (Abelin and Klinger, 1948). The authors concluded that the fat losses were due only in part to increased oxidation; the main factors responsible for loss of fat could be: (a) the inhibition of the synthesis of fat from carbohydrate, by the balance between synthesis and breakdown being shifted towards the latter, and (b) diminished ability of the organ, due to toxic action of thyroxine, to retain fat. The authors also claimed that experimental hyperthyroidism might not decrease the cholesterol of the whole body, in fact, this might even increase, but there was a pronounced loss of cholesterol in the adrenals.

A number of reports have appeared concerning the beneficial effects of dietary fat for the hyperthyroid animal and have been reviewed by Ershoff (1949). It had been found that increasing the fat content of the diet counteracted in part the rise in BMR and the reduction in liver glycogen following thyroid feeding. Available data indicated that the protective effects of fat were due at least in part, to certain unsaturated fatty acids. Ershoff stated that the growth promoting effect of fat on the hyperthyoid rat was due to some factor other than the correction of a fatty acid deficiency and suggested the possibility that fat might stimulate the synthesis of an antithyrotoxic factor by the intestinal flora.

Greenberg and Deuel (1950) indicated that immature rats on a fat-low diet were particularly susceptible to thyroid feeding, as was evidenced by a greater retardation in growth and a decreased length of survival. In these experiments the thyroid preparation was given at a level of only 0:5 per cent, compared with the levels of 0.25 and 0.50 per cent employed by Ershoff (1949). Greenberg and Deuel also noticed that the depressing effects of the thyroid preparation were entirely suppressed by cottonseed oil, in contradistinction to the partial effect of soybean oil in the tests where casein was the dietary protein.

The level of cholesterol in blood is elevated in hypothyroidism and reduced in hyperthyroidism (Schmidt and Hughes, 1938). In rabbits, changes in serum cholesterol due to thyroidectomy were found by Fleischmann et al. (1940) to resemble the changes in hypothyroid human beings after withdrawal of thyroid treatment. Thyroidectomy in the rabbit was followed by a sharp rise from the preoperative level of from 60 to 100 mg. per cent to levels of from 140 to 280 mg. per cent. The plasma cholesterol level had been shown by Marx et al. (1950) to depend on the concentration of circulating thyroid hormone. Furthermore, the cholesterol content of the diet did not seem to influence plasma cholesterol significantly in the hyperthyroid rat.

Thyroidectomy of adult rhesus monkeys had no effect on the serum cholesterol level, whereas in young animals a sharp rise of cholesterol content of the serum followed thyroidectomy (Fleischmann et al., 1943). Fleischmann and Shumaker (1942) determined total body cholesterol and serum cholesterol in thyroidectomized rabbits and rats, in rats treated with thyroxine, and in normal controls, and found that changes in the concentration of cholesterol in the serum do not parallel changes in the concentration of cholesterol in the body. The authors concluded that thyroxine influences the shift of cholesterol from the blood plasma into the tissues rather than having a specific effect on cholesterol metabolism.

Foldes and Murphy (1946) observed that cholesterol and phospholipids were increased in hypothyroidism. Hoffmann and Hoffmann (1944) believe that the thyroid hormone's first action is upon the phospholipids.

**Body Fluids, Salts and Proteins.** Magnus-Levy (1897) reported that nitrogen excretion was low in hypothyroidism and was increased in hyperthyroid individuals or in normal animals fed thyroid substance. Boothby et al. (1925) showed that in human myxedema, there was an increased storage of protein, "deposit protein," in the body fluids and not in the cell protoplasm. Thyroid administration caused rapid oxidation of the extra protein and its elimination in the urine along with the extra salts and water held in combination with the protein.

Diminution in the rate of release of nitrogen from the tissues of thyroidectomized rats was observed in eviscerated animals (Bondy, 1949) and *in vitro* preparations of liver slices and diaphragm (Kline, 1949). Treatment of the animals with thyroxine restored the rates to normal. Hoberman (1950) showed that both synthesis and catabolism of protein were retarded in fasting.

Feeding desiccated thyroid to intact and thyroidectomized rats significantly decreased the ability of the liver to synthesize alanine from pyruvate and ammonia (Canzanelli et al., 1947). In rats on a low protein diet, Axyavchik (1949) found that the administration of thyroid hormones caused the rate of deamination of alanine to reach a rate of four times that to which it had fallen on the inadequate diet.

Myxedema causes a reduction in plasma volume which is restored to normal on treatment with thyroid (Thompson, 1926). In hyperthyroidism the blood volume tends to be above normal (Bigson and Harris, 1939).

Hypothyroidism was shown by Aub et al. (1927) to cause a decrease in excretion rate of calcium and phosphorus in feces and urine but had little effect upon the concentrations of the two elements in the blood. Layani et al. (1947) reported the effect of long-continued administration of desiccated thyroid to guinea pigs. Many bony changes were indicative of withdrawal of Ca and P together with fibrosis, but no marked osteoclastic activity. Serum Ca was elevated, as was the phosphate. The bone marrow was found markedly hyperactive, but there was no change in blood hemoglobin. Brush (1945) observed that thyroxine delayed the healing of fractures in the rat. Thyroxine, however accelerated proliferation, differentiation and resorption of cartilage and bone at the tibial ephysis. As a result, the width of the cartilage disc was reduced and the number and size of the trabecula below the plate was decreased. Longitudinal growth of the animal remained normal.

Robertson (1941) suggested that thyroxine probably directly affected the renal threshold for Ca, the threshold value being raised in myxedema, and lowered by the excessive thyroid secretion in thyrotoxicosis. The increase in P elimination in thyrotoxicosis, and the diminution in myxedema were probably secondary to the changes in Ca excretion. Noback et al. (1949) stated that the development of ossification centers in the rat appeared to be controlled primarily by the thyroid with no modification of the effect by estrogen or androgen.

**Nervous System.** An effect of the thyroid on the central nervous system has been well established. Thyroidectomy leads to a decrease in mental activity and to physical sluggishness. Irritability, hyperactivity of the sweat glands, and increased peristaltic activity of the intestines, in hyperthyroidism, indicate that the thyroid has an effect on both the autonomic and central nervous system (Turner, 1948). Thyroxine causes stimulation of the respiratory centers in the medulla as shown by increased rate of breathing. Thyroidectomy renders the respiratory centers less sensitve to carbon dioxide, which results in diminished rate of breathing (Brody, 1941). The tonus of both cardiac and skeletal muscle declines after thyroidectomy or as a result of hypothyroidism as indicated by enlargement of the heart accompanied by a lowered rate and amplitude (Lerman et al., 1933).

**Growth.** Gudernatsch (1912) showed that thyroid feeding caused an acceleration in the metamorphosis of tadpoles. Thyroidectomy was shown to cause retardation of growth in different species: rats (Hammett, 1923), dogs (Binswanger, 1935), sheep and goats (Simpson, 1924), leading to a condition comparable to myxedema. Simpson (1928) made a histological study of the skeletal muscles of sheep following early thyroidectomy and showed that the normal development in cytoplasm was prevented, and that the muscle cells resembled those seen in young animals. Thyroidectomized young animals fail to attain normal growth and sexual development (Blivaiss, 1947). In adult animals, thyroidectomy has little effect (Marine, 1935). The influence of age on the effects of thyroidectomy varies in different species of animals.

Cunningham (1898) produced hyperthyroidism by (1) subcutaneous injections of thyroid extracts in dogs and rabbits; (2) feeding fresh sheep thyroid to chickens, rabbits, dogs, cats, monkeys, and man; and (3) feeding desiccated thyroid extract to chickens, rabbits, dogs, cats, monkeys, and man. Toxic symptoms, similar to those in exophthalmic goiter in man, were produced by desiccated thyroid and extract but not by fresh thyroid.

In severe hyperthyroidism, when the basal metabolism was considerably elevated, marked losses of weight occurred. There is, however, the possibility that mild hyperthyroidism in a young animal might accelerate growth (Moussu, 1899; Blaxter et al., 1949). Hammett (1924) emphasized the importance of dosage in thyroid feeding. Increase in thyroid activity of normal animals may result in either an acceleration or a retardation in growth processes, the specific effect being entirely dependent upon the size of the dosage.

## Experimental Hyperthyroidism in Poultry

**Effect on Growth.** Crew and Huxley (1923) fed dried thyroid to 12 chicks and found no significant difference between the control and experimental group. Feeding thyroid to fowls resulted in excessive molting and depigmentation of regenerated feathers (Zavadovski, 1925). Cole and Hutt (1928) found that while feeding raw and desiccated thyroid to fowls caused molting, it did not result in depigmentation of new feathers. Feather growth in chicks was improved by adding thyroactive iodocasein to the feed (Irwin, Reineke and Turner, 1943). Forced early molting in turkeys, as a result of feeding relatively high dosages of thyroprotein, was not followed by natural molting later in the year (Kosin and Wakely, 1948). The effect of thyroid feeding on plumage type, feather pigmentation and feather growth has been reviewed by Schneider (1939), Blivaiss (1947) and Fleischmann (1947).

Day-old Rhode Island Red chicks receiving 0.025 to 0.05 per cent of thyroprotein for a 12-week period made greater gains and required less feed per gram of gain than did the controls (Parker, 1943). At 4 weeks of age there was no difference in weight between the experimental and control groups, but at 8 weeks the experimental chicks were consistently heavier than the controls. At 12 weeks, however, the chicks on the lowest level of thyroactive iodocasein (0.025 per cent) weighed the most. Parker suggested that the levels of thyroprotein conducive to greatest gains change with age or stage of development of the chick.

Irwin, Reineke and Turner (1943) reported that feeding thyroprotein (below 45 gm. per 100 pounds of feed) will accelerate the growth of White Rock chicks to a limited extent, providing dosage is low. Feeding a level of 0.1 per cent iodinated casein in the diet of chicks resulted in a slight growth depression and a decrease in subcutaneous fat (Turner, Irwin and Reineke, 1944). At least 4 weeks were required to restore carcass fat when the birds were put on a thyroprotein-free diet.

Wheeler, Hoffman and Graham (1948) fed a lower level of thyroprotein (10 gm. per 100 pounds feed) to Rhode Island chicks. Thyroprotein-fed males were significantly heavier at 12 weeks of age but the females were not. Also, the treated males were significantly more uniform in body weight than the controls. The treated birds used feed more efficiently during the first 6 weeks. Similar results on New Hampshire and White Plymouth chicks were reported by Quisenberry and Krueger (1948).

Glazener and Shaffner (1948) reported that rapid-growing strains of Barred Plymouth Rock and New Hampshire Red chickens have higher thyroid activity than slow-growing strains. However, the slow-growing Plymouth Rocks showed a greater growth response to iodinated casein than the rapid-growing strains. Glazener, Shaffner and Jull (1949) fed thyroprotein at the levels of 5, 10, 15 and 20 gm. per 100 pounds of feed to cockerels from a rapid-growing and a slow-growing strain of both New Hampshire and Barred Plymouth Rock chickens. Thyroprotein depressed the growth rate of the rapid-growing strains of New Hampshire and accelerated the growth of the slow-growing New Hampshire and the strains of Barred Rock, both of which grew less rapidly than the rapid-growing New Hampshire. Five grams of thyroprotein per 100 pounds of feed proved to be optimal for growth and feed utilization for the strains used.

**Effect on Egg Production.** In 1925, Crew reported that feeding desiccated thyroid to laying hens resulted in an increase in egg production, from 6.7 eggs to 34 eggs in a 6 months period. Excessive feeding of thyroid to fowl resulted in rapid loss of weight, cessation of egg production volume and in paling of the comb (Sainton and Peynet, 1926; Asmundson, 1931). However, small doses fed over a long period of time did not affect egg production (Geacomini and Taibell, 1927). White Leghorn hens, in their second year of production, were fed on increasing levels of thyroprotein (5, 10, and 20 gm per 100 pounds of feed). Ten gm. per 100 pounds of feed was approximately the optimum dosage and resulted in a yearly egg production of 20.6 per cent compared to 22.6 per cent for the control group (Turner, Irwin and Reineke, 1945). Continuous feeding of thyroprotein (10 gm. per 100 pounds of feed) to laying hens for a long period of time, up to 7 years, increased the rate of egg production over that of normal controls of the same age (Turner et al., 1945, 1946; Turner and Kempster, 1947, 1948, 1949). The experiments also showed that the principal effect of thyroprotein feeding was the maintenance of egg production during hot weather when a decline normally occurs that parallels the seasonal decline in metabolism (Brody, Funk and Kempster, 1938; Winchester, 1940; Turner, Kempster, Hall and Reineke, 1945; Schultze and Turner, 1945). Furthermore, Turner (1948) showed that feeding thyroprotein to White Plymouth Rock hens maintained a higher egg production not only during summer months but also throughout the year.

The thyroid hormone directly or indirectly stimulates the pituitary to secrete more of the gonadotrophic hormones stimulating ova and yolk production as well as gonadal hormone (estrogen and androgen) (Turner and Kempster, 1948). The effect of the thyroid hormone is more pronounced upon the secretion of LH or ICSH rather than of FSH (Kumaran and Turner, 1949). If, in birds as in mammals, LH influences the secretion of progesterone and in turn progesterone influences the ovulation process (Rothchild and Fraps, 1949) increased egg production might be explained. However, since FSH is primarily concerned with the maturation of the ova, a limitation in the secretion rate of this factor would limit egg production. The combination of the thyroid hormone with a factor which would stimulate greater FSH secretion should be effective in maintaining higher egg production.

Hutt (1947) was not able to increase the egg production of White Leghorn pullets by feeding thyroprotein at the rate of 10 gm. per 100 pounds of feed. There was a decrease in egg production in the early weeks of treatment. Hutt and Gowe (1948) fed thyroprotein to a group of White Leghorn pullets about 8 months old starting the end of January, for a 31-week period. The results showed that the feeding of thyroprotein significantly lowered egg production for a period of about 8 weeks. Thereafter no significant difference was observed.

Hoffman and Wheeler (1948) used Rhode Island Red pullets in their experiments. A group of 14 month-old pullets was fed thyroprotein (10 gm. per 100 pounds of feed) during the warmer months of the year (June through October). Thyroprotein treatment resulted in a significant loss in body weight. The treated birds laid at the rate of 45 per cent as compared to 50 per cent for the controls during the entire experimental period. The difference was not significant but there was a significant decrease in the egg production of the treated groups during the last half of the period. During this period (August 11 to October 25), the treated birds laid 22.3 eggs per bird (32 per cent) as compared with 30.8 (44 per cent) for the controls.

In experiments designed to study the effect of feeding thyroprotein on egg shell quality and hatchability, Godfrey (1949) observed that egg production, as measured by the average number of eggs per bird, was practically the same in both the treated and the control group. However, the feeding of thyroprotein did not prevent the normal seasonal decline in egg production.

Thyroprotein supplement (15 gm. per 100 pounds of feed), for a period of 10 weeks, in the feed of White Leghorn pullets resulted in a decrease in egg production. The average production rate was reduced by 11 per cent in the second week, followed by a further, and more gradual, decrease until by the end of the 10-week period the egg production had decreased by 22 per cent as compared with a reduction of only 5 per cent for the control birds. Also there was a loss in body weight and a decrease in feed consumption (Berg and Bearse, 1951). The authors pointed out that in their experiments a higher level of thyroprotein was used, the experiments were conducted at different seasons of the year, the feeding period of 10 weeks was of shorter duration than in most previously reported trials, and that the environmental temperatures varied from those existing where other investigators conducted similar tests.

**Effect on Egg Shell Quality and Hatchability.** Asmundson (1931), and Asmundson and Pinsky (1935) found that hens given desiccated thyroid gland produced significantly heavier shells than did the same hens when not receiving thyroid. According to Gutteridge and Pratt (1946), Gutteridge and Novikoff (1947), and Hoffmann and Wheeler (1948), the egg shells of hens fed thyroprotein were significantly stronger, as measured by the specific gravity of the egg, during the summer months than those of the controls. Hutt and Gowe (1948) could not verify these results and concluded that thyroprotein had no measurable effect upon strength of egg shells in May, June, and July when shell quality was expressed as breaking strength of shell or specific gravity of the egg.

Wilson (1949) confirmed the results of Hoffmann and Wheeler (1948), and concluded that thyroprotein increased shell thickness. Similar results were obtained by Godfrey (1949) showing that the feeding of thyroprotein had produced a slight improvement in egg shell quality as measured by 14-day incubation weight-loss, specific gravity, and egg shell thickness. Godfrey hypothesized that birds which normally produce poor egg shells, as expressed by 14-day incubation weight loss, had a low level of thyroid activity and thus might respond to the feeding of thyroprotein, whereas birds which produced thicker shells did not respond because of greater thyroid activity. Similar results had been reported by Berg and Brease (1951). They observer that birds which under apparently normal environmental and nutritional conditions were unable to produce shells as thick as other birds of the same population responded to the addition of thyroprotein to the feed by producing thicker shells, whereas the birds which normally produced relatively thick shells did not respond to the drug in a like manner.

There is no evidence that thyroprotein feeding has an effect upon hatchability (Godfrey, 1949).

## Relationship Between Thyroid Activity and Vitamins

**Fat-Soluble Vitamins.** More studies have been made of the relation between the thyroid and vitamin A than the other members of the fat-soluble vitamins. However, our present knowledge of such relationship is still far from being complete and further investigations are needed.

*Vitamin A and Carotene.* Claims of increased requirements for vitamin A with elevated basal metabolic rate (BMR) in hyperthyroidism have been frequent (Abelin, 1933; v. Euler and Klussman, 1932; Sure and Buchanan, 1937), although contradictory data have also been recorded. When groups of normal and hyperthyroid rats containing uniform stores of vitamin A were fed a diet low in vitamin A, the amounts of this factor remaining in the liver were usually as high in the hyperthyroid group as in the controls (Logaras and Drummond, 1938; Baumann and Moore, 1939). Hyperthyroid rats fed moderate amounts of fish liver stored approximately as much vitamin A as control rats (Johnson and Baumann, 1947).

V. Euler and Klussmann (1932) were the first to notice that the action of thyroxine on metabolism was antagonized by vitamin A or by carotene. Carotene partly protected rats from losing weight on the administration of thyroxine. There was an indication that thyroxine reduced the storage of vitamin A and carotene in the liver. Heavy dosage of vitamin A (3000 I. U. daily) counteracted the effect of thyroxine treatment in rats (Logaras and Drummond, 1938). However, no such action was detectable when the intake of the vitamin was just sufficient to supply the requirements of the animal. In humans, daily administration of 200,000 to 400,000 I. U. of vitamin A for periods up to 51 weeks, counteracted the effect of thyrotoxicosis (Simkins, 1947).

Explanations for the existing relationship between vitamin A and the thyroid hormone were given by Belasco and Murlin (1940) and by Sadhu and Brody (1947). They suggested that thyroxine iodine was taken up by the double bond of the vitamin A which removed the metabolism-stimulating effects of thyroxine, and that the iodinated vitamin A depressed the thyrotrophic hormone secretion of the pituitary.

Feeding vitamin A was found by Freudenberger and Clausen (1935) to reduce the thyroid weight of young rats. According to Schulze and Hundhausen (1939), large amounts of vitamin A tended to produce thyroid hypoplasia with an increase in the amount of thyrotrophic hormone in the anterior pituitary.

In 1935, Abelin presented evidence that large dosage of vitamin A would counteract to a marked extent the influence of thyroxine on the BMR. Sheets and Struck (1942) reported that in rats pretreated with vitamin A, the feeding of desiccated thyroid raised the BMR only 25 per cent, as compared with 58 per cent for rats which received no vitamin A. However, after the BMR had increased in the latter, vitamin A administration had no significant effect. Likewise, it was found that vitamin A had no effect on oxygen consumption of normal rats. Thus, it seemed that vitamin A could prevent a rise in BMR due to thyroid administration, but was powerless to reduce the BMR once it had been raised.

Chicks receiving 0.2 per cent thyroprotein in their diet, required 100 units of vitamin A to produce growth equivalent to that obtained on 50 units per day with normal chicks (Cooper et al., 1950). This indicated an increased vitamin A requirement for growth in the hyperthyroid state, and showed that the metabolic rate as well as growth rate influenced vitamin A utilization in the chick. Heimer et al. (1949) stated that vitamin A storage in the liver of rats on a vitamin A-free diet was highest in thyroidectomized animals, intermediate in thyroxine-treated animals, and lowest in the controls. Growth of the control rats was most marked and probably accounted for the lower stores of vitamin A during a period when only endogenous vitamin A was available to the animals. It was suggested that thyroid activity, in this instance, was indirect, in that growth was greatest in the euthyroid state.

Several workers proposed that the thyroid gland has an effect on the conversion of carotene to vitamin A in the body besides its effect on the absorption of both substances from the intestines. Cama and Goodwin (1949) demonstrated that thiouracil inhibited, and desiccated thyroid counteracted the inhibition of the drug.

Kaplansky and Balaba (1946) showed that thyroprotein catalyzed the conversion of carotene to vitamin A *in vivo*. Drill and Truant (1947) indicated that the thyroid hormone influenced the reactions involved in the conversion of carotene to vitamin A, since normal rats could be maintained with supplements of either vitamin A or carotene, whereas thyroidectomized rats could not be maintained with carotene, exhibiting occular changes and weight loss, as well as increased mortality. According to Johnson and Baumann (1948), hyperthyroid or hypothyroid weanling rats depleted of vitamin A appeared equally able to store administered vitamin A, but the hyperthyroid animals stored more in the liver and the hypothyroid less when carotene was fed. This latter situation could be reversed by injection of thyroxine.

That the conversion of carotene into vitamin A in the intestines is not controlled by the thyroid gland, has been claimed by several investigators. Adult rabbits kept on a carotene-rich diet showed no difference in the plasma vitamin A levels when treated with desiccated thyroid (Goodwin, 1948). Smith et al. (1948) were unable to detect carotene in the blood of thyroidectomized or thiouracil-treated goats. In contrast to the results reported by Kaplansky and Balaba (1946), Cama and Goodwin (1948 and 1949) could not observe a conversion of carotene to vitamin A when incubated under varying pH, temperature and time, alone or with thyroprotein. Similar results were reported by Lowry and Lowry (1950). Allen et al. (1948) found no evidence of increased conversion of carotene to vitamin A in calves fed 1.5 to 3.0 gm. thyroprotein per 100 pounds of body weight.

*Vitamin D.* Elmer (1938) reported that in slight avitaminosis D the thyroid picture was one of tall epithelium and liquefied colloid, or hyperfunction. In severe deficiency the changes simulated those of hypofunction. Massive doses of vitamin D produced intense hyperplasia with numerous mitosis in the thyroids of rabbits (Carriere et al., 1939). Prolongation of the treatment led to degeneration of the thyroid. According to Salter (1940), in young vitamin D-deficient rats the iodine content of the thyroid gland was lowered. Also, control rats in the dark had thyroids with a higher iodine content than test rats being irradiated with ultraviolet light or receiving ergosterol. A deficiency of vitamin D was found by Sure (1938) to be the only one causing a pathological decrease in the weight of the thyroid gland.

Schechet (1951) found that the addition of 5 grains of desiccated thyroid to the rachitogenic diet of rats during the assay period of vitamin D by the "line test," produced the best growth, as well as the most uniform lines. The addition of 0.025 per cent thyroprotein appeared about equal in its ability to promote growth, and definitely aided in calcification response.

*Vitamin E.* Singer (1936) described a marked hypoplasia of the thyroid in rats 12 to 18 months of age maintained on a vitamin E-low diet Sarrie (1937) observed a similar condition at the end of the lactation period in the young of vitamin E-low mothers, a condition that did not exist when the young were transferred to foster mothers receiving a stock ration. However, Telford et al. (1938) could find no histological evidence of subnormality of the thyroid in paralyzed suckling rats of mothers deficient in vitamin E or in rats deprived of the factor chronically for over 22 months.

Biddulph and Meyer (1942) reported that the thyroids of male rats deficient in vitamin E for 6, 12, and 15 months were more active, as shown by histological study, and were heavier than the thyroids of normal controls of the same age. Analysis of the vitamin E-deficient and normal diets and the drinking water showed that the vitamin E-deficient rate received only about one-fourth as much iodine as the normal rats. The addition of sufficient iodine to the E-deficient diet to provide the same amount as was contained in the normal diet maintained normal thyroid weight and histology and the metabolic rate at the normal level.

Huter (1947) fed from 5.5 to 12.5 mg. alpha-tocopherol acetate per kg. daily for 22 to 96 days to young dogs. The males developed these symptoms in the thyroid: abnormal increase in weight and volume, no deficiency in iodine and histological changes, especially increased epithelium. The effects were much less marked in the females.

Wheeler and Perkinson (1949) stated that there was a definite relationship between thyroid state and the time and incidence of death of E-deficient chicks. The total mortality from E-deficiency was highest in the hyperthyroid group (receiving 0.04 per cent thyroprotein), intermediate in the euthyroid group and lowest in the hypothyroid group (receiving 0.1 per cent thiouracil). The mean age at death was 15, 20, and 28 days, respectively, for each of these groups, indicating the relative rate of depletion of the vitamin E stores. Forty-eight per cent of the hyperthyroid chicks were dead before any of the animals in the other groups had started to show deficiency symptoms, and 35 per cent of the euthyroid chicks were dead by the time any of the hypothyroid chicks died.

**Water-Soluble Vitamins.** — *B-vitamins.* Much attention has been focused on the B-vitamins since the increased requirements for those vitamins in hyperthyroidism had been early recognized (Drill, 1943). These vitamins, where their function has been elucidated, have been found to be associated with the enzyme systems which yield energy to the organism. The thyroid gland, through its hormones, appears to exert a regulatory influence on these energy-yielding metabolic processes (Betheil et al., 1947; Mann and Stare, 1950). Therefore, it is logical to assume that an increased metabolic rate in hyperthyroidism may necessitate an increased dietary intake of the B-vitamins.

Cowgill and Palmieri (1933) found that daily administration to pigeons of disiccated thyroid, sufficient to produce a state of hyperthyroidism, caused an increase in the amount of vitamin B required to maintain the normal urge to eat. The authors concluded that in the pigeon, as in the dog, there was some relation between the amount of vitamin B required for maintenance of body weight and the metabolism of the organism. Sure and Smith (1934) observed that the loss of weight associated with experimental hyperthyroidism could be prevented by the administration of a high B-vitamin diet (yeast). The weight of the hyperthyroid animals was maintained as long as no deficiency of the B-vitamins occurred. As soon as the yeast was removed from the diet, a drop in food intake took place, followed by a secondary loss of weight (Drill, 1941). Drill et al. (1943) fed desiccated thyroid to dogs over long periods and found that high B-vitamin diet would delay but not prevent the eventual appearance of abnormal liver function.

*Thiamine.* Himwich et al. (1932) reported that occasional administration of thiamine to dogs (exhibiting anorexia) during experimentally-induced hyperthyroidism, was followed by restoration of the urge to eat, and that the administration of desiccated thyroid to dogs subsisting on a thiamine-deficient diet shortened the interval required for the development of anorexia. Subcutaneous injection of thiamine gave protection from the loss of weight produced in rats by thyroxine injections (Peters and Rossiter, 1939). Also, pretreatment with thiamine lessened the rate at which thyroid-fed rats lost weight.

Williams and Kendall (1943) studied two normal women who were given large doses of desiccated thyroid for 241 days. When adequate amounts of thiamine were given, the BMR was elevated, but it decreased during thiamine restriction and was increased again when an adequate intake of thiamine was restored. In rats, 30 to 100 ug. crystalline thiamine counteracted the toxicity of as high a daily dose as 0.2 mg. of thyroxine (Sure and Buchanan, 1937a). Loss of weight and elevation of BMR caused by feeding desiccated thyroid (6.5 mg. daily) were counteracted by one mg. thiamine supplementation in the diet (Alardyce et al., 1947). Among a group of patients with thyrotoxicosis, the levels of thiamine in the blood were lower than normal due to loss in the excreta and to increased combustion of food (Williams et al., 1943). Drill (1938) reported that the thiamine content of the whole liver was reduced 35.7 per cent in the hyperthyroid rat receiving 100 mg. of thyroid gland daily. However, the normal and hyperthyroid animals tested eliminated the same amount of thiamine in the urine while being injected with 500 ug. of thiamine per day.

The decreased excretion of thiamine in milk reported by Hibbs and Krauss (1947) during thyroprotein feeding was considered by the authors as an indication that increased thiamine as well as increased nutrient intake might be required in thyroprotein feeding.

Injection of thiamine increased the cocarboxylase content of the tissues of both normal and hyperthyroid animals. After 7 days of pretreatment with thiamine, the cocarboxylase content of the tissues fell more rapidly in hyperthyroid than in normal animals (Peters and Rossiter, 1939).

*Riboflavin.* In counteracting the effect of desiccated thyroid feeding in rats (weight loss and elevation of BMR), Allardyce et al. (1947) found that riboflavin was the most effective of the B-vitamins used in that work. According to Hibbs and Krauss (1947), there was no change in the riboflavin content of the milk of thyroprotein-fed cows in the period of normal grain feeding; however, when the grain was limited the riboflavin content of the milk decreased.

Tipton (1950) found that riboflavin deficiency produced decreases in the succinic oxidase activity of liver homogenates from hooded rats; but no change, or possibly a slight increase, in cytochrome oxidase activity. Homogenates of livers of animals given thyroid powder or thyroxine for 7 to 10 days showed increased succinic and cytochrome oxidase activities. In riboflavin deficiency the response of succinic oxidase to thyroid was decreased, that of cytochrome oxidase being very variable.

*Pyridoxine, Nicotinic Acid, Pantothenic Acid, Inositol, and Folic Acid.* Drill and Overman (1942) found that pyridoxine and Ca-pantothenate could effectively replace the $B_2$ complex (yeast) addition to the diet of hyperthyroid rats. The rats regained their lost weight and maintained weight while still receiving thyroid gland. In the chick, Monroe and Turner (1949) reported that Ca-pantothenate hastened the inactivation of thyroxine more than did nicotinic acid or thiamine. This finding was in accord with the observation of Glanzmann and Meier (1945), who found that pantothenic acid exercised a greater protective effect in thyrotoxicosis than did any other known vitamin. However, Allardyce et al. (1947) found that the effectiveness of the B-vitamins, in counteracting the effect of desiccated thyroid feeding in rats, was in the following order, from best to poorest: riboflavin, thiamine, para-amino benzoic acid, nicotinamide, Ca-pentothenate, and pyridoxine. The authors also observed that the response was affected by the age of the rat, its sex, and whether or not it had been subjected to similar treatment before. Younger rats responded less than did older rats and the females exhibited greater changes in BMR, while males showed greater changes in weight.

The feeding of thyroid (200 mg. per day) to rats receiving a diet low in nicotinic acid (20 mg. per 100 gm. of ration) resulted in a marked diminution in the coenzyme I content of both the liver and kidney cortex. However, increased dosages of nicotinic acid could restore the normal coenzyme I content in the hyperthyroid rat, and thus counteract the deleterious effect of the increased basal metabolic rate (Katzenelbogen et al., 1941). However, Monroe and Turner (1949) found that nicotinic acid prolonged the duration of the action of thyroxine in chicks. This might be explained by the findings of Martin et al. (1941) that nicotinic acid decreased the peristaltic action of the stomach and small intestines.

Drill and Overman (1942) reported that Ca-pantothenate was required in larger quantity during experimentally induced hyperthyroidism. Meier and Glanzmann (1945) stated that pantothenic acid worked synergistically with the thyroid hormone, as shown by its effect in three cases of hypothyroidism in children. Experiments with rats showed that pantothenic acid exercised a greater protective effect in thyrotoxicosis than did any other known vitamin. Feeding a crude ration, Abelin (1946) observed a beneficial effect when massive doses of Ca-pantothenate were administered to hyperthyroid rats. On the other hand, Ershoff and Hershberg (1945) were unable to alleviate the symptoms resulting from thyroid administration by greatly increasing the Ca-pantothenate level of the ration. These results were confirmed by Betheil et al. (1947).

Although Ershoff and Hershberg (1945) suggested that the occurrence of alopecia during the course of hyperthyroidism indicated a possible increased requirement of inositol, Betheil et al. (1947) found that further addition of inositol to a thyroid-containing ration exerted no antithyroid effect.

Martin (1947) presented evidence for increased folic acid requirement in hyperthyroidism. He found that sulfonamides fed to rats produced hypothyroidism and granulocytopenia, if the rats were on purified diet. The granulocytopenia was prevented or cured by adding folic acid to the diet, and it was presumed that a folic acid deficiency resulted from the effect of the sulfonamides on the intestinal flora. It was also belived that the reduced metabolic rate resulting from the use of sulfonamides prevented the folic acid deficiency from becoming apparent. When 10 ug. of thyroxine were injected daily, leucopenia resulted regularly. Hague et al. (1948) reported that thyroxine caused high mortality in chicks fed a ration low in folic and pantothenic acids, suggesting that these two vitamins appear to be needed in order to counteract the toxic effects of thyroxine. Betheil and Lardy (1949), however, rats fed 0.38 per cent desiccated thyroid.
demonstrated that folic acid had no beneficial effect on the growth of

*Vitamin* $B_{12}$. In 1948, Rickes et al. and Smith reported the isolation from liver of a red crystalline compound termed vitamin $B_{12}$, which was active in microgram quantities for the remission of pernicious anemia in relapse. Amounts as small as 6 ug. of vitamin $B_{12}$

per kg. of diet stimulated the growth of chicks as much as dietary supplements consisting of crude sources of this factor (Ott et al., 1948).

With regard to the relation of vitamin $B_{12}$ to metabolic processes, it had been fairly well established that the requirement or effectiveness of the vitamin would vary with the protein intake, and similar relations to fat and carbohydrate were suggested (Zucker and Zucker, 1950). Vitamin $B_{12}$ and folic acid were found to be involved in chemical reactions which lead to the formation of nucleic acids in living cells, as demonstrated by studies in animals (Koch-Weser and Popper, 1949) and humans (Vilter et al., 1950). In young, growing female rats, fed diets high in carbohydrate, vitamin $B_{12}$ (0.5 ug. subcutaneous, 3 times a week) exerted a greater effect on weight gain than was exerted on littermate males on the same diet. This suggested that vitamin $B_{12}$ was involved in the conversion of carbohydrate into fat (McCollum and Chow, 1950). Bosshardt et al. (1950) suggested that vitamin $B_{12}$, like thiamine, might play an essential role in the catabolism of carbohydrate or the carbohydrate-like residue of protein, or it might have a function in the conversion of intermediates, formed in the metabolism of carbohydrate and protein, to fats (other than the essential fatty acids) that are required for optimum growth. Vitamin $B_{12}$ was shown by Drill and McCormick (1949) to have a marked lipotropic effect and to prevent the accumulation of fat in the liver of the rat, and the increase of weight of the liver which occurred with the high-fat diet. Charkey et al. (1950) suggested that one function of vitamin $B_{12}$ was to enhance anabolic processes which removed amino acids from the blood to form fixed tissues. Furthermore, a greater efficiency in utilization of blood components leading to lower blood levels could well result in reduction of renal wastage and this in turn would be reflected in greater weight gain.

Vitamin $B_{12}$ had been found to be involved in the methylation of homocystine to methionine (Stokstad and Jukes, 1950). In the absence of vitamin $B_{12}$, methionine gave a growth response but homocystine did not, in chicks from vitamin $B_{12}$-depleted hens. When vitamin $B_{12}$ was given by injection, both methionine and homocystine were active. Evidence for the existence of a relationship between vitamin $B_{12}$ and choline in nutrition was also presented by the same authors. The addition of vitamin $B_{12}$ to a purified diet, containing 20 per cent casein as the protein supplement, decreased the choline requirements for optimum growth.

Vitamin $B_{12}$ was found to be a growth factor for the hyperthyroid rat. A level of 0.1 to 0.2 and 0.125 ug. vitamin $B_{12}$ per rat per day was effective in ameliorating the growth retardation which resulted from the feeding of 0.25 per cent desiccated thyroid (Betheil and Lardy, 1949; Emerson, 1949). In chicks, vitamin $B_{12}$ administered orally (1.5 ug. per 100 gm. of ration) or parenterally (0.5 ug. bird day) completely counteracted a thyrotoxic condition produced by feeding a basal ration containing 0.05 per cent thyroprotein (Nichol et al., 1949). Sure and Easterling (1950) found that 0.2 to 0.5 ug. daily of vitamin $B_{12}$ not only protected against the entire collapse produced by subcutaneous injections of 0.2 mg. daily of DL-thyroxine, but also permitted a considerable growth to take place. Daily subcutaneous injections of vitamin $B_{12}$ (0.2 ug.) partially overcame the inhibition of growth in rats as a result of feeding 0.1 per cent thyroprotein, and increased the total food consumption above that of the controls (Meites and Shay, 1951). Similar results had been reported by Frost et al. (1949) and Meites and Newland (1950).

Crystalline vitamin $B_{12}$ gave a highly significant growth response in hyperthyroid male rats, but was less effective in the females. Even in males, however, the growth response was distinctly less than that obtained with various preparations of liver and fish solubles (Bolene et al., 1950). Ershoff (1949) reported that when immature female rats were fed purified rations containing casein as the dietary protein, a marked retardation in growth was observed following the administration of massive doses of desiccated thyroid. The effect was completely counteracted by the administration of liver residue but not by supplements of any of the known nutrients, including vitamin $B_{12}$. Lewis et al. (1950) stated that the failure of rats to respond completely to vitamin $B_{12}$ on a sucrose-casein diet (with vitamins, etc.) containing desiccated thyroid, or thyroprotein could be overcome by substituting defatted corn meal, corn starch, or dextrine for sucrose. The effect was attributed to facilitation of intestinal synthesis of other required substances.

Rupp et al. (1951) indicated that when vitamin $B^{12}$ (3 ug. twice daily) was administered to force-fed rats on constant food intake and made hyperthyroid by injection of thyroxine (150 ug. daily), the weight loss was identical with that of hyperthyroid animals not receiving vitamin $B_{12}$. The nitrogen loss resulting from the catabolic action of thyroxine, however, was significantly smaller in thyroxine treated rats receiving vitamin $B_{12}$ than in the thyroxine-treated controls.

*Vitamin C.* Several workers have demonstrated the increased requirement for vitamin C in hyperthyroidism. Sure and Theis (1939) reported that there was marked reduction in ascorbic acid content in thymus, kidney, liver and heart in hyperthyroid rats. Johnson et al. (1938) stated that ascorbic acid in rabbit plasma decreased on the oral administration of desiccated thyroid associated with loss in weight.

With smaller doses they continued to lose weight, stopped eating and eventually died.

Ascorbic acid administration, alone, caused increased liver, kidney, and thyroid respiration (Belasco and Murlin, 1940). When thyroxine was injected simultaneously with ascorbic acid, the metabolism of liver and kidney tissue wes even greater than would have resulted from thyroxine injection only. Ascorbic acid in no way alleviated the depressed thyroid respiration that was produced by thyroxine treatment.

According to Svirbely (1935), large amounts of ascorbic acid promoted the general vitality and appearance of guinea pigs on a thyroid diet. It was shown, also, that the ascorbic acid content of the organs depended on the amount of ascorbic acid fed and decreased as the metabolic rate of the tissues was increased.

**Other Growth Stimulating Substances Used in Experimental Hyperthyroidism**

*Liver.* Kunkel et al. (1948) found that liver contained an unidentified factor essential for a maximum rate of growth in the rabbit. Liver concentrates have also been shown to improve the growth performance of monkeys (Cooperman et al., 1946; Ruegamer et al., 1947) and of the mink and fox (Schaefer et al., 1948 a and b) when purified diets containing all known vitamins were fed.

That there was present in the liver a factor(s) which was essential for the growth of rats suffering from experimentally induced hyperthyroidism, was demonstrated by Ershoff (1947) and by Betheil et al. in the same year. Immature rats fed on toxic doses of thyroid died early and showed retardation in growth (Ershoff, 1948; Bolene et al., 1950). Similar results had been obtained in rabbits (Betheil and Lardy, 1949) and in mice (Bosshardt et al., 1949).

In earlier work, Sure and Buchanan (1937) showed that a liver extract used in the treatment of pernicious anemia had a beneficial effect on hyperthyroid animals, and attributed this effect to its content of B vitamins. The antithyrotoxic substance, or substances of the liver, was shown by Ershoff (1948) to be present in considerable concentration in the water-insoluble fraction of liver (liver residue) and was apparently distinct from any other know nutrients including vitamin $B_{12}$ (Ershoff, 1950). Betheil and Lardy (1949) presented evidence of the probable dual nature of the growth factor present in whole liver powder since solvent fractionation indicated that in spite of exhaustive extraction of the liver powder only a part of the activity could be brought into solution. The authors suggested the possible occurrence of a bound form of the growh factor.

*Yeast.* Several workers have shown that some factor is present in yeast which exerts a marked antithyrotoxic effect on animals receiving thyroactive materials (Ershoff and Hershberg, 1943; Ershoff, 1947; Betheil et al., 1947). However, yeast has been found less effective than liver in promoting the growth of thyroid-fed rats (Betheil and Lardy, 1949).

*Animal Protein Factor (APF).* The term animal protein factor (APF) is generally applied to the factor or factors necessary for the maintenance of normal hatchability and growth of young chicks subsisting on a practical ration which does not contain animal protein supplement (Zucker and Zucker, 1950). Subsequent usage has branched out in several directions; the term has been applied to a factor needed by mice on a purified casein diet (Bosshardt and Zucker, 1948; Van Landingham and Lyon, 1947), by pigs on a corn-peanut meal (Cunha et al., 1949), and by *Lactobacillus casei* (Daniel, et al., 1948). It has now been shown that a deficiency common to all these conditions (except possibly the last ) could be remedied by crystalline vitamin $B_{12}$ which is therefore the principal animal protein factor (Zucker and Zucker, 1950).

However, there is currently an attempt being made to restrict the term animal protein factor to a chick growth factor demonstrated by use of rations based on soybean meal and corn which have been typically used in much of the chicken work. The term animal protein factor complex was used by Stokstad et al. (1949) for a group of factors required with such a diet. Their experiments indicated that crystalline $B_{12}$ did only part of the job, for an APF supplement made from cultures of *Streptomyces aureofaciens* produced a growth response unobtainable with vitamin $B_{12}$. Similar results were reported by Carlson et al. (1949), Menge et al. (1949), Combs et al. (1950), and Sunde et al. (1950). Later, Stokstad and Jukes (1950) reported that growth responses in chicks on a corn-soybean diet were also produced by cultures in which the aureomycin, as measured by antibiotic potency, was destroyed by alkaline hydrolysis.

Animal protein factor supplements were found to be effective in counteracting the growth-depressant effect of toxic levels of thyroprotein (Bolene et al., 1950).

*Other Substances with APF Properties.* Distillers' solubles, fishmeal, and fish solubles were reported to produce additional growth in experimental animals. Stokstad et al. (1949) found that these products did not produce additional growth above that obtained with vitamin $B_{12}$.

Antibiotics. As defined by Waksman (1949), "An antibiotic or an antibiotic substance is a substance produced by microorganisms, which has the capacity of inhibiting the growth and even of destroying other microorganisms."

Many reports have shown that crystalline aureomycin (Stokstad and Jukes, 1950; Jukes et al., 1950; Whitehill et al., 1950; Groschke and Evans, 1950), streptomycin (Moore et al., 1946; Lueoke et al., 1950 and 1951), and penicillin and terramycin (Sieburth et al., 1951) were effective in promoting growth of chicks, turkeys and pigs.

Several possible explanations have been offered for the growth-stimulating properties of antibiotics (Moore et. al., 1946; Sieburth et al., 1951; Linkswiller et al., 1951); (a) the inhibition of toxin-producing bacteria; (b) the reduction in total numbers of intestinal bacteria and lowering of competition between host and microflora for nutrients; and (c) the selective inhibition of a group of the microflora permits increased growth of other microorganisms that synthesize unidentified essential nutrients.

## EXPERIMENTAL PROCEDURES AND RESULTS

It is clear from the review of the literature that our knowledge of the relationship between nutrition and thyroid gland function has been greatly advanced during the past decade. A better understanding of the mechanisms of the biological actions of the vitamins and the discovery of new growth stimulating factors are helping to solve the problems associated with the nutrition of the hyperthyroid individual. Yet, in many cases, controversial results have been reported which give evidence of the demand for further investigations in this field.

The present work has been carried out in an effort to meet the increased nutrient requirements in experimentally induced hyperthyroidism. Results of such work might be helpful wherever thyroactive materials are used in the nutrition of animals.

### Experimental Methods — General

In all experiments hereinafter reported, unless otherwise stated, chicks of the same breed (White Plymouth Rock) were used. Chicks were purchased from the Poultry Department and a local hatchery on the first day after hatching and kept in battery brooders with raised screen floors, with feed and water available at all times. After 4 weeks, the birds were transferred to batteries in another building to give them more head space. The room temperature throughout the first 4 weeks ranged from 75° to 85°F., thereafter the birds were transferred, the temperature did not exceed 70°F. It should be pointed out that in the period following the fourth week, the temperature was under control only during the cold weather.

The control ration fed to the chicks in all experiments, except where otherwise noted, had the following composition:

| | Parts by weight |
|---|---|
| Yellow corn meal | 45 |
| Wheat shorts | 15 |
| Soybean oil meal | 15 |
| Alfalfa meal | 10 |
| Meat scraps (50 per cent protein) | 7 |
| Wheat bran | 5 |
| Steamed bone meal | 0.5 |
| Common salt | 1 |
| Fish liver oil (400 D, 2000 A) | 0.25 |

The chicks were individually weighed and wing-banded before being assigned at random to the experimental groups. No attempt was made to determine the number of each sex at the beginning of the experiments, sex being determined at the end of the period.

Figures shown in the tables of results represent the average for the survival at the end of the experiment.

The thyroprotein used in this work is the product bearing the trade name Protamone, manufactured by the Cerophyl Laboratory in Kansas City, Missouri.

The body weight was considered as a criterion for comparison of the effects of different supplements.

## Techniques and Results of the Individual Experiments

**The Effect of Feeding Different Levels of Thyroprotein to Day-old Chicks.** Day old chicks were assigned at random into five groups of 30 chicks each and fed the following diets for 6 weeks:

1. Control feed
2. Control + 0.01 per cent thyroprotein
3. Control + 0.02 per cent thyroprotein
4. Control + 0.03 per cent thyroprotein
5. Control + 0.04 per cent thyroprotein

Weekly weights were taken of individual chicks and at the end of the experimental period sex was determined.

The results show that male and female chicks differ in their response to thyroprotein feeding (Table 1 and Fig. 1). While the growth rate of male chicks was affected only when the thyroprotein level reached 0.03 per cent, a lower level (0.02 per cent) had a depressing effect on body weight of the female chicks. A higher level of thyroprotein (0.04 per cent) caused retardation in growth in both sexes as compared with that of the controls.

Comparing the averages in different groups, it is seen that, under the conditions of this experiment, the low level of thyroprotein (0.01

TABLE 1--EFFECT OF DIFFERENT LEVELS OF THYROPROTEIN* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Average weight in successive weeks 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 12 | M | 48.67 | 79.83 | 119.83 | 182.92 | 303.83 | 434.00 | 589.67 |
| | | 13 | F | 45.38 | 77.15 | 111.31 | 171.62 | 276.62 | 389.69 | 527.69 |
| | | 25 | Av. | 47.03 | 78.49 | 115.57 | 177.27 | 290.23 | 411.85 | 558.68 |
| 2 | Control + 0.01% Thyroprotein | 9 | M | 46.22 | 76.11 | 112.56 | 188.44 | 310.44 | 431.22 | 581.89 |
| | | 17 | F | 45.82 | 77.18 | 111.47 | 179.71 | 284.59 | 390.82 | 520.35 |
| | | 26 | Av. | 46.02 | 76.70 | 112.02 | 184.08 | 297.52 | 411.02 | 551.12 |
| 3 | Control + 0.02% Thyroprotein | 11 | M | 47.36 | 70.91 | 102.73 | 177.09 | 289.45 | 416.55 | 570.00 |
| | | 14 | F | 45.86 | 64.86 | 91.93 | 153.93 | 251.14 | 352.93 | 479.29 |
| | | 25 | Av. | 46.61 | 67.89 | 97.33 | 165.51 | 270.30 | 384.74 | 524.65 |
| 4 | Control + 0.03% Thyroprotein | 11 | M | 48.27 | 71.00 | 106.48 | 175.73 | 295.64 | 415.45 | 552.36 |
| | | 11 | F | 45.27 | 64.45 | 94.91 | 152.82 | 252.65 | 355.55 | 480.18 |
| | | 22 | Av. | 46.77 | 67.73 | 100.55 | 164.28 | 274.14 | 385.50 | 516.27 |
| 5 | Control + 0.04% Thyroprotein | 13 | M | 47.77 | 74.92 | 109.00 | 182.23 | 283.54 | 380.46 | 493.85 |
| | | 12 | F | 45.67 | 68.25 | 100.17 | 162.92 | 252.75 | 335.83 | 451.25 |
| | | 25 | Av. | 46.72 | 71.59 | 104.59 | 172.58 | 268.15 | 358.15 | 472.55 |

* Thyroprotein (Protamone): Cerophyl Co., Kansas City, Mo.

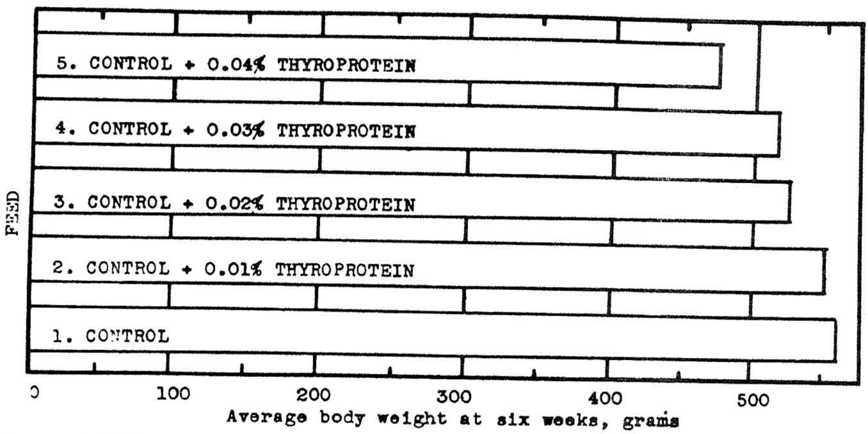


Figure 1. Effect of different levels of thyroprotein on body weight of White Plymouth Rock chicks.

per cent) did not alter the growth rate of chicks with starting age of one day. However, a level of 0.02 per cent or higher of thyroprotein in the feed caused a decreased rate of gain in body weight which paralleled the increase in thyroprotein level.

**The Effect of Animal Protein Supplement (APF) on Chicks Fed Different Levels of Thyroprotein.** An experiment was carried out as with the previous one except that 0.5 per cent APF was incorporated in the diets which contained the different levels of thyroprotein.

APF produced a growth response in chicks greater than the maximum response obtained with the control feed. The effect was more pronounced in males than in females. (Table 2 and Fig. 2).

TABLE 2--EFFECT OF APF* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS FED DIFFERENT LEVELS OF THYROPROTEIN

| Group No. | Feed | No. | Sex | Average weight in successive weeks 0 | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 12 | M | 48.67 | 79.83 | 119.83 | 182.92 | 303.83 | 434.00 | 589.67 |
| | | 13 | F | 45.38 | 77.15 | 111.31 | 171.62 | 276.62 | 389.69 | 527.69 |
| | | 25 | Av. | 47.03 | 78.49 | 115.57 | 177.27 | 290.23 | 411.85 | 558.68 |
| 2 | Control + 0.5% APF | 12 | M | 47.00 | 96.92 | 147.00 | 239.50 | 366.75 | 494.17 | 635.83 |
| | | 15 | F | 44.87 | 88.07 | 134.73 | 216.27 | 334.53 | 446.33 | 555.87 |
| | | 27 | Av. | 45.94 | 92.50 | 140.87 | 227.89 | 350.64 | 470.25 | 595.85 |
| 3 | Control + 0.5% A&F + 0.01% Thyroprotein | 13 | M | 47.62 | 92.31 | 142.92 | 236.54 | 354.62 | 459.38 | 630.31 |
| | | 14 | F | 47.07 | 85.43 | 131.57 | 214.36 | 327.50 | 432.46 | 557.51 |
| | | 27 | Av. | 47.35 | 88.37 | 137.25 | 225.45 | 341.06 | 445.80 | 593.91 |
| 4 | Control + 0.5% APF + 0.02% Thyroprotein | 14 | M | 46.43 | 86.97 | 135.93 | 205.86 | 348.62 | 478.00 | 637.29 |
| | | 13 | F | 44.69 | 79.15 | 123.69 | 190.85 | 308.85 | 423.46 | 558.62 |
| | | 27 | Av. | 45.56 | 82.97 | 129.81 | 198.36 | 328.74 | 450.73 | 597.96 |
| 5 | Control + 0.5% APF + 0.03% Thyroprotein | 12 | M | 47.25 | 96.33 | 151.17 | 249.00 | 382.82 | 505.58 | 642.00 |
| | | 14 | F | 47.64 | 86.71 | 128.86 | 215.43 | 325.64 | 425.15 | 549.57 |
| | | 26 | Av. | 47.45 | 91.52 | 140.02 | 232.22 | 354.03 | 465.36 | 595.79 |
| 6 | Control + 0.5% APF + 0.04% Thyroprotein | 13 | M | 48.62 | 80.92 | 127.31 | 223.77 | 357.92 | 471.92 | 618.15 |
| | | 14 | F | 47.71 | 79.86 | 116.64 | 186.57 | 300.29 | 397.07 | 523.71 |
| | | 27 | Av. | 48.17 | 80.39 | 121.98 | 205.17 | 329.11 | 434.50 | 570.93 |

* APF: Animal Protein Factor Supplement (Lederle)

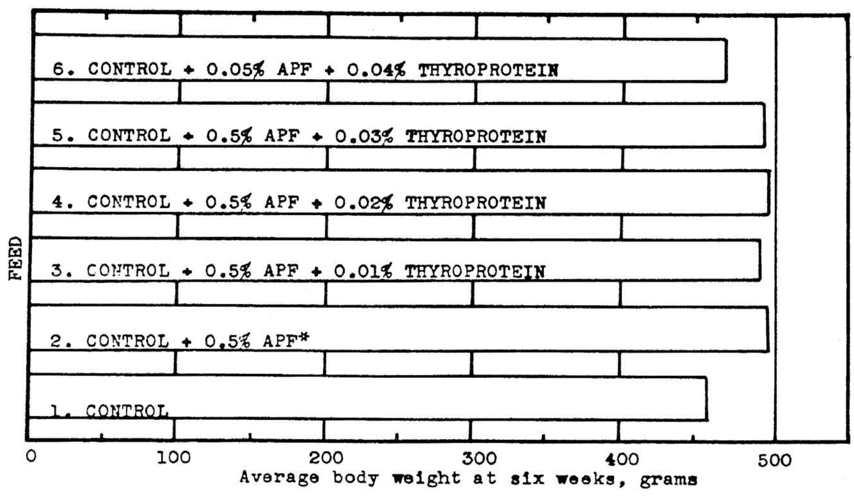


Figure 2. Effect of APF and different levels of thyroprotein on body weight of White Plymouth Rock chicks.
*APF is animal protein factor supplement (Lederle).

A level of 0.5 per cent in the diets of chicks was effective in counteracting any retardation of growth caused by feeding a level of thyroprotein up to 0.04 per cent in the diet, the weight of the birds was lower than those where no thyroprotein was fed, but the difference did not approach significance. APF not only guarded against loss in weight, but also evinced an additional growth response above the controls.

From the results of this experiment, it was decided to use the 0.04 per cent level in the following experiments unless stated otherwise.

**Effect of Increasing the Level of APF on Body Weight of Hyperthyroid Chicks.** The present experiment was carried out to test the assumption that the level of APF (0.5 per cent) used in the previous experiment was effective in producing maximum growth response in chicks receiving 0.04 per cent thyroprotein.

Chicks (day-old) were assigned at random to five groups for 8 weeks on the following diets:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 0.5 per cent APF
4. Control + 0.04 per cent thyroprotein + 0.5 per cent APF
5. Control + 0.04 per cent thyroprotein + 1.0 per cent APF

The results furnish evidence that increasing the level of APF to 1.0 per cent had no beneficial effect over that obtained with the 0.5 per

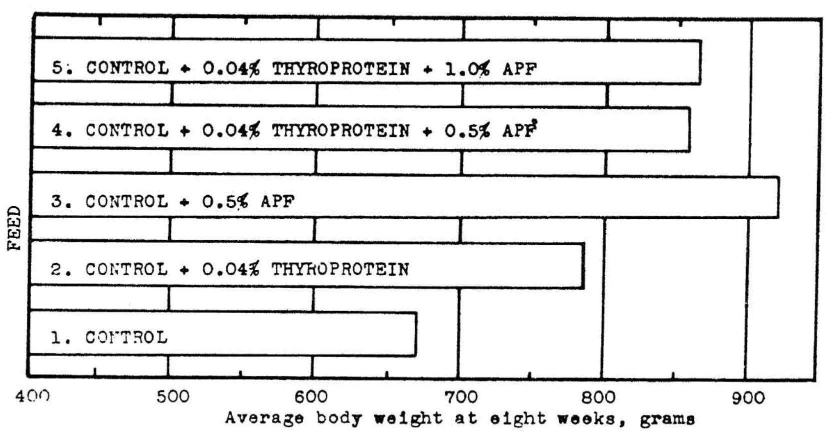


Figure 3. Effect of thyroprotein and two levels of APF on body weight of White Plymouth Rock chicks.

cent level. The average weight of hyperthyroid birds receiving 0.5 per cent APF was slightly less than that of birds receiving no thyroprotein but the difference was not significant. (Table 3 and Fig. 3).

TABLE 3--EFFECT OF THYROPROTEIN AND APF* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Average weight in successive weeks 0 | 2 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 10 | M | 50.90 | 123.10 | 295.30 | 519.90 | 707.40 |
| | | 10 | F | 50.60 | 115.10 | 266.80 | 466.80 | 635.60 |
| | | 20 | Av. | 50.75 | 119.10 | 281.05 | 493.35 | 671.50 |
| 2 | Control + 0.04% Thyroprotein | 15 | M | 46.93 | 120.67 | 282.33 | 513.40 | 797.33 |
| | | 11 | F | 47.91 | 123.27 | 286.09 | 507.64 | 774.64 |
| | | 26 | Av. | 47.42 | 121.97 | 284.21 | 510.52 | 785.99 |
| 3 | Control + 0.5% APF | 12 | M | 49.17 | 129.17 | 312.33 | 563.67 | 917.25 |
| | | 13 | F | 51.92 | 139.83 | 322.75 | 575.92 | 928.42 |
| | | 25 | Av. | 50.55 | 134.50 | 317.54 | 569.80 | 922.84 |
| 4 | Control + 0.04% Thyroprotein + 0.5% APF | 15 | M | 49.53 | 124.87 | 312.27 | 543.53 | 903.13 |
| | | 8 | F | 48.63 | 120.88 | 288.00 | 514.38 | 812.75 |
| | | 23 | Av. | 49.08 | 122.88 | 300.14 | 528.96 | 857.94 |
| 5 | Control + 0.04% Thyroprotein + 1.0% APF | 17 | M | 49.35 | 123.06 | 310.71 | 553.12 | 925.88 |
| | | 13 | F | 48.31 | 124.46 | 299.77 | 496.23 | 803.85 |
| | | 30 | Av. | 48.83 | 123.76 | 305.24 | 524.68 | 864.87 |

* APF: Animal protein factor supplement (Lederle) contains 4-5 mg. aureomycin per gm. in addition to vitamin $B_{12}$ and other unknown factors.

Also it could be seen that thyroprotein did not have an effect on birds during the first 6 weeks of the experiment. However, the following 2 weeks, the thyroprotein-fed birds became heavier than the controls. At the end of 8 weeks the average weights were 671.50 and 785.99 for the controls and the thyroprotein-fed birds, respectively.

**Comparative Effects of APF, Vitamin $B_{12}$ and Other B-Vitamins.** Chicks were fed the control diet for the first week and then individually wing-banded and distributed among ten groups, which received the following diets for 6 weeks:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 0.5 per cent APF
4. Control + 0.5 per cent APF + 0.04 per cent thyroprotein
5. Control + vitamin $B_{12}$ (3 ug/100 gm. of feed)
6. Control + vitamin $B_{12}$ + 0.04 per cent thyroprotein
7. Control + B-vitamins (thiamine, 0.3; riboflavin, 0.6; niacin, 5.0; Ca-pantothenate, 2.0; pyridoxine, 0.4; p-aminobenzoic acid, 10; choline chloride, 150; inositol, 100; folic acid, 0.05;

and biotin, 0.02 mg. per 100 gm. of feed)
8. Control + B-vitamins + 0.04 per cent thyroprotein
9. Control + B-vitamins + 0.5 per cent APF
+ 0.04 per cent thyroprotein

The vitamin $B_{12}$ as well as the other B-vitamins added to the control diet was equivalent to twice the requirements of those factors for maximum growth in chicks (Nichol et al., 1949).

It is noticed that the average weight of the group that received thyroprotein was but slightly less than that of the control. (Table 4 and Fig. 4). The males exhibited a slower gain in weight as a result of thy-
controls and the thyroprotein-fed group, respectively. On the other hand, the average weight of thyroprotein-fed females was slightly high-
roid feeding, the average weights being 580.86 and 551.00 grams for the
er than that of the controls.

TABLE 4--EFFECT OF THYROPROTEIN, APF* AND B-VITAMINS ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Average body weight in successive weeks 1 | 3 | 5 | 7 |
|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. |
| 1 | Control | 14 | M | 64.07 | 182.21 | 375.86 | 580.86 |
| | | 8 | F | 56.00 | 157.00 | 312.50 | 488.13 |
| | | 22 | Av. | 60.35 | 169.61 | 344.18 | 534.50 |
| 2 | Control + 0.04% Thyro-protein | 5 | M | 62.00 | 167.40 | 320.40 | 551.00 |
| | | 15 | F | 57.07 | 165.20 | 323.20 | 522.27 |
| | | 20 | Av. | 59.54 | 166.30 | 321.80 | 536.64 |
| 3 | Control + 0.5% APF | 8 | M | 60.63 | 170.63 | 388.88 | 728.88 |
| | | 15 | F | 56.47 | 161.00 | 334.73 | 604.33 |
| | | 23 | Av. | 58.55 | 165.82 | 361.81 | 666.61 |
| 4 | Control + 0.5% APF + 0.04% Thyroprotein | 5 | M | 61.20 | 183.80 | 373.40 | 663.40 |
| | | 16 | F | 61.63 | 172.25 | 358.31 | 630.56 |
| | | 21 | Av. | 61.42 | 178.03 | 365.86 | 646.98 |
| 5 | Control + Vit. B12 (3 mg./100 g. feed) | 15 | M | 62.33 | 176.93 | 384.47 | 690.80 |
| | | 10 | F | 62.10 | 175.20 | 368.10 | 638.30 |
| | | 25 | Av. | 62.22 | 176.07 | 376.29 | 664.55 |
| 6 | Control + Vit. B12 + 0.04% Thyroprotein | 10 | M | 58.90 | 159.80 | 348.10 | 633.60 |
| | | 13 | F | 60.62 | 147.62 | 307.38 | 542.85 |
| | | 23 | Av. | 59.76 | 153.71 | 327.74 | 588.23 |
| 7 | Control + B-vits. (x2)** | 9 | M | 65.00 | 167.22 | 362.33 | 646.33 |
| | | 16 | F | 62.88 | 155.31 | 329.06 | 572.88 |
| | | 25 | Av. | 63.94 | 161.27 | 345.70 | 609.61 |
| 8 | Control + B-vits. + 0.04% Thyroprotein | 19 | M | 62.79 | 163.53 | 372.58 | 672.05 |
| | | 6 | F | 58.67 | 143.33 | 315.33 | 559.50 |
| | | 25 | Av. | 60.73 | 153.43 | 343.96 | 615.78 |

* APF: Animal protein factor supplement

** Thiamin, 0.3; riboflavin, 0.6; niacin, 5.0; Ca-pantothenate, 2.0; pyridoxine, 0.4; p-aminobenzoic acid, 10; choline chloride, 150; inositol, 100; folic acid, 0.05; and biotin, 0.02 mg. per 100 g. of feed.

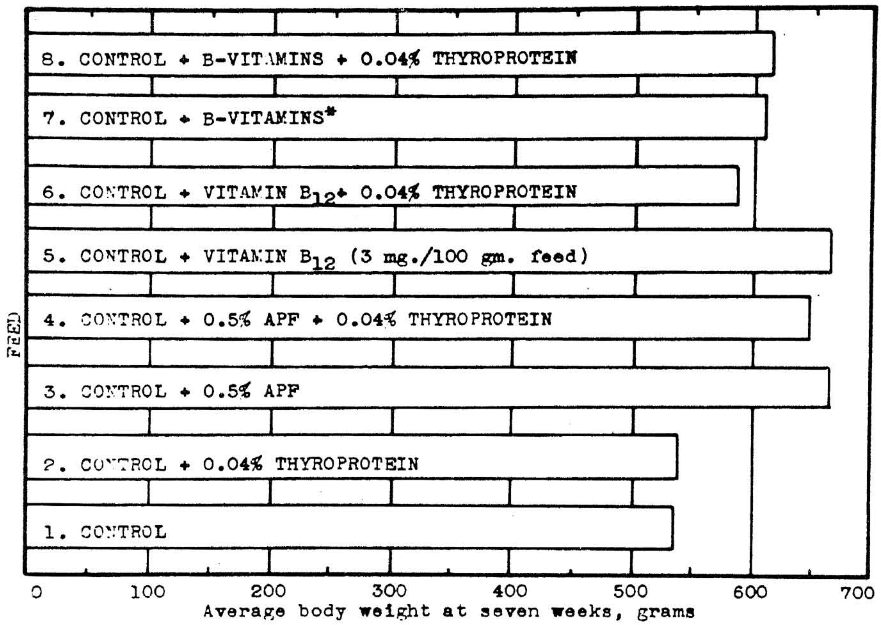


Figure 4. Effect of thyroprotein, APF, vitamin $B_{12}$, and other B-vitamins on body weight of White Plymouth Rock chicks (starting age, 1 week).
*Thiamin 0.3, riboflavin 0.6, niacin 5.0, Ca-pantothenate 2.0, pyridoxine 0.4, p-aminobenzoic acid 10, choline CL 100, folic acid 0.05, and biotin 0.02 mg. per 100 gm. of feed.

APF supplementation caused a significant increase in weight of both sexes and protected against a loss in weight that might have resulted from feeding thyroprotein. It was also observed that males showed a loss, while females an increase, in weight following thyroprotein feeding in addition to APF. However, the average weights for the sexes combined, in both groups, did not differ significantly.

The addition of vitamin $B_{12}$ to the control diet resulted in an increased growth response. This result suggested that the control diet in this experiment was low in vitamin $B_{12}$ since the amount of this vitamin added was not large enough to be considered excessive. Vitamin $B_{12}$, when administered to thyroprotein-fed chicks, fully protected the males against loss in weight, but did not have that effect on females. This is evident when the weights of hyperthyroid chicks receiving vitamin $B_{12}$ were compared with those weights of chicks receiving vitamin $B_{12}$ only. However, chicks that received thyroprotein and vitamin $B_{12}$ were significantly heavier than the controls or those fed thyroprotein alone.

Addition of B-vitamins to the diet of hyperthyroid chicks protected against loss in weight as shown in Table 4. When no vitamin $B_{12}$ was included among the supplementary B-vitamins (other than $B_{12}$) growth was less than that obtained with vitamin $B_{12}$ alone. This might indicate that vitamin $B_{12}$ was a limiting factor in the control diet.

**Vitamin D Requirement in Hyperthyroidism.** The present experiment was performed to find if thyroprotein feeding would interfere with vitamin D function and result in a change in the ash content of bones. The chick method for assay of vitamin D of the A.O.A.C. was followed. Day-old White Leghorn chicks were fed for 3 weeks the A.O.A.C. rachitogenic diet either alone or with supplements as follows.

1. Basal (rachitogenic)
2. Basal + 0.02 per cent thyroprotein
3. Basal + 9 I. U. vitamin $D_3$ (per 100 gm. of feed)
4. Basal + 9 I. U. vitamin $D_3$ + 0.02 per cent thyroprotein
5. Basal + 15 I. U. vitamin $D_3$
6. Basal + 15 I. U. vitamin $D_3$ 0.02 per cent thyroprotein
7. Basal + 25 I. U. vitamin $D_3$
8. Basal + 25 I. U. vitamin $D_3$ + 0.02 per cent thyroprotein
9. Basal + 25 I. U. vitamin $D_3$ + 0.04 per cent thyroprotein

According to Campbell and Emslie (1947), the three levels of vitamin supplement used in this experiment (9, 15, and 25 I. U.) would produce low, medium and heavy calcification in bones, respectively.

Forty chicks were used in each group. At the end of 3 weeks, chicks were killed and the left tibia of each bird removed and cleaned of adhering tissue. After alcohol and ether extractions, the bones were dried, weighed and ashed to constant weight. It can be seen that a level of

TABLE 5--EFFECT OF THYROPROTEIN AND VITAMIN D ON BONE FORMATION OF CHICKS FED RACHITOGENIC DIET

| Group No. | Diet | Per Cent Ash (tibia) |
|---|---|---|
| 1 | Basal (rachitogenic)* | 33.92 |
| 2 | Basal + 0.2% Thyroprotein | 34.75 |
| 3 | Basal + 9 I. U. Vitamin $D_3$** | 40.10 |
| 4 | Basal + 9 I. U. Vitamin $D_3$ + 0.02% Thyroprotein | 40.19 |
| 5 | Basal + 15 I. U. Vitamin $D_3$ | 40.78 |
| 6 | Basal + 15 I. U. Vitamin $D_3$ + 0.02% Thyroprotein | 41.29 |
| 7 | Basal + 25 I. U. Vitamin $D_3$ | 44.62 |
| 8 | Basal + 25 I. U. Vitamin $D_3$ + 0.02% Thyroprotein | 44.64 |
| 9 | Basal + 25 I. U. Vitamin $D_3$ + 0.04% Thyroprotein | 43.42 |

* A. O. A. C. rachitogenic diet
** Per 100 gm. of feed

0.02 per cent thyroprotein produced a slight, but consistent, increase in bone ash (Table 5 and Fig. 5). However, group 9 gave evidence of increased requirement for the vitamin when fed the higher level of thyroprotein (0.04 per cent). Feeding 0.02 per cent thyroprotein to chicks receiving 25 I. U. vitamin $D_3$ per 100 gm. of feed (Group 8) gave an ash content (44.64 per cent) identical to that of Group 7, where no thyroprotein was fed (44.62 per cent). Increasing the amount of thyroprotein (0.04 per cent) resulted in bones with lower ash content (43.42 per cent).

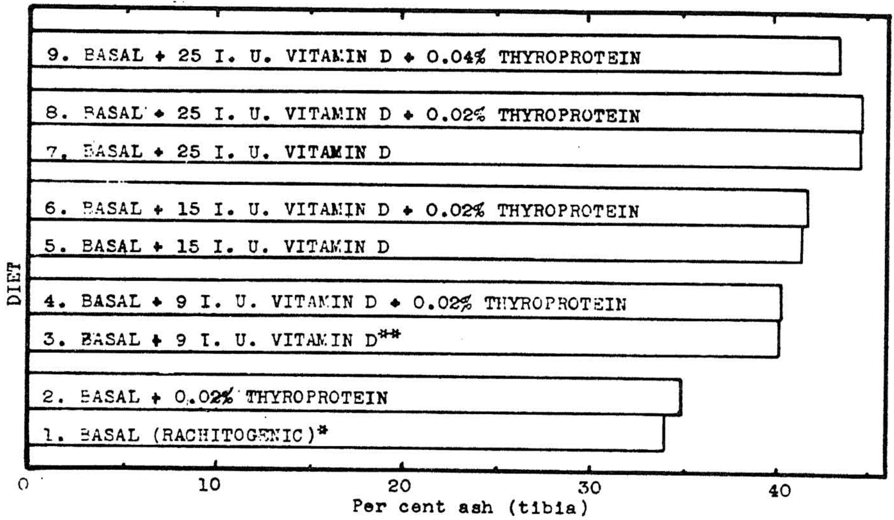


Figure 5. Effect of thyroprotein and vitamin D on bone formation of chicks fed rachitogenic diet.
*A.O.A.C. Rachitogenic diet.
**Vitamin $D_3$ units per 100 gm. of feed.

**The Effect of Liver Residue.** Different liver preparations are reputed to have growth stimulating effects in normal and hyperthyroid animals. It was of interest to test the effect of an extracted liver residue (Wilson) alone, and in combination with other growth stimulating materials (APF and antibiotics).

Day-old chicks were assigned at random to five groups fed the following diets:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 5.0 per cent liver residue
4. Control + 0.04 per cent thyroprotein + 5.0 per cent liver residue
5. Control + 0.04 per cent thyroprotein + 0.05 per cent APF

Chicks were weighed individually every 2 weeks until 6 weeks of age.

It is seen that the level of 5.0 per cent liver residue produced a growth-promoting effect in both normal and hyperthyroid chicks (Table 6 and Fig. 6). At the end of 6 weeks the average weight of chicks fed thyroprotein alone was 414.04 gm. compared with 492.91 gm. when liver residue was used as a supplement.

TABLE 6--EFFECT OF THYROPROTEIN, LIVER RESIDUE* AND APF** ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. of Chicks | Average body weight in successive weeks 0 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|
| | | | gm. | gm. | gm. | gm. |
| 1 | Control | 16 | 42.24 | 86.42 | 220.56 | 415.19 |
| 2 | Control + 0.04% Thyroprotein | 24 | 42.54 | 85.38 | 220.29 | 414.04 |
| 3 | Control + 5% Liver Residue | 21 | 41.90 | 97.90 | 256.81 | 485.67 |
| 4 | Control + 0.04% Thyroprotein + 5% Liver Residue | 22 | 42.00 | 95.18 | 260.91 | 492.91 |
| 5 | Control + 0.04% Thyroprotein + 5% Liver Residue + 0.5% APF | 22 | 40.86 | 113.68 | 297.64 | 530.36 |

* Extracted Liver Residue (Wilson)
** APF: Animal Protein Factor Supplement

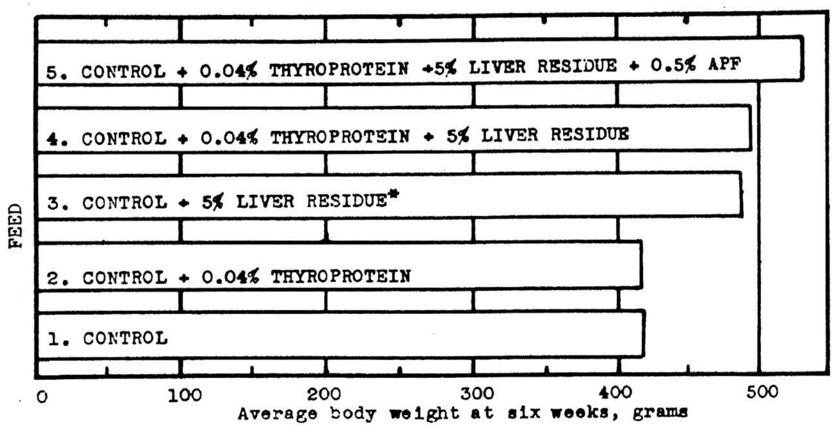


Figure 6. Effect of thyroprotein, liver residue and APF on body weight of White Plymouth Rock chicks.
*Extracted liver residue (Wilson).

The fifth group in this experiment was included to find if the growth response obtained by feeding the liver residue was maximum, or if the APF, due to its contents of other factors, could still produce an additional growth response. A comparison between groups 4 and

5 showed that the addition of APF to the diet of hyperthyroid chicks receiving liver residue will cause further increase in the growth response.

**The Effect of Antibiotics.** Some antibiotic supplements containing vitamin $B_{12}$ and also pure antibiotics became available and were included in the present work.

The two most potent of the antibiotic supplements produced by Merck and Co., namely, MK-42 and MK-45, were chosen. Product MK-42 contained 30 gm. streptomycin and product MK-45 contained 15 gm. penicillin per pound. Each substance contained, in addition, 12.5 mg. vitamin $B_{12}$ per pound.

The effect on growth as a result of supplementation of the diet of both normal and hyperthyroid chicks with these substances was investigated in the following experiments:

Experiment A

Five groups of day-old chicks were fed the following diets for eight weeks:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 0.04 per cent thyroprotein + 0.1 per cent MK-42
4. Control + 0.04 per cent thyroprotein + 0.1 per cent cent MK-45
5. Control + 0.04 per cent thyroprotein + 0.05 per cent MK-42 + 0.05 per cent MK-45

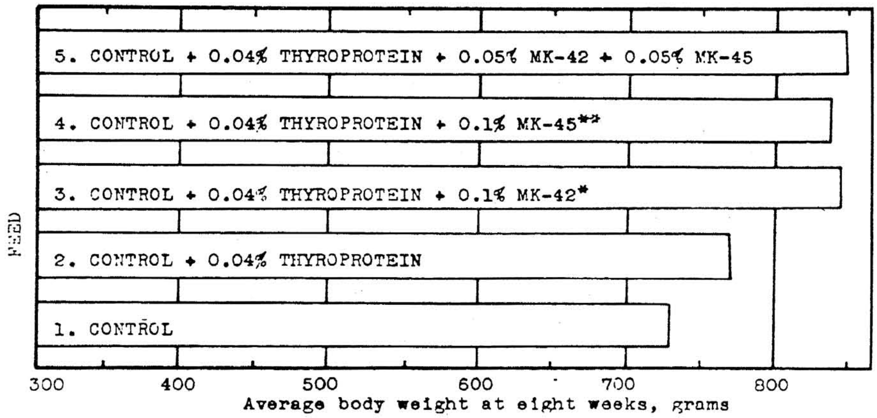


Figure 7. Effect of thyroprotein and antibiotic supplements on body weight of White Plymouth Rock chicks.

*Supplement MK-42 (Merck) contains 30 gm. streptomycin and 12.5 mg. $B_{12}$ per pound of feed.

**Supplement MK-45 (Merck) contains 15 gm. penicillin and 12.5 mg. $B_{12}$ per pound of feed.

In this experiment as well as in the following experiments, the control feed was supplemented with vitamin $B_{12}$ (20 ug. per pound of feed).

A comparison of the final weights of the different groups showed that both compounds (MK-42 and MK-45) had a similar growth-stimulating effect in chicks fed either the control diet or one containing thyroprotein (Table 7 and Fig. 7).

It was also noticeable that the thyroprotein produced a slight increase in growth response over that of the controls.

When the two compounds were added together in the feed of hyperthyroid chicks (Group 5), the effect did not exceed that produced by either compound alone.

TABLE 7--EFFECT OF THYROPROTEIN AND ANTIBIOTIC SUPPLEMENTS* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Average weight in successive weeks 0 | 2 | 4 | 6 | 8 |
|---|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 10 | M | 37.30 | 114.10 | 316.00 | 482.70 | 778.30 |
| | | 15 | F | 35.87 | 101.40 | 287.67 | 423.00 | 701.00 |
| | | 25 | Av. | 36.59 | 107.75 | 301.84 | 452.85 | 739.65 |
| 2 | Control + 0.04% Thyroprotein | 9 | M | 36.44 | 100.00 | 293.67 | 508.11 | 805.89 |
| | | 14 | F | 36.64 | 101.43 | 286.21 | 485.93 | 732.79 |
| | | 23 | Av. | 36.54 | 100.72 | 289.94 | 497.02 | 769.34 |
| 3 | Control + 0.04% Thyroprotein + 0.1% MK-42[1] | 9 | M | 37.00 | 119.89 | 335.33 | 546.44 | 899.22 |
| | | 17 | F | 38.06 | 122.41 | 320.00 | 499.24 | 788.00 |
| | | 26 | Av. | 37.53 | 121.15 | 327.67 | 522.84 | 843.61 |
| 4 | Control + 0.04% Thyroprotein + 0.1% MK-45[2] | 5 | M | 36.20 | 127.00 | 335.00 | 509.80 | 870.00 |
| | | 19 | F | 36.89 | 134.32 | 324.26 | 492.37 | 804.00 |
| | | 24 | Av. | 36.55 | 130.66 | 329.63 | 501.09 | 837.00 |
| 5 | Control + 0.04% Thyroprotein + 0.05% MK-42 + 0.05% MK-45 | 15 | M | 38.27 | 134.73 | 366.13 | 540.40 | 916.07 |
| | | 14 | F | 37.36 | 132.50 | 340.43 | 495.29 | 780.14 |
| | | 29 | Av. | 37.82 | 133.62 | 353.28 | 517.85 | 848.11 |

* Antibiotic supplements containing vitamin $B_{12}$ (Merck)
[1] Supplement MK-42 contains 30 g. streptomycin and 12.5 mg. $B_{12}$ per pound
[2] Supplement MK-45 contains 15 g. penicillin and 12.5 mg. $B_{12}$ per pound

Experiment B

In the previous experiment no attempt was made to include a group that received the antibiotic supplements without thyroprotein. One week-old chicks were fed for 8 weeks on the diets indicated below:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45
4. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45 + 0.04 per cent thyroprotein

The hyperthyroid birds maintained an average weight throughout the experimental period, almost identical to, or a little heavier, than that of the controls. The addition of the combination of the two substances (MK-42 and MK-45) maintained a higher average weight than in both the controls and thyroprotein-fed birds. The weight of the birds receiving the antibiotic-containing supplements was not affected by thyroprotein feeding for the two averages were 840.89 and 850.77 grams, with and without thyroprotein, respectively (Table 8 and Fig. 8).

TABLE 8--EFFECT OF THYROPROTEIN AND ANTIBIOTIC SUPPLEMENTS* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Average weight in successive weeks 1 | 3 | 5 | 7 |
|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. |
| 1 | Control | 12 | M | 61.08 | 190.08 | 410.00 | 708.50 |
| | | 14 | F | 53.71 | 158.14 | 350.79 | 587.00 |
| | | 26 | Av. | 57.35 | 174.11 | 380.40 | 647.75 |
| 2 | Control + 0.04% Thyroprotein | 12 | M | 59.25 | 190.75 | 406.00 | 691.42 |
| | | 19 | F | 53.53 | 168.74 | 353.53 | 618.26 |
| | | 31 | Av. | 56.39 | 179.75 | 379.77 | 654.84 |
| 3 | Control + 0.05% MK-42[1] + 0.05% MK-45[2] | 17 | M | 57.76 | 200.65 | 428.41 | 786.82 |
| | | 16 | F | 53.44 | 183.88 | 369.19 | 655.31 |
| | | 33 | Av. | 55.60 | 192.27 | 398.80 | 721.07 |
| 4 | Control + 0.05% MK-42 + 0.05% MK-45 + 0.04% Thyroprotein | 15 | M | 57.50 | 146.33 | 401.75 | 761.33 |
| | | 17 | F | 57.40 | 140.60 | 371.35 | 670.35 |
| | | 32 | Av. | 57.45 | 143.47 | 386.55 | 715.84 |

* Antibiotic supplements containing vitamin B12 (Merck)
1 Supplement MK-42 contains 30 g. streptomycin and 12.5 mg. $B_{12}$ per pound
2 Supplement MK-45 contains 15 g. penicillin and 12.5 mg. $B_{12}$ per pound

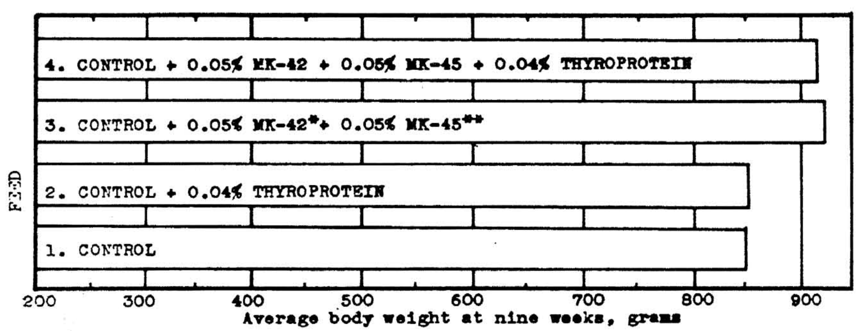


Figure 8. Effect of thyroprotein and antibiotic supplements on body weight of White Plymouth Rock chicks.
*Supplement MK-42 (Merck) contains 30 gm. streptomycin and 12.5 mg. $B_{12}$ per pound of feed.
**Supplement MK-45 (Merck) contains 15 gm. penicillin and 12.5 mg. $B_{12}$ per pound of feed.

**Effect of Antibiotic Supplements, Liver Extract and APF.** The possibility that liver extract and APF could cause an additional growth response to that obtained by the antibiotic supplements, suggested the present experiment. Chicks were put on experiment the day after being hatched. The experiment lasted for 6 weeks and had 6 groups fed the diets indicated below:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45 + 2.0 per cent liver residue
4. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45 + 2.0 per cent liver residue + 0.04 per cent thyroprotein
5. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45 +2.0 per cent liver residue + 0.2 per cent APF
6. Control + 0.05 per cent MK-42 + 0.05 per cent MK-45 + 2.0 per cent liver residue + 0.2 per cent APF + 0.04 per cent thyroprotein

TABLE 9--EFFECT OF THYROPROTEIN, ANTIBIOTIC SUPPLEMENTS*, LIVER RESIDUE** AND APF ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Body weight in successive weeks 0 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. |
| 1 | Control | 11 | M | 37.73 | 131.00 | 304.73 | 545.91 |
| | | 12 | F | 35.42 | 108.96 | 244.25 | 446.25 |
| | | 23 | Av. | 36.35 | 119.98 | 274.49 | 496.08 |
| 2 | Control + 0.04% Thyroprotein | 12 | M | 35.58 | 101.75 | 252.33 | 507.92 |
| | | 12 | F | 36.00 | 93.33 | 218.83 | 437.17 |
| | | 24 | Av. | 35.79 | 97.54 | 235.58 | 472.55 |
| 3 | Control + 0.05% MK-42[1] + 0.05% MK-45[2] + 2% Liver Extract | 13 | M | 37.46 | 138.00 | 345.31 | 594.92 |
| | | 14 | F | 36.36 | 119.21 | 282.21 | 506.28 |
| | | 27 | Av. | 36.91 | 128.61 | 313.76 | 550.61 |
| 4 | Control + 0.05% MK-42 + 0.05% MK-45 + 2% Liver Residue + 0.04% Thyroprotein | 12 | M | 38.42 | 147.08 | 355.92 | 592.58 |
| | | 15 | F | 36.33 | 128.87 | 287.67 | 494.53 |
| | | 27 | Av. | 37.38 | 137.98 | 321.80 | 543.56 |
| 5 | Control + 0.05% MK-42 + 0.05% MK-45 + 2% Liver Residue + 0.2% APF | 13 | M | 37.46 | 138.11 | 349.46 | 634.08 |
| | | 12 | F | 36.75 | 129.08 | 311.92 | 541.17 |
| | | 25 | Av. | 37.11 | 133.70 | 330.69 | 587.63 |
| 6 | Control + 0.05% MK-42 + 0.05% MK-45 + 2% Liver Residue + 0.2% APF + 0.04% Thyroprotein | 14 | M | 35.93 | 146.50 | 363.21 | 620.93 |
| | | 13 | F | 34.77 | 131.23 | 304.38 | 523.92 |
| | | 27 | Av. | 35.35 | 138.87 | 333.80 | 572.43 |

* Antibiotic supplements containing vitamin $B_{12}$ (Merck)
** Liver Residue (Wilson)
1 Supplement MK-42 contains 30 g. streptomycin and 12.5 mg. $B_{12}$ per pound
2 Supplement MK-45 contains 15 g. penicillin and 12.5 mg. $B_{12}$ per pound

It can be seen that although the thyroprotein-fed chicks in group 2 did not gain as much weight as the controls, the difference between the two groups was not significant (Table 9 and Fig. 9). Antibiotic supplements plus liver residue (group 3) stimulated further growth in controls and thyroprotein-fed chicks. The increased growth rate remained the same when thyroprotein was added (group 4). Furthermore, additional growth, over that observed in group 3, was caused by the incorporation of a 0.2 per cent APF in the feed (group 5). Birds in this group had a higher average weight than in the other groups. Approximately the same increase in weight was shown in group 6 where the same diet as in group 5 was fed, plus 0.04 per cent thyroprotein.

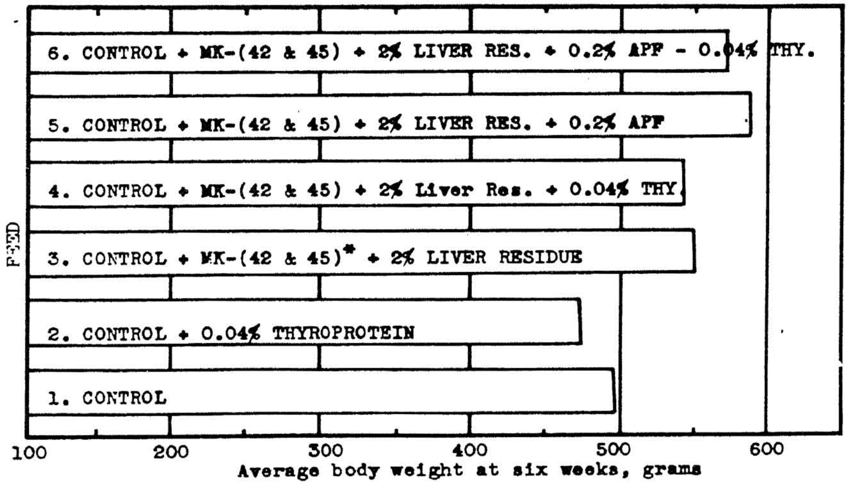


Figure 9. Effect of thyroprotein, antibiotic supplements and APF on body weight of White Plymouth Rock chicks.
*Same supplements and amounts as indicated in Figures 7 and 8.

**The Effect of a Streptomycin Mixture.** The antibiotic supplements used in the above experiments contain, besides antibiotics, vitamin $B_{12}$ and other unknown factors. Some pure antibiotics became available and their growth-stimulating effect upon the hyperthyroid chicks was investigated.

A streptomycin mixture (Merck) with a potency of 0.39 gram streptomycin base per gram of the mixture was fed in two levels (200 and 400 mg. per kg. of feed) in diets of day-old chicks for a period of 6 weeks. Chicks were grouped and fed the diet indicated below:

1. Control
2. Control + 0.04 per cent thyroprotein
3. Control + streptomycin mixture (200 mg. per kg. of feed)
4. Control + streptomycin mixture (200 mg. per kg. of feed) + 0.04 per-cent thyroprotein
5. Control + streptomycin mixture (400 mg. per kg. of feed)
6. Control + streptomycin mixture (400 mg. per kg. of feed) + 0.04 per-cent thyroprotein

TABLE 10--EFFECT OF THYROPROTEIN AND STREPTOMYCIN* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Body weight in successive weeks 0 | 2 | 4 | 6 |
|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. |
| 1 | Control | 11 | M | 42.09 | 121.55 | 312.27 | 498.45 |
| | | 14 | F | 41.57 | 109.86 | 258.57 | 429.43 |
| | | 25 | Av. | 42.24 | 115.71 | 285.42 | 463.94 |
| 2 | Control + 0.04% Thyroprotein | 8 | M | 41.29 | 119.50 | 307.75 | 506.25 |
| | | 9 | F | 39.56 | 110.11 | 281.78 | 399.33 |
| | | 17 | Av. | 40.41 | 114.81 | 294.77 | 452.79 |
| 3 | Control + Streptomycin Mixture (200 mg/kg feed) | 13 | M | 47.77 | 138.08 | 336.77 | 554.00 |
| | | 15 | F | 43.27 | 116.87 | 277.40 | 432.87 |
| | | 28 | Av. | 45.52 | 127.48 | 307.09 | 493.44 |
| 4 | Control + Streptomycin Mixture (200 mg/kg feed) + 0.04% Thyroprotein | 10 | M | 45.10 | 132.70 | 327.80 | 542.70 |
| | | 12 | F | 44.92 | 119.25 | 279.33 | 457.00 |
| | | 22 | Av. | 45.01 | 125.98 | 303.57 | 499.85 |
| 5 | Control + Streptomycin Mixture (400 mg/kg feed) | 12 | M | 45.67 | 147.92 | 331.25 | 575.33 |
| | | 14 | F | 41.64 | 120.21 | 274.00 | 461.93 |
| | | 26 | Av. | 43.66 | 134.07 | 302.63 | 518:63 |
| 6 | Control + Streptomycin Mixture (400 mg/kg feed) + 0.04% Thyroprotein | 10 | M | 45.80 | 140.00 | 325.40 | 535.20 |
| | | 14 | F | 44.71 | 125.36 | 277.57 | 437.07 |
| | | 24 | Av. | 45.26 | 132.68 | 301.49 | 486.14 |

* Streptomycin mixture (Merck), each gm. = 0.39 gm. streptomycin base

Thyroprotein feeding did not significantly affect the average weight of birds by the end of the experiment when compared with the controls (Table 10 and Fig. 10).

Both levels of streptomycin produced greater growth response than did the control feed alone. However, there was no significant difference between the effects of the two levels of streptomycin.

Addition of thyroprotein to the diets of streptomycin-fed chicks did not affect the growth rate as there was no significant difference in weight between groups 3-6 in the experiment.

Comparing group 2 (thyroprotein alone) with groups 4 and 6, it could be seen that streptomycin addition to the diet of hyperthyroid chicks allowed not only a normal but also an additional growth response.

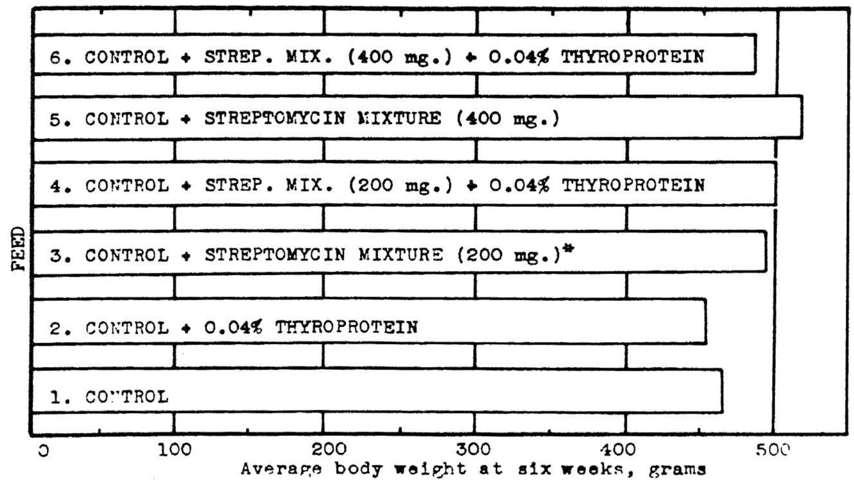


Figure 10. Effect of thyroprotein and streptomycin on body weight of White Plymouth Rock chicks.
*Streptomycin mixture (Merck), each gram equals 0.39 gram of streptomycin base (mg./kg. of feed).

**Effect of Aureomycin.** Crystalline aureomycin Cl was used in the following three experiments to test its effects on the growth rate of the hyperthyroid chick. A level of 50 mg. aureomycin per kg. of feed was given and the experiments were carried until the birds reached 7 weeks of age. The chicks were put on experiments at the age of 2 and three weeks. The diets fed were as follows:

1. Control
2. Control + aureomycin
3. Control + 0.01 per cent thyroprotein
4. Control + aureomycin + 0.01 per cent thyroprotein
5. Control + 0.02 per cent thyroprotein
6. Control + aureomycin + 0.02 per cent thyroprotein

The chicks were started on experiment at the age of 2 weeks and individually weighed each week for the following 4 weeks. The average weights are of the total numbers in the groups since no attempt was made to determine the number of each sex (Table 11 and Fig. 11).

Aureomycin supplement caused a greater response than did control feed alone. At the age of 6 weeks the average weights were 482.08 and 514.13 grams for the controls (group 1) and the aureomycin-fed (group 2), respectively.

The low level of thyroprotein (0.01 per cent) seemed to have a growth-stimulating effect when fed to 2 week-old chicks. Although the difference was not high enough to be significant, the thyroprotein-fed chicks (group 3) maintained a higher average weight than the controls (group 4).

When the diet of the chicks receiving the low level of thyroprotein was supplemented with aureomycin, the average body weight at 7 weeks of age was above that of the controls or the thyroprotein-fed chicks (groups 1 and 2).

Group 5 (0.02 per cent thyroprotein) and group 6 (0.02 per cent thyroprotein plus aureomycin) maintained average body weights throughout the experiment that were equal to those obtained in the

TABLE 11--EFFECT OF THYROPROTEIN AND AUREOMYCIN* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Average weight in successive weeks 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| | | | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 25 | 89.88 | 154.00 | 244.76 | 362.64 | 482.08 |
| 2 | Control + Aureomycin | 24 | 88.44 | 157.88 | 251.28 | 386.40 | 514.13 |
| 3 | Control + 0.01% Thyroprotein | 23 | 90.26 | 151.57 | 251.83 | 383.96 | 500.22 |
| 4 | Control + Aureomycin + 0.01% Thyroprotein | 25 | 91.00 | 157.80 | 243.48 | 384.52 | 523.48 |
| 5 | Control + 0.02% Thyroprotein | 24 | 92.36 | 151.95 | 247.04 | 374.28 | 489.44 |
| 6 | Control + Aureomycin + 0.02% Thyroprotein | 24 | 93.68 | 161.48 | 254.80 | 387.20 | 516.36 |

* Aureomycin CL (Lederle), 50 mg. per kg. of feed

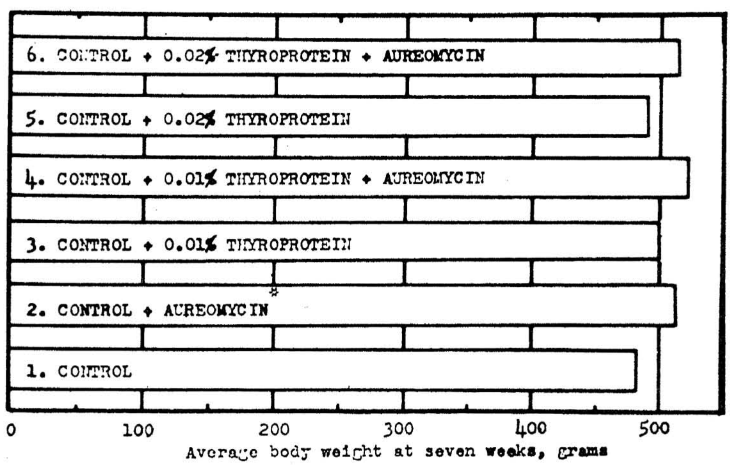


Figure 11. Effect of thyroprotein and aureomycin on body weight of White Plymouth Rock chicks.
*Aureomycin CL (Lederle), 50 mg. per kg. of feed.

control group. However, comparing the results of the two levels of thyroprotein, the higher level (group 5) resulted in an average weight slightly lower than in group 3 (0.01 per cent thyroprotein) but the difference was not significant.

Supplementation of the diet that contained 0.02 per cent thyroprotein (group 6) with aureomycin resulted in greater growth response than that obtained without aureomycin (group 5) or in the controls (group 1). The level of aureomycin used in this experiment was effective in stimulating growth in hyperthyroid chicks fed 0.01 or 0.02 per cent thyroprotein.

Two similar experiments were performed, using the diets as before but starting the chicks at age 2 and 3 weeks, respectively. At the end of the experimental periods, chicks were weighed, killed, the sex determined and the thyroids and gonads weighed (Tables 12 and 13 and Figs. 12 and 13).

From Table 12 it can be seen that aureomycin supplement (group 2) produced a significant increase in body weight of chicks over the controls (group 1). Both levels of thyroprotein (0.01 and 0.02 per cent) stimulated a greater growth response than that obtained by the control feed alone. Addition of aureomycin to thyroprotein-containing diets made them more effective in producing greater growth response than thyroprotein alone.

However, by testing the mean of each diet against the mean of the control diet, it was found that only in group 2 (aureomycin) was there a significantly greater final body weight.

When the chicks were started on experiment at the age of 3 weeks, the effect of the various supplements differed from that obtained with chicks started at the age of 2 weeks. Aureomycin did not produce a significant effect on growth rate, for the average body weights were 602.19 and 614.64 grams for the controls (group 1) and the aureomycin-fed chicks (group 2), respectively.

Thyroprotein feeding resulted in a slight decrease in rate of growth when a low level of 0.01 per cent was used. The effect was even more pronounced when a 0.02 per cent level was fed. This resulted in an average body weight of 523.26 grams compared with 602.19 grams for the controls. However, the addition of aureomycin to chicks receiving either level of thyroprotein (groups 4 and 5) counteracted the retardation of growth due to thyroprotein feeding.

Comparing the general effect of the same diets in relation to the starting age of the chicks, there was an indication that the chicks do not react to the diets in the same way. It would be better to begin supplementation with aureomycin at age 2 weeks, rather than wait until the chicks are 3 weeks old.

TABLE 12--EFFECT OF THYROPROTEIN AND AUREOMYCIN* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS**

| Group No. | Feed | No. | Sex | Average body weight in successive weeks 2 | 3 | 4 | 5 | 6 | 7 |
|---|---|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. | gm. | gm. |
| 1 | Control | 9 | M | 100.22 | 152.00 | 228.00 | 320.22 | 418.00 | 579.56 |
| | | 8 | F | 97.38 | 155.63 | 235.75 | 312.63 | 409.88 | 551.25 |
| | | 17 | Av. | 98.80 | 153.82 | 231.88 | 316.43 | 413.94 | 565.41 |
| 2 | Control + Aureomycin* | 12 | M | 101.00 | 188.58 | 291.83 | 407.50 | 459.50 | 702.40 |
| | | 8 | F | 93.63 | 170.50 | 261.13 | 361.50 | 476.50 | 607.25 |
| | | 20 | Av. | 97.32 | 179.54 | 276.48 | 384.50 | 513.00 | 654.84 |
| 3 | Control + 0.01% Thyroprotein | 9 | M | 98.89 | 158.89 | 240.67 | 339.33 | 472.33 | 612.56 |
| | | 7 | F | 99.43 | 150.14 | 240.86 | 334.00 | 450.00 | 578.86 |
| | | 16 | Av. | 99.16 | 154.52 | 240.77 | 336.67 | 461.17 | 595.71 |
| 4 | Control + 0.01% Thyroprotein + Aureomycin | 12 | M | 99.83 | 178.92 | 269.92 | 371.83 | 518.75 | 661.00 |
| | | 6 | F | 93.33 | 173.83 | 256.83 | 349.83 | 478.67 | 603.00 |
| | | 18 | Av. | 96.58 | 176.38 | 263.38 | 360.83 | 498.71 | 632.00 |
| 5 | Control + 0.02% Thyroprotein | 9 | M | 101.78 | 152.11 | 239.89 | 329.44 | 462.11 | 590.78 |
| | | 55 | F | 100.20 | 156.40 | 242.80 | 322.00 | 450.80 | 586.20 |
| | | 14 | Av. | 100.99 | 154.26 | 241.35 | 325.72 | 456.46 | 588.49 |
| 6 | Control + 0.02% Thyroprotein + Aureomycin | 10 | M | 101.70 | 172.50 | 254.50 | 344.10 | 468.70 | 614.30 |
| | | 10 | F | 94.60 | 163.80 | 245.20 | 335.60 | 454.80 | 584.30 |
| | | 20 | Av | 98.15 | 168.15 | 249.85 | 339.85 | 461.75 | 599.30 |

* Aureomycin CL (Lederle), 50 mg. per kg. of feed
** Starting age, 2 weeks

TABLE 13--EFFECT OF THYROPROTEIN AND AUREOMYCIN* ON BODY WEIGHT OF WHITE PLYMOUTH CHICKS**

| Group No. | Feed | No. | Sex | Average body weight in successive weeks 3 | 4 | 5 | 7 |
|---|---|---|---|---|---|---|---|
| | | | | gm. | gm. | gm. | gm. |
| 1 | Control | 14 | M | 168.50 | 261.86 | 373.14 | 616.07 |
| | | 10 | F | 170.20 | 263.70 | 372.70 | 588.30 |
| | | 24 | Av. | 169.35 | 262.78 | 372.92 | 602.19 |
| 2 | Control + Aureomycin | 15 | M | 160.47 | 267.33 | 391.87 | 635.87 |
| | | 10 | F | 162.00 | 260.80 | 379.87 | 593.40 |
| | | 25 | Av. | 161.24 | 264.07 | 385.79 | 614.64 |
| 3 | Control + 0.01% Thyroprotein | 13 | M | 172.92 | 267.15 | 385.08 | 602.23 |
| | | 9 | F | 160.33 | 246.56 | 350.56 | 549.56 |
| | | 22 | Av. | 166.63 | 256.86 | 567.82 | 575.90 |
| 4 | Control + 0.01% Thyroprotein + Aureomycin | 8 | M | 166.38 | 263.38 | 383.63 | 606.00 |
| | | 14 | F | 159.93 | 255.07 | 372.07 | 568.29 |
| | | 22 | Av. | 163.16 | 259.23 | 377.85 | 587.15 |
| 5 | Control + 0.02% Thyroprotein | 8 | M | 171.88 | 258.75 | 368.38 | 555.75 |
| | | 8 | F | 159.75 | 236.50 | 320.13 | 490.38 |
| | | 16 | Av. | 165.82 | 247.63 | 344.26 | 523.06 |
| 6 | Control + 0.02% Thyroprotein + Aureomycin | 8 | M | 170.38 | 263.88 | 382.63 | 601.50 |
| | | 9 | F | 151.33 | 237.22 | 343.00 | 533.22 |
| | | 17 | Av. | 160.86 | 250.55 | 362.82 | 567.36 |

* Aureomycin CL (Lederle), 50 mg. per kg. of feed
** Starting age, 3 weeks

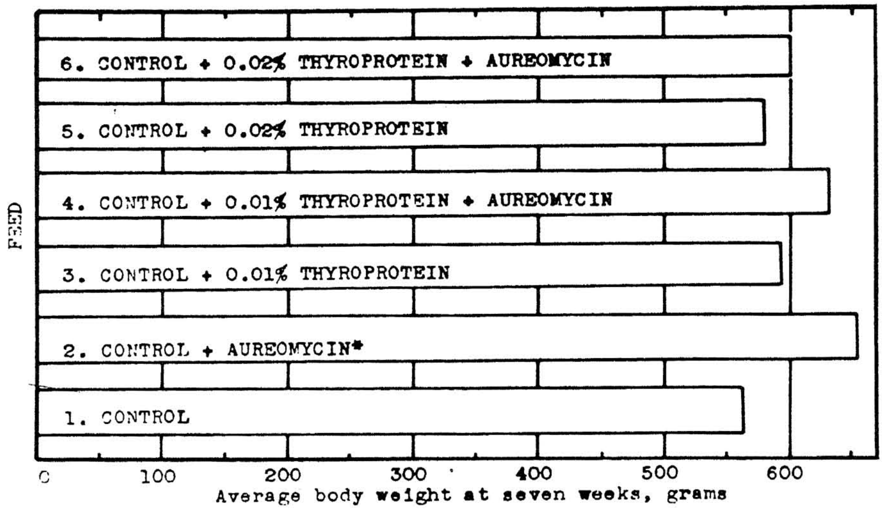


Figure 12. Effect of thyroprotein and aureomycin on body weight of White Plymouth Rock chicks (starting age, 2 weeks).
*Aureomycin CL (Lederle), 50 mg. per kg. of feed.

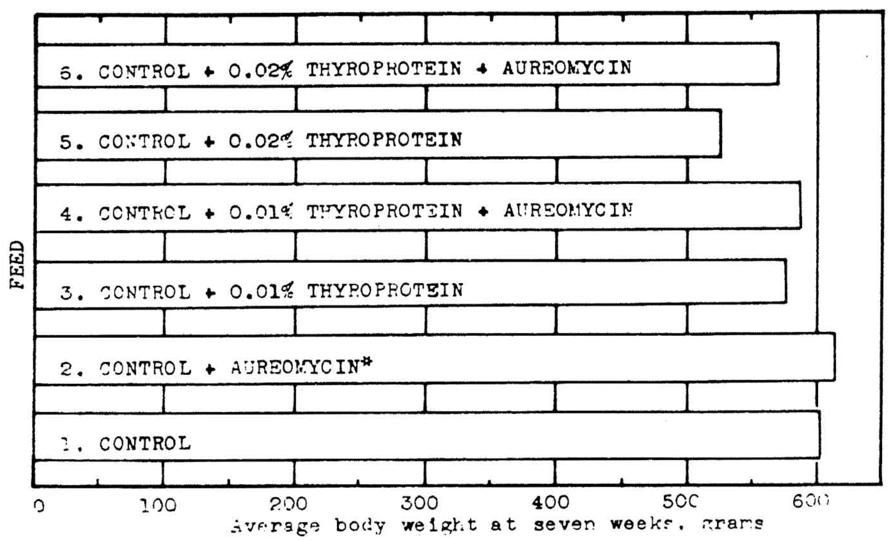


Figure 13. Effect of thyroprotein and aureomycin on body weight of White Plymouth Rock chicks (starting age, 3 weeks).
*Aureomycin CL (Lederle), 50 mg. per kg. of feed.

**Effect of Thyroprotein and Aureomycin on Weight of Thyroid Gland and Gonads.** It became of interest to find out if the different diets used in the above two experiments might affect the weights of thyroid glands and gonads. Immediately after the birds were killed, the glands and organs were dissected relatively free of fat and connective tissue and weighed with a precision balance (Roller-Smith Co.).

Aureomycin seems to have a depressing, but questionable, effect on the weight of the thyroid gland (Tables 14 and 15). When the proportional weight of the thyroid per 100 grams of body weight was considered as a basis for comparison, chicks that started at the age of 2 weeks showed less response to aureomycin administration than did the chicks that were started at the age of 3 weeks. For the two ages the values for the controls and the aureomycin-fed chicks were 7.5 vs. 6.8 and 8.2 vs. 6.2 mg., respectively.

Chicks, at the age of 2 weeks, appeared to be more sensitive to thyroprotein feeding than 3 weeks old chicks. Either the average absolute weight or the proportional weights of the thyroids per 100 grams of body weight were smaller for both levels of thyroprotein, when the starting age was 2 weeks.

Addition of aureomycin to thyroprotein-containing feeds did not alter the change in weight that resulted from feeding thyroprotein

TABLE 14--EFFECT OF THYROPROTEIN AND AUREOMYCIN ON WEIGHT OF THYROID GLAND AND GONADS OF WHITE PLYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Body* Weight gm. | Thyroid Weight mg. | Gonads Weight mg. |
|---|---|---|---|---|---|---|
| 1 | Control | 9 | M | 579.56 | 38.9 (6.7)** | 158.9 (24.2)** |
| | | 8 | F | 551.25 | 45.9 (8.3) | 178.9 (32.5) |
| | | 17 | Av. | 565.41 | 42.4 (7.5) | |
| 2 | Control + Aureomycin | 12 | M | 702.42 | 42.9 (6.1) | 150.6 (21.4) |
| | | 8 | F | 607.25 | 45.9 (7.6) | 168.5 (27.8) |
| | | 20 | Av. | 654.82 | 44.4 (6.8) | |
| 3 | Control + 0.01% Thyroprotein | 9 | M | 612.56 | 12.4 (2.0) | 129.2 (21.1) |
| | | 7 | F | 578.86 | 11.1 (1.9) | 186.6 (32.2) |
| | | 16 | Av. | 595.71 | 11.8 (2.0) | |
| 4 | Control + 0.01% Thyroprotein + Aureomycin | 12 | M | 661.00 | 9.2 (1.4) | 133.3 (20.2) |
| | | 6 | F | 603.00 | 8.9 (1.5) | 181.5 (30.1) |
| | | 18 | Av. | 632.00 | 9.0 (1.5) | |
| 5 | Control + 0.02% Thyroprotein | 9 | M | 590.78 | 9.3 (1.6) | 125.1 (21.2) |
| | | 5 | F | 586.20 | 9.1 (1.6) | 202.2 (34.5) |
| | | 14 | Av. | 588.49 | 9.2 (1.6) | |
| 6 | Control + 0.02% Thyroprotein + Aureomycin | 10 | M | 614.30 | 10.3 (1.7) | 143.3 (23.2) |
| | | 10 | F | 584.30 | 10.8 (1.8) | 190.8 (32.7) |
| | | 20 | Av. | 599.30 | 10.5 (1.8) | |

* Average body weight at 7 weeks (starting age, 2 weeks)

TABLE 15--EFFECT OF THYROPROTEIN AND AUREOMYCIN ON WEIGHT OF THYROID GLAND AND GONADS OF WHITE PHYMOUTH CHICKS

| Group No. | Feed | No. | Sex | Body* Weight gm. | Thyroid Weight mg. | Gonads Weight mg. |
|---|---|---|---|---|---|---|
| 1 | Control | 14 | M | 616.07 | 46.7 (7.6)** | 150.6 (24.4)** |
| | | 10 | F | 588.30 | 51.7 (8.8) | 207.9 (35.3) |
| | | 24 | Av. | 602.19 | 49.2 (8.2) | |
| 2 | Control + Aureomycin | 15 | M | 635.87 | 34.0 (5.3) | 131.5 (20.7) |
| | | 10 | F | 593.40 | 41.5 (7.0) | 156.2 (26.3) |
| | | 25 | Av. | 614.64 | 37.8 (6.2) | |
| 3 | Control + 0.01% Thyro-protein | 13 | M | 602.23 | 12.5 (2.1) | 146.3 (24.3) |
| | | 9 | F | 549.56 | 13.6 (2.5) | 189.3 (34.5) |
| | | 22 | Av. | 575.90 | 13.1 (2.3) | |
| 4 | Control + 0.01% Thyro-protein + Aureomycin | 8 | M | 606.00 | 12.5 (2.1) | 109.8 (18.1) |
| | | 14 | F | 568.29 | 13.7 (2.4) | 157.8 (27.8) |
| | | 22 | Av. | 587.15 | 13.1 (2.3) | |
| 5 | Control + 0.02% Thyro-protein | 8 | M | 555.75 | 12.3 (2.2) | 136.2 (24.5) |
| | | 8 | F | 490.38 | 12.4 (2.4) | 179.0 (36.5) |
| | | 16 | Av. | 523.06 | 12.4 (2.3) | |
| 6 | Control + 0.02% Thyro-protein + Aureomycin | 8 | M | 601.50 | 9.1 (1.5) | 118.8 (19.8) |
| | | 9 | F | 533.22 | 12.8 (2.4) | 147.6 (27.7) |
| | | 17 | Av. | 567.36 | 11.0 (2.0) | |

* Average body weight at 7 weeks (starting age, 3 weeks)
** Weight per 100 gm. of body weight

alone. However, some contradictory results were obtained in groups 4 and 6 (Table 14) which were attributed to experimental error.

The effects of thyroprotein, aureomycin, or a combination of both on the weights of the organs of chicks were not evident. The result might be due to the great variability in the gonads and the relatively small number of chicks used in these experiments.

## DISCUSSION

Nutrition has now gained prominence in the study of hyperthyroidism. In both natural and experimental hyperthyroidism, the supplementation of the diet with vitamins and growth-promoting factors, has been found to alleviate the detrimental symptoms associated with this condition. However, the mechanisms by which these supplements exert their effects are not fully understood. But with the current advance in knowledge of the interrelationships between vitamins, enzymes and endocrine systems, it may not be long until the different problems associated with the use of thyroactive materials in animal experimentation are solved.

In many cases, reports concerned with the beneficial responses to different supplementation in the nutrition of the hyperthyroid animal have revealed contradictory results. However, a number of factors should not be overlooked when comparing the results of different experiments. It has been discovered that the differences in thyroid hormone secretion rate and consequently in the response to thyroprotein feeding, could be attributed to differences in breed, species, age, and environmental conditions. Other two important factors in that respect are the basal diet used and the amount of nutrients stored in the tissues of the experimental animals at the beginning of the experimental period. As a result, groups of animals from different sources respond in a different manner to thyroprotein feeding or to the dietary supplementation. Transfer of nutrients through the egg to the chick has been repeatedly shown to permit essentially normal growth on rations which are low in the particular factor unless the hens have been previously depleted (Bolene et al., 1950). It seems likely that an analogous transfer may occur in the case of the factor(s) antagonistic to the growth-depressing effect of a high level of thyroprotein.

Some of the factors mentioned above could be responsible for the variations observed in the response of chicks, of the same age, to the same level of thyroprotein. A level of 0.04 per cent thyroprotein in the diet of day-old chicks while showing a depressing effect in some experiments, caused an increase in body weight over that of the controls in other experiments. With a starting age of one week, the chicks seemed to be more tolerant to the level of 0.04 per cent thyroprotein.

When the thyroprotein levels were reduced to 0.01 and 0.02 per cent, a stimulus to the growth rate over that of the controls resulted if chicks were started on the experiment at the age of two weeks but not at the age of three weeks. Chicks at the age of two weeks, appeared to be more sensitive to thyroprotein feeding than three-week old chicks. The weights of the thyroids were smaller for both levels of thyroprotein (0.01 and 0.02 per cent) when the starting age was 2 weeks.

Few reports have appeared in the literature dealing with the effect of thyroprotein feeding on growing White Plymouth Rock chicks. Irwin, Reineke, and Turner (1943) reported that low dosage of thyroprotein would accelerate the growth of White Plymouth chicks. Glazener and Shaffner (1948) showed that, in chicks, there was a strain difference which controlled the response of White Plymouth chicks to thyroprotein feeding. The slow-growing strains showed a greater growth response to thyroprotein than did the rapid-growing strains. This might contribute to the variations in response to thyroprotein feeding observed in the present work.

The supplementation used in this work seemed to provide a suitable means for the sustenance of the hyperthyroid chick. These different substances have been reported to accelerate the growth rate of animals receiving rations which supply adequate nutrients according to the present feeding standards.

A level of 0.5 per cent APF in the diet of the chicks caused a growth response greater than the maximum response obtained with the control feed. The same level of APF was effective in counteracting retardation of growth caused by feeding a level of thyroprotein up to 0.04 per cent. Increasing of the level of APF to 1.0 per cent had no beneficial effect over that obtained with the 0.5 per cent level. Working with rats, Bolene et al. (1950), found APF (2 per cent) to be effective in counteracting the growth-depressing effect of toxic levels of thyroprotein (0.25 per cent).

In normal chicks, the growth response produced by adding APF (0.5 per cent) was similar to that obtained with vitamin $B_{12}$ supplement (3 ug. per 100 gm. of feed). Stokstad et al. (1949) found that vitamin $B_{12}$ was only partially as effective as the APF, but the basal ration they used differed from the one employed in the present work. However, in the hyperthyroid chick, the average weight of chicks supplemented with APF exceeded that of the vitamin $B_{12}$-supplemented group. This is an indication of the complex nature of APF as revealed by other workers (Carlson et al., 1949; Combs et al., 1950; Stokstad and Jukes, 1950). Prinzie (1951) demonstrated the importance of the composition of the control feed as a factor influencing the effect of vitamin $B_{12}$ supplement in hyperthyroidism. Vitamin $B_{12}$ (3 ug. injected subcutaneously three times per week) exhibited a protective action against high doses of thyroprotein (0.25 per cent), provided that the growing rats received a ration high in soybean meal content (29 per cent). When the rats were fed a ration that contained wheat meal instead of soybean meal, vitamin $B_{12}$ was not effective.

A group of the B vitamins (thiamine, riboflavin, niacin, Ca-pantothenate, pyridoxine, para-aminobenzoic acid, choline, folic acid, and biotin) was included in the diet of the control as well as the hyperthyroid chicks. The supplement caused an increased growth rate in the controls and protected completely against any loss in weight that might result from feeding thyroprotein. The two groups that were supplemented with the B vitamins did not differ in their final average weight whether thyroprotein was included in their diet or not. Under the condition of the experiment, this result suggests that the levels of the vitamins used might meet the requirements of these vitamins when chicks were fed a level of 0.04 per cent thyroprotein. It should be noted that many of the individual vitamins listed above had been shown to possess an antithyrotoxicosis effect, though the amounts used in some reports seem excessive when compared with the dosage in this work (Allardyce et al., 1947). The average weight of the hyperthyroid chicks was more when the feed was supplemented with the B-vitamins (other than $B_{12}$) than when the supplement given was vitamin $B_{12}$ alone. In the case of the control chicks the reverse was observed.

Vitamin $B_{12}$ supplement caused an increase in weight of hyperthyroid chicks fed control feed. This is in agreement with the work of Sure and Easterling (1950). However, when vitamin $B_{12}$ was added to the diets of the controls and the hyperthyroid groups, the average weight of the latter was below that of the former.

That there is an interrelationship between the thyroid gland and vitamin D had been reported by Sure in 1938. It was found that a deficiency of vitamin D was the only vitamin to cause a decrease in the weight of the thyroid gland. In the present investigation a level of 0.02 per cent of thyroprotein did not alter the amount of ash in the tibia of chicks receiving different levels of vitamin D. The percentage ash in thyroprotein fed chicks was even slightly higher than where no thyroprotein was added. These results are in accord with the work of Schechet (1951) on rats. The addition of 0.025 per cent thyroprotein was found to be about equal in its ability to promote growh, and aided in the calcification response. Nevertheless, in the present work, there was an indication that increasing the level of thyroprotein to 0.04 per cent might require an additional supplement of vitamin D since the ash percentage was lower than when 0.02 per cent of thyroprotein was fed.

An extracted liver residue, at the level of 5 per cent, had a growth promoting effect in both normal and hyperthyroid chicks. Similar results had been reported in rats (Ershoff, 1948; Bolene et al., 1950), in rabbits (Betheil and Lardy, 1949) and in mice (Bosshardt et al., 1949). Attempts have been made to isolate from liver the factor(s) that have the antithyrotoxic effect (Betheil and Lardy, 1949; Ershoff, 1950).

The addition of APF to the diet of hyperthyroid chicks receiving liver residue caused further increase in the growth response. This finding suggested that APF contained additional growth promoting factors not present in the liver residue used. Betheil and Lardy (1949) presented evidence for the probable dual nature of the growth factor present in whole liver powder since solvent fractionation indicated that in spite of exhaustive extraction of liver powder only a part of the activity could be brought into solution.

The growth-promoting effect of different antibiotic supplements and pure antibiotics has been reported by a number of workers, suggesting the application of their use in the diet of the hyperthyroid chick. Two supplements that contained vitamin $B_{12}$ (12.5 mg. per pound) in addition to either streptomycin (30 gm. per pound) or penicillin (15 gm. per pound), were found to have a similar growth stimulating effect in chicks fed either the control diet or one containing thyroprotein.

Antibiotic supplements plus liver residue stimulated further growth in controls or thyroprotein fed chicks. Furthermore, additional growth was caused by the incorporation of a 0.02 per cent APF in the feed. An explanation for the effect of the antibiotic supplements in hyperthyroidism could be in their provision of additional unidentified supplements through the selective inhibition of certain groups of microorganisms and in the stimulation of growth of other useful microorganisms.

Another antibiotic, streptomycin, was found to produce greater growth response than did the control feed alone. This confirms the reports of Moore et al. (1946) and Lueoke et al. (1950). Addition of thyroprotein (0.04 per cent) to the diets of streptomycin-fed chicks did not affect the growth rate. The level of streptomycin required for maximum growth and to guard against weight loss when feeding thyroprotein was found to be 200 mg. streptomycin mixture (78 mg. streptomycin base) per kg. of feed.

Aureomycin Cl (50 mg. per kg. of feed) was found to elicit a significant increase in growth response over that of the control feed. Furthermore, aureomycin stimulated a growth response above that observed when chicks were fed low levels of thyroprotein (0.01 and 0.02 per cent). No such results were accomplished when the starting age was three weeks.

## SUMMARY AND CONCLUSIONS

1. A study has been carried out to investigate the effect of thyroprotein feeding on growing White Plymouth chicks and the possibility of meeting the nutritional requirements of the hyperthyroid animal by supplementation of the feed with additional vitamins and growth stimulating factors.

2. A level of 0.04 per cent thyroprotein (18 gm. per 100 pounds) in the diet of day-old chicks did not have a consistent effect on body weight. In some experiments this level of thyroprotein showed a depressing effect while in other experiments it caused a slight increase in body weight. Factors responsible for such discrepancies in the results have been discussed.

3. Lower levels of thyroprotein (0.01 and 0.02 per cent) gave an almost significant stimulus to the growth rate over that of the controls when chicks were started on the experiment at the age of two weeks but not at the age of three weeks. Chicks at the age of two weeks appeared to be more sensitive to thyroprotein feeding than three-weeks-old chicks. The weights of the thyroids were smaller for both levels of thyroprotein (0.01 and 0.02 per cent) when the starting age was two weeks.

4. APF at a level of 0.5 per cent in the diet caused a growth response greater than the maximum response obtained with the control feed. The same level of APF was effective in promoting a growth rate over that of the controls when thyroprotein constituted as much as 0.04 per cent of the feed. Increasing the level of APF to 1.0 per cent had no beneficial effect over that obtained with the 0.5 per cent level.

5. Vitamin $B_{12}$ (3 ug. per 100 gm. of feed) was equally as effective as 0.5 per cent APF in promoting growth in chicks receiving control feed. However, in the hyperthyroid chicks, APF was more effective in growth stimulation.

6. A supplement that contained B-vitamins (other than vitamin $B_{12}$) caused an increased growth rate in the controls and completely protected against any loss in weight that might result from feeding thyroprotein.

7. Thyroprotein at a level of 0.02 per cent did not alter the amount of ash in the tibia of chicks on a rachitogenic diet, and receiving different levels of vitamin D. The percentage ash in thyroprotein-fed chicks was even slightly higher than where no thyroprotein was added. Nevertheless, there was an indication that increasing the level of thyroprotein to 0.04 per cent might necessitate giving an additional supplement of vitamin D since the ash percentage was lower than when 0.02 per cent of thyroprotein was fed.

8. An extracted liver residue, at the level of 5 per cent, had a growth promoting effect in both normal and hyperthyroid chicks. The addition of APF to the diet of hyperthyroid chicks receiving liver residue caused further increase in the growth response, suggesting that APF contained additional growth-promoting factors not present in the liver residue.

9. Two supplements that contained vitamin $B_{12}$ (12.5 mg. per pound) in addition to either streptomycin (30 gm. per pound) or penicillin (15 gm. per pound), were found to have a growth stimulating effect in chicks fed either the control diet or one containing thyroprotein. Both supplements plus liver residue stimulated further growth in controls or thyroprotein-fed chicks. Furthermore, additional growth was caused by the incorporation of 0.2 per cent APF in the feed.

10. Streptomycin mixture (200 ug. per kg. of feed) stimulated an additional growth response in normal chicks. Addition of thyroprotein (0.04 per cent) to the diets of streptomycin-fed chicks did not affect the growth rate.

11. Aureomycin Cl (50 mg. per kg. of feed) was found to elicit an increase in growth response over that of the control feed and to stimulate growth above that observed when chicks were fed low levels of

thyroprotein (0.01 and 0.02 per cent). No such results were obtained when the starting age was three weeks.

12. There seemed to be evidence that the antibiotics might affect the weight of the thyroid gland. Aureomycin had a slightly depressing effect on the weight of the thyroid gland. The effect was more noticeable when the starting age of the chicks was two rather than three weeks.

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