

**EFFECT OF CERTAIN VITAMIN DEFICIENCIES IN GROWING
CHICKS WITH AND WITHOUT THYROXINE INJECTIONS.**

By

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INTRODUCTION

The importance of the role of vitamins in the animal body remained obscure to investigators in the field of nutrition until after the beginning of the present century. However, during the past two decades, very rapid progress has been made. At present, the chemical structure is known for 15 different vitamins which are required for the maintenance, growth, and reproduction of animals.

Similarly, the importance of the endocrine glands in the regulation of the physiological functions of the body was recognized only in recent years. At present, the endocrine glands are known to function as the controlling centers of the various physiological processes that occur in the animal body. With the extensive investigations of the effects of different vitamins and hormones in the animal body, it is of particular interest, at the present time, to consider the interrelations between the vitamins and hormones in their functioning. The present study was undertaken, therefore, to study the interrelationship of certain vitamins of the B-complex (riboflavin, folic acid, pantothenic acid, and vitamin B₁₂) and thyroxine, the hormone of the thyroid glands.

The importance of riboflavin, folic acid, pantothenic acid, and vitamin B₁₂ in poultry nutrition is well established. Adequate amounts of all of these vitamins

are required for rapid growth and good livability in the chick. Riboflavin, folic acid, and pantothenic acid are also required for normal reproduction in hens. In addition, a deficiency of pantothenic acid results in nutritional dermatitis in chicks and hens. Riboflavin deficiency also results in a curled toe paralysis condition. Folic acid is also required for normal feather formation and hemoglobin formation in the chick.

The thyroid gland is known to function as a controlling physiological mechanism for growth, maturation, and reproduction of chickens. The thyroid hormone (thyroxine) controls the metabolic rate and the general oxidative processes in the body. It is also interrelated with other endocrine glands. Reproduction is effected by the thyroid function, probably through the gonads and pituitary. Dysfunction of the thyroid gland interferes with thyroxine production, resulting in a depression of the metabolic rate, impaired physical and mental responses, lethargy, and sterility. On the other hand, excessive thyroxine production or administration causes elevation of the metabolic rate, nervousness, increased activity, and sometimes results in toxicity.

At the present time, considerable experimental work is being conducted in which the action of certain chemical compounds, known to have specific effects on the functioning of the thyroid glands, are being studied in relation

to their effect on the market quality of poultry meat. These substances not only effect the degree of fattening but also effect the feed utilization.

A few investigators have reported an inter-dependence between certain of the vitamins and hormones. Since both the hormones and the vitamins are regulatory in nature, it is considered probable that many more interrelations exist between these two most important types of regulatory substances in the body. With this in mind, work was initiated, involving the study of possible interrelationships of thyroxine and four of the B-complex vitamins in chick nutrition. Riboflavin, folic acid, pantothenic acid, and vitamin B₁₂ were selected for study.

REVIEW OF LITERATURE

Jackson (1925, 1929) and Stephens (1941) have clearly shown that malnutrition in laboratory animals profoundly modifies the structure and function of endocrine glands, resulting in hypofunction of the entire endocrine system. Endocrine-nutritional relationships have been reviewed by Guilbert in 1942. It was pointed out that undernutrition generally produces a phenomenon of inanition which acts through the pituitary gland and thereby affects all other glands. Phillips, Friedman, and Turner (1939) reviewed the relation of nutrition to reproduction. They remarked that "food restriction that causes marked growth retardation in young or weight losses in adults, results in cessation of estrus in the female, loss of sexual libido, mortality of sperm, and testicular atrophy in the male". Breneman (1940) observed that restriction of food to chicks resulted in a suppression of body growth and produced testes that were smaller in proportion to body weight than those of chicks receiving a full diet. Evans (1928), Guilbert and Hart (1930), Pearson (1937), Orent and McCollum (1931), and Hertz (1945) have shown serious nutritional disturbances to depress reproductive capacity. Gomez Mont et al. (1947) observed that in partially starved rats, the hypertrophy and hyperplasia of the thyroid gland induced by thiouracil is less marked and develops later

than in normally fed controls. Guilbert (1942) reported that an iodine deficiency in the diet of animals causes reproductive failure primarily through development of a goitrous condition in the developing fetus. Abelin (1930), working with rats, found that increasing the casein content of a thyrotoxic diet decreased the severity of the symptoms.

Effects of vitamin A deficiency on the thyroid glands of the rat have been reported by many investigators.

McCarrison (1931) noted hypertrophy of the glands in rats and abnormal distention of the follicles in vitamin A deficiency. Coplan and Sampson (1935) reported that vitamin A deficiency produced definite hypertrophy of the thyroid glands in the female rat and consistent atrophy in the male. A discussion on the excess requirement of vitamin A in human hyperthyroidism has also been presented by Simkins (1947). Lipsett and Winzler (1947) found that the thyroid glands of vitamin A deficient rats, were relatively heavier than those of the control animals, and that vitamin A deficiency decreased the rate of formation of thyroxine. Their results indicated that iodine metabolism was abnormal in the vitamin A deficient rat. Sadhu and Truscott (1948) observed that hypervitaminosis A was accompanied by a decrease in protein-bound iodine in the liver and thyroid glands, and an increase in protein-bound iodine in the blood serum, pituitary and skeletal muscles. They suggested that these results were due to a

decreased hepatic destruction of thyroxine, with a consequent hyperthyroxinemia. The latter depresses the thyrotropic hormone secretion, producing the observed decrease in both thyroid weight and in protein-bound iodine in the thyroid glands.

The work of Ershoff et al. (1945) is an example of a vitamin-hormone relationship in which they pointed out that vitamin A in rats stimulates rapid growth through the pituitary growth hormone.

In addition to the observations of Abelin et al. (1930), Abelin (1933) and Von Euler and Klussman (1932), Logaras and Drummond (1938), and Sure and Buchanan (1937b) also obtained evidence of an increased requirement of vitamin A in the rat during experimental hyperthyroidism. The same was found to be true for the ascorbic acid requirements of the guinea pig by Svirbley (1935), and Sure and Theis (1939) in rats.

Handler and Follis, Jr. (1948) further stated that thyroid feeding hastened the death of choline deficient rats showing no necrosis or fibrosis of the liver. They found that rats, fed diets deficient in both cystine and choline, were more susceptible to hyperthyroidism than those receiving diets adequate in either of these nutrients.

Gertz (1946) postulated that the disturbance of the normal hormonal mechanism, associated with a vitamin deficiency, may represent either failure in the hormone

production, a loss of responsiveness on the part of the tissues involved, or decreased hormone destruction. Thus anestrus in rats resulting from feed restriction (Marrian et al. 1929, Drill et al. 1944), impairment of stilbestrol response in the folic acid deficient chicks (Hertz, 1945), and persistent estrus in B-complex deficient rats to which estrogens have been intrasplenically administered, are examples of the possible reactions, mentioned above, showing definite functional relationships between vitamins and hormones. According to Drill et al. (1943) excessive doses of thyroxine or desiccated thyroid cause anestrus and loss of body and ovarian weight in the adult rat and prevent gonadal development in the young animal. They stated that these effects can be prevented in adult rats by the administration of increased amounts of thiamin or yeast. Ershoff (1945) reported that these nutrients are without effect in the young animals.

Hertz (1945) stated that a highly specific dietary factor (folic acid) may be shown to be both quantitatively and qualitatively involved in a hormonal response resulting in new tissue formation. This situation is somewhat analogous to the necessity for adequate iodine ingestion for normal thyroid function. Drill (1938) presented extensive data indicating that the thyroid-fed rat has an increased requirement for thiamin, pyridoxine, and pantothenic acid. He further suggested that the failure

of the thyrotoxic rat to utilize estrogen optimally is related to its deficiency of B-complex factors.

Ershoff (1947) stated that administration of liver completely counteracted the retardation of growth and inhibition of ovarian development observed in immature rats fed large amounts of desiccated thyroid. He concluded that liver contained some factor other than riboflavin, calcium pantothenate, or folic acid, which is required for normal growth and ovarian development in the immature thyroid-fed rat.

Sure and Smith (1934) demonstrated that retardation of loss of weight can be secured in animals receiving toxic doses of pure crystalline thyroxine (Squibb's) by administration of a potent vitamin B₁ concentrate. Sure and Buchanan (1937a) found that after the heat stable components of the vitamin B complex had been provided by autoclaved northwestern dehydrated baker's yeast, pure crystalline vitamin B₁ was an excellent anti-thyrotoxic agent in daily doses of 30 to 100 gamma, capable of counteracting the toxicity of as high a daily dose as 0.2 mg. of thyroxine.

Coggeshall and Greene (1933) found that in their studies the liver glycogen in rats was reduced more by the administration of thyroxine than by feeding dried thyroid gland. Himwich, Goldfarb, and Cowgill (1931, 1932) found that dogs fed desiccated thyroid needed more vitamin

B-complex as judged by the onset of anorexia and loss of weight. This was confirmed in the pigeon by Cowgill and Palmieri (1933). Frazier and Friedman (1935) found a lower percentage of glycogen in the liver during hyperthyroidism, which could not be raised by the feeding of sugar. Drill (1937) suggested that the loss of glycogen in the liver during hyperthyroidism might be due to the destruction of, or inability to utilize, the B-complex vitamins supplied by yeast as a result of administration of desiccated thyroid. He noted that rats, on a normal diet containing 2.1 to 2.4 International Units of vitamin G per day and receiving 0.1 mg. of thyroxine subcutaneously per day, eventually lost weight and showed low liver glycogen values.

Betheil et al. (1947) suggested that the increased metabolic rate, which results from the administration of a thyrotoxic material, may necessitate an increased dietary intake of members of the vitamin B-complex. The results of many workers confirm such a hypothesis (Abelin, 1930), Cowgill and Palmieri (1933), and Himwich et al. (1931, 1932).

The partial protective effect of vitamin B₁ against thyrotoxicity, reported by Sure and Smith (1934), was later confirmed by Sure and Buchanan (1937a) and Drill and Sherwood (1938). Drill and Overman (1942) found that pyridoxine and calcium pantothenate were required in larger quantities during experimentally induced

thyrotoxicosis. Abelin (1945, 1946) observed a beneficial effect when massive doses of calcium pantothenate were administered to hyperthyroid rats receiving a crude ration. This suggested a functional relationship between pantothenic acid and thyroxine.

Betheil et al. (1947) confirmed the presence in yeast of an anti-thyrotoxic factor as previously reported by Brill (1937), Sure and Smith (1934), and Sure and Buchanan (1937a). They also found liver to be a better source of the anti-thyrotoxic material than yeast and reported that this factor was not one of the then recognized B-complex vitamins.

Allardyce et al. (1947), working with rats, observed that the administration of riboflavin, thiamin, and pyridoxine counteracted the effect of desiccated thyroid so far as the elevation of the basal metabolic rate and loss of weight were concerned. Riboflavin proved to be the most effective of the three vitamins used. These workers found that, without riboflavin supplements, the basal metabolic rate of male and female rats dropped below the original level. These animals lost weight, despite the continued administration of desiccated thyroid over a long period of time. When the series of thyroid administrations were repeated after an interval of time, the elevation of the basal metabolic rate was less in all cases. Apparently the rats adjust themselves fairly

rapidly to the action of added desiccated thyroid and develop a certain tolerance. Although the rats were able to overcome the action of daily doses of desiccated thyroid without vitamin supplements, the inclusion of vitamins facilitated a more rapid recovery. The effectiveness of the vitamins was in the following order, from greatest to least: riboflavin, thiamin HCl, para-aminobenzoic acid, nicotinic acid amide, calcium pantothenate, and pyridoxine.

Martin (1947) stated that when rats, on stock diets containing sulfaguanidine, were given thyroxine, an acute leucopenia developed. The simultaneous oral treatment of these rats with "folic acid" concentrate resulted in a nullification of the tendency toward a leucopenia. On the stock sulfanamide diet, there was a marginal intake of folic acid attributed to a reduced synthesis of this vitamin in the intestinal tract. He concluded that thyroxine, and the concomitant high metabolic rate, increased the demand of the animal for "folic acid."

Ershoff and Hershberg (1945) working with rats, however, were unable to alleviate the symptoms resulting from thyroid administration by greatly increasing the calcium pantothenate level of the ration. They further found that rats fed a purified ration, very high in the members of vitamin B-complex known to be required, grew poorly when desiccated thyroid was added to the diet. The feeding of a yeast-containing ration resulted in much better growth

and survival. This indicated that some factor present in yeast, other than the vitamins added, exerted an anti-thyrototoxic effect. Their results indicated that rats, fed purified diets, are extremely sensitive to thyroid feeding. This sensitivity manifested itself in sudden death, apparently due to cardiac failure. Length of survival was markedly prolonged in thyroid-fed rats receiving B-complex vitamins in the form of yeast. These workers stated, in this regard, that the marked sensitivity of the hyperthyroid organism to adrenalin is of particular importance.

Raab (1941) demonstrated that doses of adrenalin, relatively innocuous for the normal rat, proved fatal within three to five minutes when injected into animals that had been pretreated with thyroxine for three days. Similarly the concentration of adrenalin and adrenalin-like substances in the heart muscle following an injection of adrenalin was markedly increased if the animals were pretreated with desiccated thyroid.

Ershoff (1948) stated that immature female rats failed to survive when fed purified rations containing both pancreas and desiccated thyroid. He further observed that length of survival was significantly prolonged if either pancreas or thyroid were eliminated from the experimental ration. The deleterious effects of pancreas in the thyroid fed rat were not due to increased digestion or

absorption of thyroactive material.

Marx et al. (1948) and Ershoff and Marx (1948) indicated in their recent works that cholesterol may also prolong the survival time in immature rats, fed desiccated thyroid in a diet also containing desiccated whole liver, yeast, extracted liver residue, the benzol-insoluble fraction of extracted liver residue and xanthine.

Robblee et al. (1948) stated that a thyrotoxic condition in the chick, induced by feeding desiccated thyroid or iodinated casein, was effectively counteracted either by supplementing the diet with condensed fish solubles or by the injection of reticulogen (a concentrated liver extract used in the treatment of pernicious anemia). An increased growth response was obtained upon addition of either desiccated thyroid or iodinated casein to a ration containing adequate amounts of the known and unidentified growth factors.

Ershoff (1948), in his recent studies with rats, observed that oral administration of desiccated whole liver prolonged survival and counteracted the retardation in growth of immature rats fed toxic doses (0.5% of ration) of desiccated thyroid. The findings of Ershoff and McWilliams (1948) indicate that the beneficial effect of extracted liver residue on growth and survival of immature rats, fed massive doses of desiccated thyroid, is equally evident in rats fed massive doses of thyroxine, thyroglobulin or

iodinated casein. The effects were particularly striking in the rats receiving thyroxine. According to Ershoff (1948) the protective factor(s) was present in the benzol-insoluble fraction of extracted liver residue. He further suggested that the growth promoting and survival-prolonging effects of liver in the immature hyperthyroid rat are due to separate factors other than the presently recognized nutrients, including vitamin B₁₂.

Nichol et al. (1949) found that vitamin B₁₂ administered orally or parenterally completely counteracted a thyrotoxic condition in chicks produced by feeding a basal ration containing 0.05 percent iodinated casein.

Haque et al. (1948) reported that in New Hampshire chicks fed a ration low in thiamin, pyridoxine, biotin, pantothenic acid, or riboflavin, injected thyroxine produced blackening at the base of the wing feathers. In vitamin D and E deficiency, thyroxine produced a deeper blackening than was observed in vitamin D and E deficiency without the administration of thyroxine. They further observed that thyroxine caused high mortality in chicks fed a ration low in folic acid or pantothenic acid. Pantothenic acid deficiency symptoms in chicks were partially counteracted by thyroxine injection.

The results of studies by Haque et al. (1949), working with testosterone propionate and estradiol propionate in the deficiency of certain vitamins (riboflavin, pantothenic

acid, folic acid, vitamin B, choline, niacin, vitamin D, vitamin A, thiamin, pyridoxine, and biotin), indicated that a deficiency of riboflavin in chicks produced a slightly goiterogenic effect. These workers also suggest that riboflavin is required for the formation of thyroxine by the thyroid glands in chicks.

PURPOSE

The object of this work was to study the effect of thyroxine administration in chicks fed adequate diets except for varying degrees of single vitamin deficiencies. In these experiments, riboflavin, folic acid, pantothenic acid, and vitamin B₁₂ were selected for study.

GENERAL EXPERIMENTAL PROCEDURE

Day-old New Hampshire "straight run" chicks obtained from the University of Maryland Poultry Experiment Station were used in all studies, except the study involving vitamin B₁₂. In this study, mixed chicks, hatched from eggs of hens fed a ration containing no animal protein, were obtained from the Division of Poultry Husbandry, United States Department of Agriculture, Beltsville, Maryland.

At hatching time, all chicks were wing-banded and weighed. They were reared in electrically heated batteries with raised screen floors. Feed and water were available to the chicks at all times. The body weights of individual chicks were recorded weekly. Mortality of chicks was also recorded.

All experiments were terminated when the chicks were four weeks old, except as indicated. The experimental period was preceded by a preliminary standardization period in all studies. Baby chicks ranging in body weight from 35 to 42 grams, were selected and distributed into groups of approximately 30 to 40 chicks at the start of the preliminary period. At the end of this period, the chicks were distributed into uniform groups, eliminating the very rapidly and slowly growing chicks. The preliminary period varied in length in the different studies,

ranging from one to three weeks in duration. This was done in order to modify the length of the experimental period.

In the studies dealing with riboflavin, folic acid, and pantothenic acid, a purified type ration was used. This ration (113) is adequate in all the presently recognized nutrients required by the chick for the rapid growth, with the possible exception of vitamin B₁₂. Since single vitamin deficiencies were studied in each experiment, these rations were rendered deficient in these vitamins by omitting the vitamin under study. The vitamin omitted was then added in graded amounts to the diets of the different groups of chicks in each study. In this way the effect of thyroxine injections in chicks receiving diets containing suboptimal and adequate levels of each vitamin could be studied. The chicks were fed the respective diets during the preliminary and experimental period.

In the experiment with vitamin B₁₂, all the chicks received a diet deficient in this vitamin during the two weeks preliminary period. This diet is given in table 2 (diet A). After this period the chicks were fed diet B also shown in table 2. A crude vitamin B₁₂ concentrate was added in graded amounts to the respective diets of the different groups of chicks. Diet B is also deficient in vitamin B₁₂.

Table 1. Composition of Basal Ration 113.

Main Ingredients	Percent	Vitamin Supplements	mg/100 gm.
Glucose (Cerelese)	61.4	Thiamin HCL	0.4
Casein (crude)	18.0	Riboflavin	0.8
Gelatin	10.0	Ca pantothenate	2.0
Soybean oil	4.0	Nicotinic acid	5.0
Salts 1M	6.0	Choline Cl	200.0
Methionine	0.5	Pyridoxine HCL	0.6
CaCO ₃	1.5	Biotin	0.02
K ₂ HPO ₄	0.9	Folic acid	0.2
NaHPO ₄	0.73	Inositol	100.0
Ca ₃ (PO) ₂	1.3	Para-amino-benzoic acid	0.2
NaCl	0.88	alpha-tocopherol	0.5
MgSO ₄ ·7H ₂ O	0.50	2-Methyl-1,4-naphthoquinone	0.1
Fe(C ₆ H ₅ O ₇) ₂ ·6H ₂ O	0.14		
MnSO ₄ ·4H ₂ O	0.041		
KI*	0.004		
ZnCl ₂	0.002		
CuSO ₄ ·5H ₂ O	0.002		
H ₃ BO ₃	0.0009		
CoSO ₄ ·7H ₂ O	0.0001		
Vitamins A in I.U.	1200 ¹		
Vitamin D ₃ in A.O.A.C. units	170 ¹		

¹ Vitamins A and D₃ are fed by dropper weekly.

* Ground and mixed separately.

Table 2. The composition of ration used in the study of Vitamin B₁₂ and thyroxine.

Ingredients	Percent of total ration	
	Ration A gm	Ration B gm
Cerelose	50.0	11.5
alpha-protein	40.0	-
Soybean meal	-	80.0
Soybean oil	3.0	2.0
Cod liver oil	0.5	0.5
D-Methionine	0.5	-
CaCO ₃	2.0	2.0
K ₂ HPO ₄	0.9	0.9
Ca ₃ (PO ₄) ₂	0.8	0.8
Na Cl	0.88	0.88
MgSO ₄ ·7H ₂ O	0.50	0.50
Ferric citrate	0.14	0.14
MnSO ₄ ·H ₂ O	0.041	0.041
KI	0.004	0.004
ZnCl ₂	0.002	0.002
H ₃ BO ₃	0.0009	0.0009
CoSO ₄ ·7H ₂ O	0.0001	0.0001
CuSO ₄ ·5H ₂ O	0.002	0.002
	mg	mg
Thiamin HCl	1.0	1.0
Riboflavin	1.0	1.0
Ca pantothenate	2.0	2.0
Niacin amide	5.0	5.0
Pyridoxine	0.60	0.60
Choline Cl	200.0	200.0
Biotin	0.02	0.02
Folic acid	0.20	0.20
Inositol	100.0	100.0
Para amino benzoic acid	0.20	0.20
Menadione	0.50	0.50
Alpha tocopherol	0.50	0.50

To ensure a uniform distribution of the vitamins, all the vitamins, except vitamins A and D, were first placed in solution and mixed with the dry ingredients. Vitamin E and D feeding oil was administered orally to each chick on 7th, 14th and 21st day in quantities to supply 1200 I.U. of vitamin E and 170 A. .A.C. units of vitamin D₃ per chick per week.

The thyroxine injections were not administered until the beginning of the experimental period in all the studies. Crystalline thyroxine¹ was dissolved in a water solution containing 4 percent sodium hydroxide, in amounts so that each cc. of the resulting solution contained either 0.2 or 2.0 milligrams of thyroxine, as indicated.

One half of chicks in all the studies were injected intramuscularly with thyroxine solution. The amount of thyroxine used varied in different experiments, as indicated under results. The number of injections also differed in the various experiments. These modifications were made in order to study the effects of injecting different amounts of thyroxine with a varying number of injections in chicks receiving graded levels of each vitamin.

The detailed procedure employed in the experiments dealing with the study of the relationship of each of the vitamins to thyroxine is described below:

A. Experiments with riboflavin and thyroxine. Ration 113, excluding riboflavin, was used as the basal ration.

Crystalline riboflavin was added to the respective diets at levels of 0.1, 0.2, 0.4, and 0.8 mg. per 100 grams of ration in all experiments. However, a level of 0.3 mg. of riboflavin per 100 grams of diet also was used in the first experiment. In this experiment a total of one milligram of thyroxine (0.5 cc. of solution) per 100 grams of body weight was administered in a single injection to each chick at the age of three weeks. Two groups of chicks were also maintained on a practical chick starter ration. The chicks of one of these groups also were injected with one milligram of thyroxine per 100 grams of body weight on the 21st day of age.

In the second and third experiments, each chick was injected with 0.1 mg. of thyroxine (0.5 cc. of solution containing 0.2 mg. per cc.) per 100 grams of body weight, during the 1st and 4th day of the second, third and fourth weeks. Each chick received a total of 0.6 mg. of thyroxine per 100 grams of body weight during the experimental period in these experiments.

B. Experiments with folic acid and thyroxine. Basal ration 113 excluding folic acid was used. Crystalline folic acid was added to the respective diets at levels of 15, 50, and 200 gamma per 100 grams of feed in all experiments. However, in the first experiment, in addition to

the above levels, diets containing 25, 35, 100, 500, and 1000 gamma of folic acid per 100 grams of diet were studied. Diets, containing 1000 gamma and 25 gamma per 100 grams, were also studied in the second and third studies, respectively. Two groups of chicks were also maintained on a practical chick starter ration in the first and second experiments.

In experiment 1, a single injection of 0.5 mg. of thyroxine (0.25 cc.) per 100 grams of body weight was given to each chick at the age of 3 weeks.

In experiment 2, three different levels of thyroxine (0.25, 0.5, and 1.0 mg. per 100 grams of body weight) were injected on the 21st day of age in chicks fed the diets containing different levels of folic acid. Similar injections were also given to the chicks fed the practical chick starter ration. This was undertaken to study the comparative effects of each level of thyroxine administered in one injection, in chicks fed similar graded amounts of folic acid.

In experiment 3, the experimental period, number of injections and the amount of thyroxine administered were modified. The experimental period was three weeks in length, beginning at the end of the first week. Six injections of 0.1 mg. thyroxine per 100 grams body weight were given on the 1st and 4th day of the 2nd, 3rd and 4th week, or a total of 0.6 mg. of thyroxine per 100 grams of

body weight. Blood hemoglobin determinations were made on certain of the injected and non-injected groups of chicks fed diets deficient and adequate levels of folic acid.

C. Experiments with pantothenic acid and thyroxine.

Basal ration 113 was used excluding the pantothenic acid. Pantothenic acid was added to the different diets at levels of 0.15, 0.5, and 2.0 mg. per 100 grams of ration in all the experiments. Additional levels of 0.3, 1.0 and 5.0 mg. per 100 grams of feed also were fed in the first experiment.

In experiment 1, a single injection of 0.5 mg. of thyroxine (0.25 cc.) per grams of body weight was given to each chick on the 21st day of experiment.

Experiment 2 was undertaken to study the effect of varying the number of injections with an equal amount of thyroxine administered in chicks fed diets containing graded levels of pantothenic acid. In this experiment, each chick was injected with a total of 1.0 mg. of thyroxine per 100 grams body weight. In one series this was given in 3 injections of 0.25, 0.25 and 0.5 mg. on the 14th, 19th, and 24th day, respectively. In a second series two injections of 0.5 and 0.5 mg. of thyroxine per 100 grams of body weight were given on the 14th and 21st day, respectively, while in a third series a single injection of 1.0 mg. per 100 grams of body weight was administered on the 21st

day. Chicks receiving diets containing similar levels of pantothenic acid were used in all these series.

In experiment 3, the number of injections, amount of thyroxine and the length of experimental period were modified as described in the third experiment involving folic acid. Six injections, each consisting of 0.1 mg. of thyroxine per 100 grams of body weight were given during a three weeks experimental period. Observations on the severity of the dermatitic symptoms of pantothenic acid deficiency were made on the 28th day of experiment.

- D. Experiments with vitamin B₁₂ and thyroxine. Prior to the experimental period, the chicks were fed diet A (table 2) for a two week preliminary standardization period. Basal ration B, the composition of which is also given in table 2, was used during the three week experimental period. This basal ration contained a protein supplement of vegetable origin and was quite deficient in vitamin B₁₂. A crude vitamin B₁₂ concentrate was added to the different diets at the levels to supply 0.375, 0.75, 1.5, and 3.0 gamma of vitamin B₁₂, respectively, per 100 grams of ration. Six injections of 0.2 mg. of thyroxine (a total of 1.2 mg. of thyroxine) per 100 grams of body weight, were given to each chick on the 1st and 4th day of the 3rd, 4th and 5th week.

RESULTS

A. Experiments with riboflavin and thyroxine. Three experiments were conducted to determine the effects of thyroxine injections upon mortality, body weight, and thyroid weight of chicks fed diets containing low or adequate amounts of riboflavin.

In experiment 1, a single injection of 1.0 mg. of thyroxine per 100 grams of body weight was given, on the 21st day, to chicks fed diets containing different levels of riboflavin. The results are presented in table 3. In experiments 2 and 3, the experimental period was lengthened to 3 weeks in duration. The level of thyroxine was also modified to 0.6 mg. per 100 grams of body weight, given in 6 injections (each of 0.1 mg. per 100 grams of body weight) during the experimental period. The results of these two experiments were combined and are presented in table 4 and figure 1. The combined results of all three experiments are shown in table 5 and figure 2.

(1) Mortality. The data given in tables 3, 4, and 5 show that the mortality was greater in all groups of chicks injected with thyroxine than in those not injected. Of the total 230 non-injected chicks in these three experiments, 10.4% died during the experimental period as compared to 26.0% for the 234 injected chicks. This higher mortality occurred either as a direct effect of the

Table 3. Effect of thyroxine injections on mortality, body weight and thyroid weight in chicks receiving different amounts of riboflavin.

(Experiment 1)

Group	Added mg/100 gm of diet	Thyroxine injected*	Number chicks at 4 weeks	Mortality during 4th week %	Average weight, gm	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	4 (7)**	42.8	89	16.6
2	0.0	+	3 (7)	57.1	76	9.6
3	0.1	-	13 (13)	0.0	139	13.2
4	0.1	+	9 (13)	30.7	140	10.3
5	0.2	-	13 (13)	0.0	230	10.2
6	0.2	+	9 (13)	30.7	232	11.0
7	0.3	-	14 (14)	0.0	215	9.9
8	0.3	+	11 (14)	21.4	219	7.8
9	0.4	-	13 (13)	0.0	237	10.1
10	0.4	+	11 (13)	15.3	225	8.7
11	0.8	-	12 (12)	0.0	245	8.9
12	0.8	+	10 (12)	16.6	230	7.7
13	Practical chick starter	-	12 (12)	0.0	185	13.2
14	" " "	+	12 (12)	0.0	201	8.3

* A single injection of 1.0 mg per 100 gm of body weight was injected on 21st day.

** Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

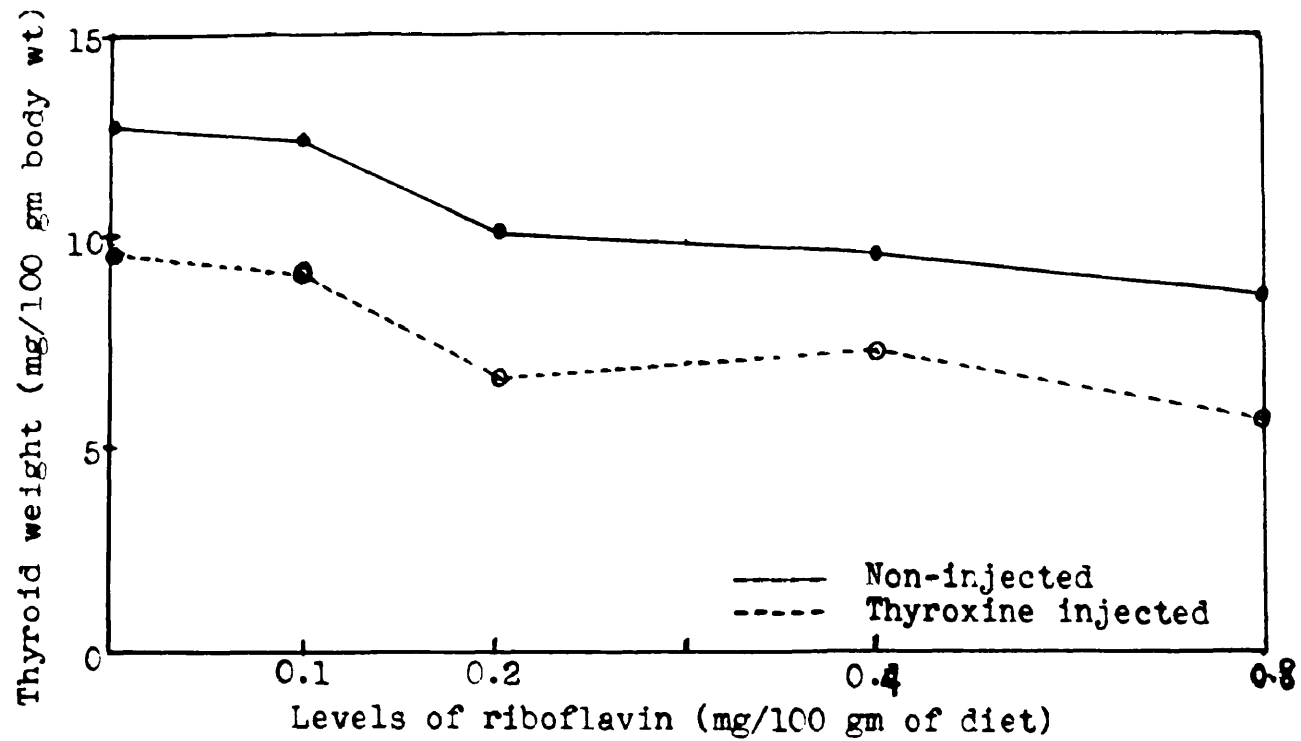


Figure 1. Average thyroid weight as influenced by thyroxine injection and level of riboflavin in the diet.

(Combined results of experiments 2 and 3)

Table 4. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of riboflavin.

(Experiments 2 and 3 combined)

Group	Added riboflavin, mg/100 gm of diet	Thyroxine injected*	Number chicks 4 weeks	Mortality during expt. period 4 weeks %	Average weight, 4 weeks gm	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	15 (30)**	50.0	69	12.6
2	0.0	+	10 (30)	66.7	79	9.6
3	0.1	-	25 (30)	16.6	110	12.3
4	0.1	+	23 (30)	23.3	131	9.1
5	0.2	-	28 (28)	0.0	184	10.1
6	0.2	+	24 (30)	20.0	207	6.3
7	0.4	-	28 (29)	3.4	221	9.6
8	0.4	+	26 (30)	13.3	223	7.1
9	0.8	-	29 (29)	0.0	223	8.9
10	0.8	+	25 (30)	16.6	228	5.6

* Six injections of 0.1 mg per 100 gms of body weight were given during 3 weeks of experimental period.

** Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

Table 5. Effect of thyroxine injections on mortality, body weight and thyroid weight in chicks receiving different amounts of riboflavin.

(Experiments 1, 2 and 3 combined)

Group	Added mg/100 gm of diet	Thyroxine injected*	Number chicks 4 weeks	Mortality during expt. period %	Average weight, 4 weeks gm	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	19 (37)*	48.6	73	13.4
2	0.0	+	13 (37)	64.8	78	9.6
3	0.1	-	38 (43)	11.6	120	12.6
4	0.1	+	32 (43)	25.5	133	9.4
5	0.2	-	41 (41)	0.0	202	10.2
6	0.2	+	33 (43)	23.2	214	7.8
7	0.4	-	41 (42)	2.3	226	9.8
8	0.4	+	57 (43)	13.9	224	7.6
9	0.8	-	41 (41)	0.0	229	8.9
10	0.8	+	35 (42)	16.6	228	6.2
11	Practical chick starter	-	12 (12)	0.0	185	13.2
12	" " "	+	12 (12)	0.0	201	8.3

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

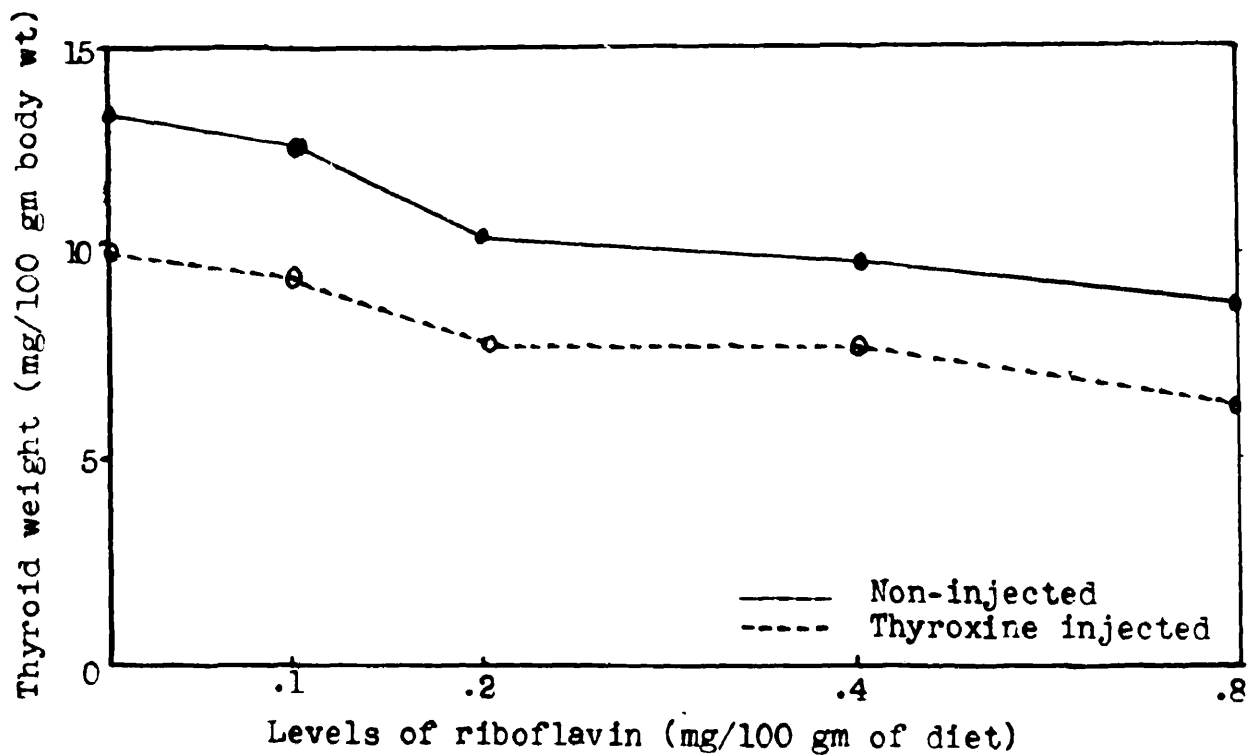


Figure 2. Average thyroid weight as influenced by thyroxine injections and level of riboflavin in the diet.

(Combined results of expts. 1, 2, and 3)

thyroxine injection itself, or due to an increased requirement for riboflavin as a result of the thyroxine injection, producing a more severe deficiency of this vitamin.

- (2) Body weight. From the data on the average body weights, given in table 4 (combined results of 2nd and 3rd experiments), it appears that the injection of thyroxine in chicks, receiving diets low or suboptimal in riboflavin, slightly increased the growth response over those not injected. Although this difference in growth is not great, it is consistent in all the groups where the level of riboflavin was not adequate. However, this difference in growth response was not observed in experiment 1 (table 3). In the first experiment the chicks were given a single injection of 1.0 mg. of thyroxine per 100 grams of body weight on the 21st day of age and the experimental period was only one week in length. In experiments 2 and 3, where the injection of thyroxine resulted in an increased growth response, the chicks were given 6 injections of thyroxine (0.1 mg. each) during a three-weeks experimental period. As the level of riboflavin was increased in all experiments, the growth response was correspondingly increased irrespective of thyroxine injections.

- (3) Thyroid glands. When thyroxine was not injected, the average weight of the thyroid glands, expressed in milligrams per 100 grams of body weight, was greatest in the chicks of those groups receiving no added riboflavin

(tables 3, 4, and 5). As the level of riboflavin in the diet was increased to the adequate level the average weight of the thyroid glands was correspondingly decreased. This is also illustrated by the data plotted in figures 1 and 2. The average thyroid weight, expressed as milligrams per 100 grams of body weight, was greater for chicks receiving diets containing 0.0 or 0.1 mg. of added riboflavin per 100 grams than for those chicks receiving a diet which contained 0.8 mg. of added riboflavin per 100 grams. This difference was found to be significant at the 1% level when the data was analyzed statistically using the "t" test. In experiments 2 and 3 (table 4 and figure 1), this also was true for those groups of chicks which were injected with thyroxine. Such an effect was not observed in experiment 1, where a much shorter experimental period was used and only one injection of thyroxine was administered.

From the results presented in figures 1 and 2, it appears that there is an inverse relationship between the average thyroid weight, when expressed in milligram per 100 grams of body weight, and the level of riboflavin in the diet. The average thyroid weight was lower in each case for the chicks of the injected groups than for those chicks of the non-injected groups receiving the same level of riboflavin. This indicated the effectiveness of the thyroxine. Only one exception to this, shown in table 5 (group 6) was noted.

- (4) Comparative effects of number of injections and length of experimental period. In experiment 1 (table 3), the chicks were given single injections of 1.0 mg. of thyroxine per 100 grams of body weight at the age of 3 weeks, with an experimental period of only one week. However, in experiments 2 and 3 (table 4) six injections of a total of 0.6 mg. of thyroxine per 100 grams of body weight were given during a three week experimental period, beginning with the second week.

The effect of thyroxine injection on mortality, was similar when the thyroxine was given either in a single injection of 1.0 mg. per 100 grams of body weight at the start of a one week experimental period or in 6 injections of 0.1 mg. per 100 grams of body weight during a three-week experimental period. Both of these levels of thyroxine represent a much higher level than is normally secreted by the chick. Nevertheless, the effects of thyroxine injection on thyroid weight and body weight became more obvious in the 2nd and 3rd experiments where the three-week experimental period was used, even though less thyroxine was injected.

- (5) Other observations. At three weeks of age, New Hampshire chicks, fed a diet deficient in riboflavin, developed white streaks of depigmentation in the primary and secondary wing feathers. These streaks were more obvious when the chicks were injected with thyroxine. Two

to three such streaks were present in the primary and secondary feathers of each wing. Other recognized symptoms of riboflavin deficiency were observed.

Chicks fed the purified diet containing 0.2 mg. or more of riboflavin per 100 grams of diet grew as rapidly or faster than the chicks fed the practical chick starter used in this experiment.

B. Experiments with folic acid and thyroxine. Three experiments were conducted to determine the effects of thyroxine injections upon mortality, body weight and thyroid weight of chicks fed diets containing low and adequate levels of folic acid. This study was further designed to observe the effects of different levels of thyroxine injections during a one week experimental period. The results obtained in these experiments are presented in tables 6 to 11 inclusive, and figure 3. In experiment 1, a single injection of 0.5 mg. of thyroxine per 100 grams of body weight was given on the 21st day to chicks fed diets containing different levels of folic acid. The results of this experiment are presented in table 6. In experiment 2, three different levels of thyroxine (0.25, 0.5, and 1.0 mg. per 100 grams of body weight each) were administered in single injections on the 21st day. The results are presented in tables 7 and 8, except for those results obtained with the 0.5 mg. dose. These results are combined with those of experiment 1 and are shown in table 6. In the third

experiment, the levels of thyroxine, number of injections and length of experimental period were modified to 0.6 mg. of thyroxine (0.1 mg. per 100 grams body weight) given in 6 injections during a three weeks experimental period. The results of this experiment are presented in table 9 and figure 3. The combined results of all the experiments are shown in table 10. The effect of injection of different amounts of thyroxine on thyroid weight in chicks is shown in table 11.

- (1) Mortality. From table 10, it is evident that there was greater mortality in all groups of chicks injected with thyroxine than in the groups of non-injected chicks. In the chicks of the non-injected groups, there was a total of 13.0 percent mortality during the experimental period, whereas, in the injected groups, there was 27.6 percent mortality. There were 199 chicks in the non-injected groups and 203 chicks in the injected groups. Although there was considerable mortality in the chicks of the non-injected groups, the mortality was over twice as high in the injected groups. As is shown in the tables, the mortality of chicks receiving low levels of folic acid was high. Furthermore the injection of thyroxine resulted in an even greater mortality of chicks receiving the low levels of folic acid.

No greater mortality was observed when higher levels of thyroxine were administered as compared to the lower

Table 6. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of folic acid.

(Combined results of experiments 1 and 2)

Group	Added folic acid, gamma/100 gm of feed	Thyroxine injected	Number chicks 4 weeks	Mortality during 4th week %	Average weight, 4 weeks gm	Average thyroid wt. ng/100 gm body wt.
1	15	-	7 (12)**	41.6	125	15.9
2	15	+	4 (12)	66.6	144	13.5
* 3	25	-	4 (5)	20.0	169	9.9
* 4	25	+	2 (5)	60.0	163	8.4
* 5	35	-	9 (9)	0.0	232	9.5
* 6	35	+	7 (9)	22.2	245	8.2
7	50	-	17 (17)	0.0	200	12.6
8	50	+	13 (13)	27.7	209	11.3
* 9	100	-	8 (9)	11.1	222	11.2
* 10	100	+	8 (9)	11.1	243	10.6
11	200	-	16 (16)	0.0	245	11.6
12	200	+	16 (16)	0.0	246	8.7
* 13	500	-	8 (8)	0.0	246	10.1
* 14	500	+	6 (7)	14.2	239	10.7
15	1000	-	14 (14)	0.0	261	9.6
16	1000	+	13 (15)	13.3	234	8.9
* 17	Practical chick starter	-	5 (5)	0.0	202	13.4
* 18	" " "	+	5 (5)	0.0	200	11.0

* Represent results of experiment 1 only.

** Figures in parentheses indicate total number of chicks at the beginning of the experimental period.

Table 7. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of folic acid.

(Experiment 2)

Group	Added folic acid, gamma/100 gm of feed:	Thyroxine injected:	Number of chicks: 4 weeks:	Mortality during 4th week: %	Average weight, 4 weeks: gm	Average thyroid wt. mg/100 gm body wt.
1	15	-	4 (5)*	20.0	128	12.1
2	15	+	1 (5)	80.0	192	14.6
3	50	-	7 (8)	12.5	192	13.4
4	50	+	5 (8)	37.4	150	13.1
5	200	-	8 (8)	0.0	242	10.4
6	200	+	7 (8)	12.5	239	8.9
7	1000	-	8 (8)	0.0	247	10.2
8	1000	+	7 (8) ^{one} missing	0.0	253	8.3
9	Practical chick starter	-	5 (5)	0.0	215	12.8
10	" " "	+	5 (5)	0.0	200	10.4

A single injection of 0.25 mg of thyroxine was given on 21st day.

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

Table 8. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of folic acid.

(Experiment 2)

Group	Added folic acid: gamma/100 gm of feed:	Thyroxine injected:	Number chicks: 4 weeks:	Mortality: during 4th week: %	Average weight: 4 weeks: gm	Average thyroid wt.: mg/100 gm body wt.
1	15	-	5 (5)*	0.0	151	10.7
2	15	+	1 (5)	80.0	144	8.3
3	50	-	8 (8)	0.0	170	10.8
4	50	+	5 (9)	33.3	171	11.8
5	200	-	8 (8)	0.0	247	11.3
6	200	+	8 (8)	0.0	234	8.3
7	1000	-	8 (8)	0.0	230	10.3
8	1000	+	5 (8)	37.5	239	8.8
9	Practical chick starter	-	4 (5)	20.0	209	10.5
10	" " "	+	3 (5)	40.0	211	10.4

A single injection of 1.0 mg of thyroxine per 100 gm of body weight was given on the 21st day.

* Numbers in parentheses indicate total number of chicks at start of experimental period.

Table 9. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of folic acid.

(Experiment 3)

Group	Added folic acid, gamma/100 gm of feed	Thyroxine injected	Number of chicks : 4 weeks	Mortality during expt. period, 4 weeks %	Average weight, gm	Average thyroid wt., mg/100 gm body wt	Hemoglobin
1	00	-	5 (14)*	62.2	92	12.1	6.40
2	00	+	3 (15)	80.0	76	10.0	4.48
3	15	-	8 (15)	46.6	192	11.9	7.93
4	15	+	14 (15)	6.6	152	8.9	6.58
5	25	-	13 (13)	0.0	239	10.1	-
6	25	+	14 (15)	6.6	229	7.0	-
7	50	-	15 (15)	0.0	307	11.0	-
8	50	+	15 (15)	0.0	290	6.8	-
9	200	-	15 (15)	0.0	285	9.8	7.61
10	200	+	14 (15)	6.6	321	6.6	7.99

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

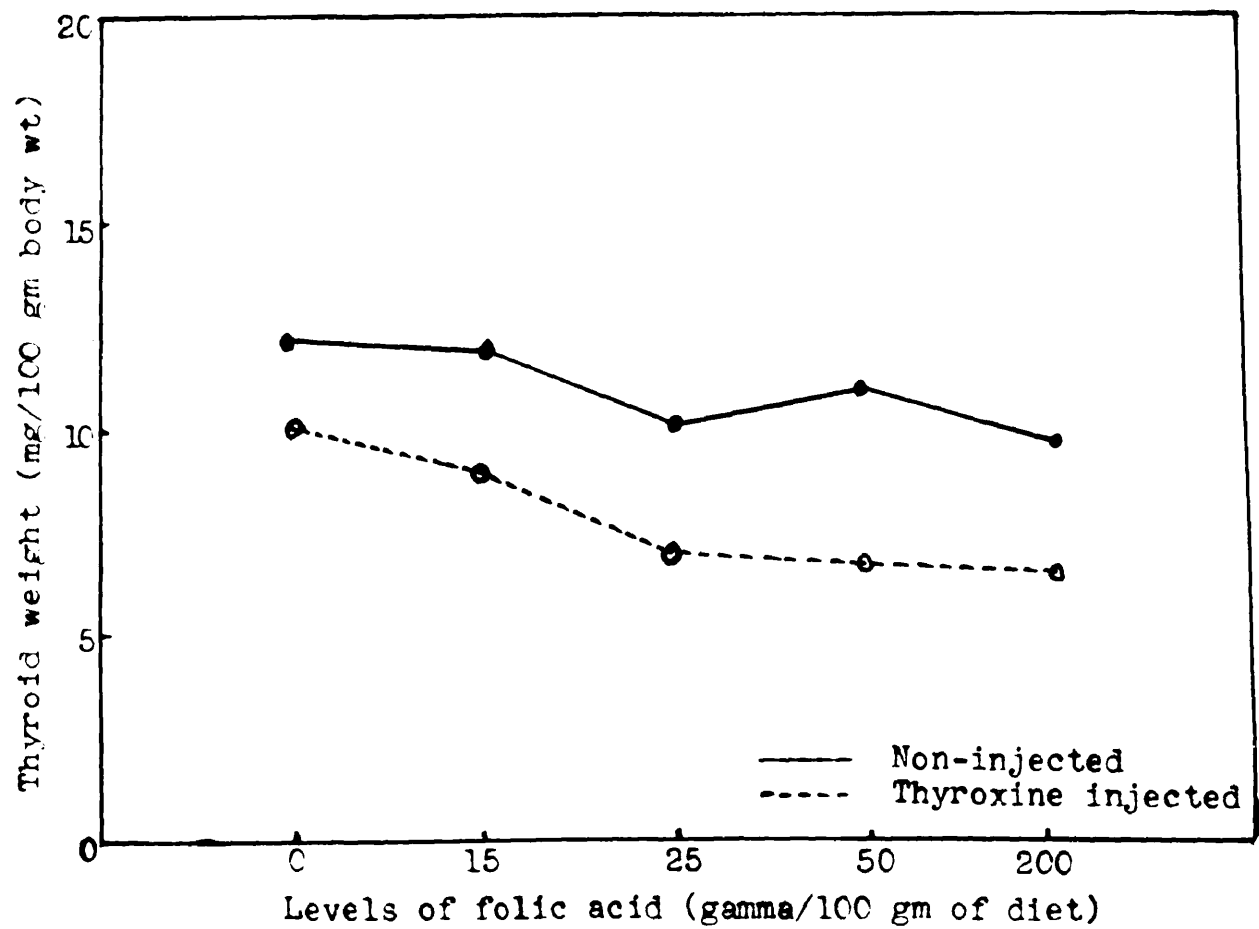


Figure 3. Average thyroid weight as influenced by thyroxine injections and level of folic acid in the diet.

(Experiment 3)

Table 10. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of folic acid.

(Combined results of experiments 1, 2 and 3)

Group	Added folic acid: μ g/100 gm of feed:	Thyroxine injected:	Number chicks: 4 weeks:	Mortality during expt. period: 4 weeks: %	Average weight, 4 weeks: gm	Average thyroid wt. mg/100 gm body wt.
1	00	-	6 (17)*	64.7	94	11.6
2	00	+	3 (18)	83.3	76	10.0
3	15	-	24 (37)	35.1	149	12.5
4	15	+	20 (37)	45.9	152	10.1
5	25	-	17 (18)	5.5	223	10.1
6	25	+	16 (20)	20.0	221	7.2
7	50	-	49 (50)	2.0	225	13.6
8	50	+	33 (50)	24.0	228	10.0
9	200	-	47 (47)	0.0	255	10.3
10	200	+	45 (47)	4.2	265	8.6
11	1000	-	30 (30)	0.0	249	9.9
12	1000	+	25 (31)	19.3	240	8.8

*Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

	Total # chicks	Mortality #	Mortality %
Non-injected chicks	199	26	13.0
Injected chicks	203	56	27.6

Table 11. Effect of injection of different amounts of thyroxine on thyroid weight in chicks receiving different levels of folic acid.

(Experiment 2 only)

Group	Thyroxine injected*	Added folic acid, gamma/100 gm of feed	Level of thyroxine injected in mg/100 gm body weight		
			1.0	0.50	0.25
1	-	15	10.7	13.9	12.1
2	+	15	8.3	13.5	14.6
3	-	50	10.8	12.6	13.4
4	+	50	11.8	11.8	13.1
5	-	200	11.3	11.6	10.4
6	+	200	8.3	8.7	8.9
7	-	1000	10.3	9.6	10.2
8	+	1000	8.8	8.9	8.3

* The chicks were injected on the 21st day of age.

levels, when given in a single injection. However, when six injections of 0.1 mg. of thyroxine per 100 grams body weight were given during a period of three weeks, no apparent effect on mortality was observed (table 9).

(2) Body weight. Little difference in body weight was observed in these experiments as a result of thyroxine injections, irrespective of amount of thyroxine or length of the experimental period. The results of the experiments shown in tables 8 and 9 suggest that the average body weight is slightly lower when thyroxine is administered to chicks receiving low levels of folic acid. This is not true, however, in the results shown in table 7.

(3) Thyroid glands. In the experiments involving an experimental period of only one week in duration (tables 6, 7, and 8) there is little correlation between the thyroid weight and level of folic acid in the diet, with the possible exception of the results shown in table 6. In this experiment the thyroid weight was slightly decreased as the level of folic acid in the diet was increased. The injection of thyroxine at the beginning of the experimental period decreased the thyroid weight indicating its potency. But again, no correlation was observed between the thyroid size of the injected chicks and the level of folic acid in their diet, except for the results shown in table 6. In this experiment, the thyroid weight decreased as the level of folic acid in diet increased.

On the other hand, when the experimental period was three weeks in duration and the thyroxine was given in six (0.1 mg. per 100 grams of body weight) injections during this period, the average thyroid weight of both the thyroxine injected and non-injected groups of chicks was consistently greater when the amount of folic acid in the diet was not adequate (table 9 and figure 3). As the folic acid content of the diet was increased the thyroid weights decreased. It is believed that the effect of a folic acid deficiency on thyroid size was more clearly shown in this experiment than in those previously described.

When folic acid was fed at a level higher than the normal requirement, there was no observed effect on thyroid size, mortality or body weight at 4 weeks of age in either the thyroxine injected or uninjected groups.

- (4) Hemoglobin. The results of hemoglobin determination on the blood are shown in table 9. The hemoglobin level was found to be slightly lower for the groups of chicks receiving the basal ration containing no added folic acid than that for those receiving the adequate levels of added folic acid. However, the injection of thyroxine resulted in a marked decrease in hemoglobin content of the blood of chicks receiving the low folic acid diet. On the other hand, when the adequate amounts of folic acid were supplied the injections of thyroxine did not effect the hemoglobin level of the blood. Since a folic acid deficiency results

in anemia in chicks, these results would indicate that the injection of thyroxine had produced an increased requirement for folic acid, thereby resulting in a more acute deficiency state.

- (5) Comparative effects of amounts of thyroxine injected and length of experimental period. The administration of a single injection of different amounts of thyroxine followed by a one week experimental period as done in experiments 1 and 2 (tables 6, 7, and 8) had similar effects on mortality and body weight of chicks fed diets with different levels of folic acid. As mentioned above, the thyroxine injection increased rate of mortality but exerted no appreciable effect on rate of growth.

Table 11 shows the effect of amount of thyroxine injected on thyroid size when an experimental period of one week was used. The injection of 0.25 mg. per 100 grams of body weight was found to be equally effective in reducing the thyroid size as 1.0 mg. of thyroxine per 100 grams of body weight. In experiment 3, however, a longer experimental period was used (from 7th to 28th day) and the thyroxine was given in 6 doses of 0.1 mg. per 100 grams of body weight during the experimental period. In this experiment the effects of the folic acid level of the diet and the thyroxine injections were observed more readily. There was a consistent decrease in thyroid size as the level of folic acid in the diet was increased.

This was true for the chicks receiving thyroxine injections, as well as for those not receiving the injections. Furthermore, as was mentioned above, greater differences in growth were observed when the three week experimental period was employed. Moreover, the mortality, as a result of the thyroxine injections, was greatly reduced since smaller quantities of thyroxine were injected at intervals during a longer period. These results indicate that the longer experimental period is important if such differences are to be studied. Apparently the injection of smaller amounts of thyroxine more frequently is just as effective as single injections of larger quantities.

C. Experiments with pantothenic acid and thyroxine.

Three experiments were conducted to study the effects of thyroxine injection upon mortality, body weight, symptoms of pantothenic acid deficiency and thyroid weight of chicks fed diets containing low and adequate levels of pantothenic acid. These experiments were also designed to study the effect at 4 weeks of age of injecting an amount of thyroxine on the 21st day as compared to the injection of the same amount in two and three injections given on the 14th and 21st day and the 14th, 19th and 24th days, respectively. In these experiments graded levels of pantothenic acid were fed to the different groups of chicks ranging from a complete deficiency to adequate levels as shown in the tables.

In experiment 1, a single injection of 0.5 mg. of thyroxine per 100 grams of body weight was given on the 21st day in chicks fed diets with different levels of pantothenic acid. The results are shown in table 12.

In experiment 2, one milligram of thyroxine per 100 grams of body weight was injected. In one series this was given on the 21st day in chicks followed by an experimental period of one week. In a second series, it was administered on the 14th and 21st day (0.5 mg. and 0.5 mg. of thyroxine per 100 grams of body weight). In a third series it was given on the 14th, 19th, and 24th day (0.25, 0.25, and 0.5 mg. of thyroxine per 100 grams of body weight). In each series chicks fed diets containing different levels of pantothenic acid were used. The results are shown in tables 13, 14 (in Appendix), and 15 and in figure 4. Since almost all of the chicks receiving the low levels of pantothenic acid in the first two series died, tables 13 and 14 which present these results are included in the appendix.

In experiment 3, 6 injections of 0.1 mg. of thyroxine per 100 grams of body weight were given to chicks fed diets with graded levels of pantothenic acid. These were given on the 1st and 4th day of the second, third and fourth weeks. The results are presented in table 16 and figure 5. Combined results of all three experiments are shown in table 17 and figure 6.

Table 12. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of pantothenic acid.

(Experiment 1)

Group	Added pantothenic acid, mg/100 gm of feed	Thyroxine injected	Number of chicks : 4 weeks	Mortality during 4th week	Average weight, 4 weeks	Average thyroid wt. ag/100 gm body wt.
1	0.0	-	0	0.0	0	0.0
2	0.0	+	0 (3)*	100.0	0	0.0
3	0.15	-	4 (6)	33.3	33	19.1
4	0.15	+	3 (6)	50.0	97	24.2
5	0.3	-	7 (8)	12.5	104	19.3
6	0.3	+	3 (8)	62.5	122	19.3
7	0.5	-	7 (7)	0.0	117	21.3
8	0.5	+	6 (7)	14.2	118	16.5
9	1.0	-	7 (9)	22.2	176	15.2
10	1.0	+	8 (9)	11.1	153	15.6
11	2.0	-	2 (8)	0.0	200	11.6
12	2.0	+	3 (8)	62.5	174	13.1
13	3.0	-	12 (12)	0.0	191	12.2
14	6.0	+	11 (12)	8.3	202	12.8

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

Table 15. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of pantothenic acid.

(Experiment 2)

Group	Added pantothenic acid, mg/100 gm of feed	Thyroxine injected*	Number chicks : 4 weeks	Mortality during expt. period, %	Average weight, gm	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	0 (7)**	100.0	0	0.0
2	0.0	+	0 (7)	100.0	0	0.0
3	0.15	-	1 (6)	83.3	96	14.3
4	0.15	+	1 (5)	83.3	100	12.0
5	0.50	-	5 (6)	16.6	202	11.8
6	0.50	+	5 (6)	16.6	207	7.5
7	2.0	-	7 (7)	0.0	209	10.9
8	2.0	+	6 (7)	14.2	220	8.2

* Three injections of 0.25, 0.25 and 0.5 mg of thyroxine per 100 gm of body weight were injected on 14th, 19th, and 24th day of the experiment.

** Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

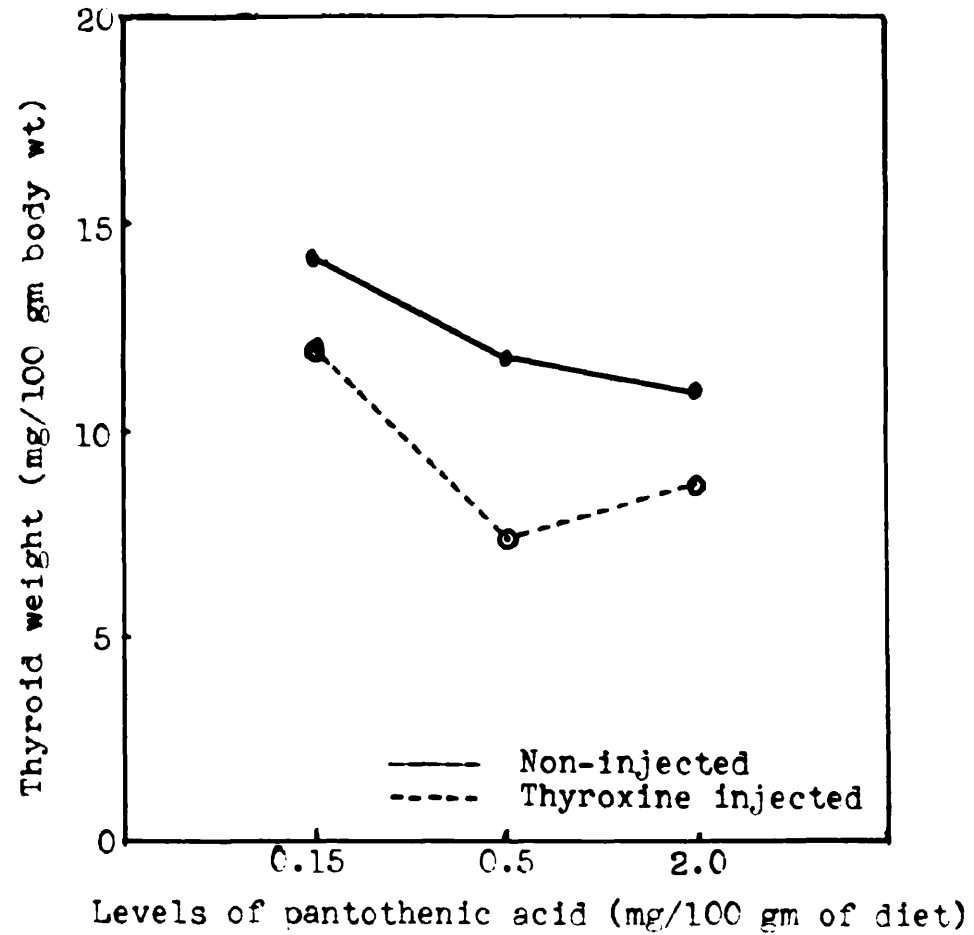


Figure 4. Average thyroid weight as influenced by thyroxine injections and level of pantothenic acid in the diet.

(Experiment 2)

Table 16. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of pantothenic acid.

(Experiment 3)

Added : Mortality : Average : Average : Thyroxine : Number : during : weight : thyroid wt. : pantothenic acid, mg/100 : injected : chicks : expt. period : 4 weeks : mg/100 gm : group : of food : 4 weeks : 1 gm : body wt.

1	2	3	4	5	6	7	8
0.0	0.0	0.15	0.15	0.0	0.0	0.0	0.0
-	+	-	+	-	+	-	+
1 (15)	0 (15)	2 (15)	0 (15)	2 (15)	0 (15)	14 (15)	13 (15)
22.3	100.0	88.6	100.0	40.0	13.3	6.6	13.3
68	0	68	0	136	136	272	280
3.6	0.0	14.5	0.0	13.0	8.4	10.7	8.1

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

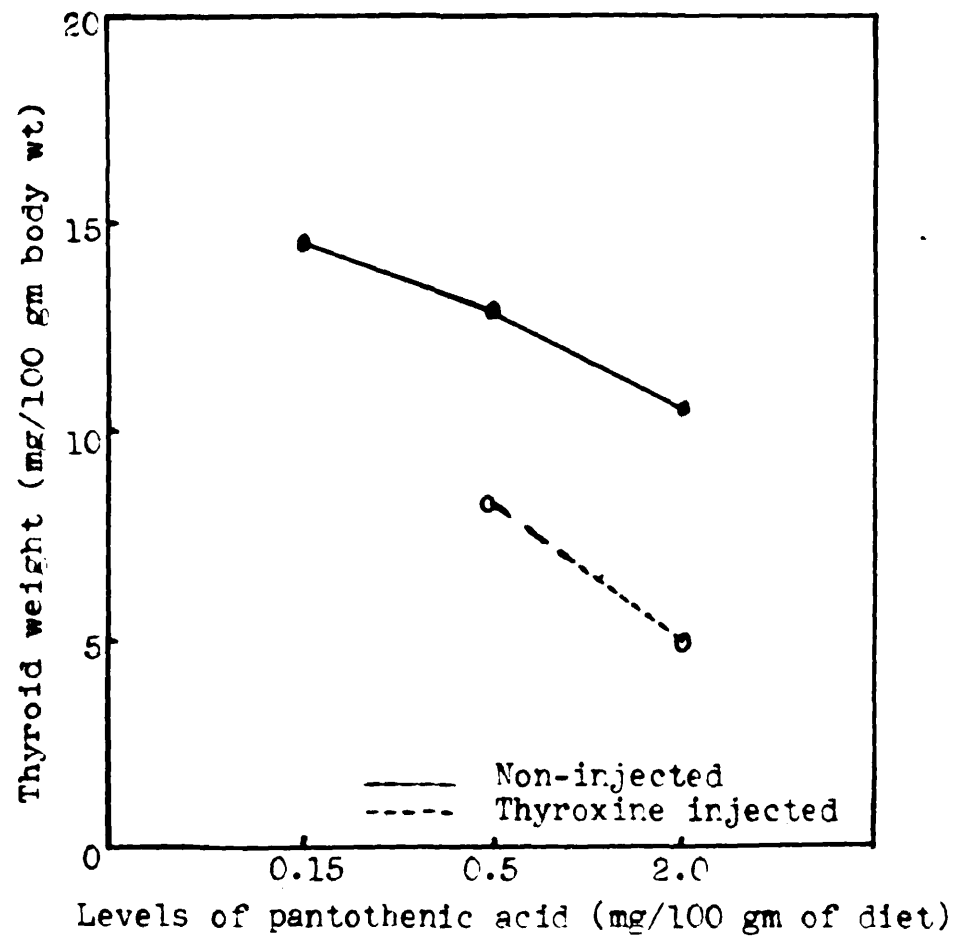


Figure 5. Average thyroid weight as influenced by thyroxine injections and level of pantothenic acid in the diet.

(Experiment 3)

Added	Number	Weight	Hydrolysis	Grouped	mg/100 gm	mg	body wt.
1	1 (20)	96.6	68	0.0	2.4		
2	0 (24)	100.0	0	0.0			
3	8 (28)	47.1	42	17.0			
4	8 (28)	86.0	108	18.0			
5	21 (28)	80.8	180	14.8			
6	22 (28)	10.8	178	8.9			
7	42 (44)	8.8	201	11.8			
8	24 (44)	28.4	224	9.2			

* Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

Table 14. Effect of thyroxine injection on mortality, body weight and hydrolysis in chicks receiving different amounts of penicillins feed. (Experiments 1, 2 and 3 combined)

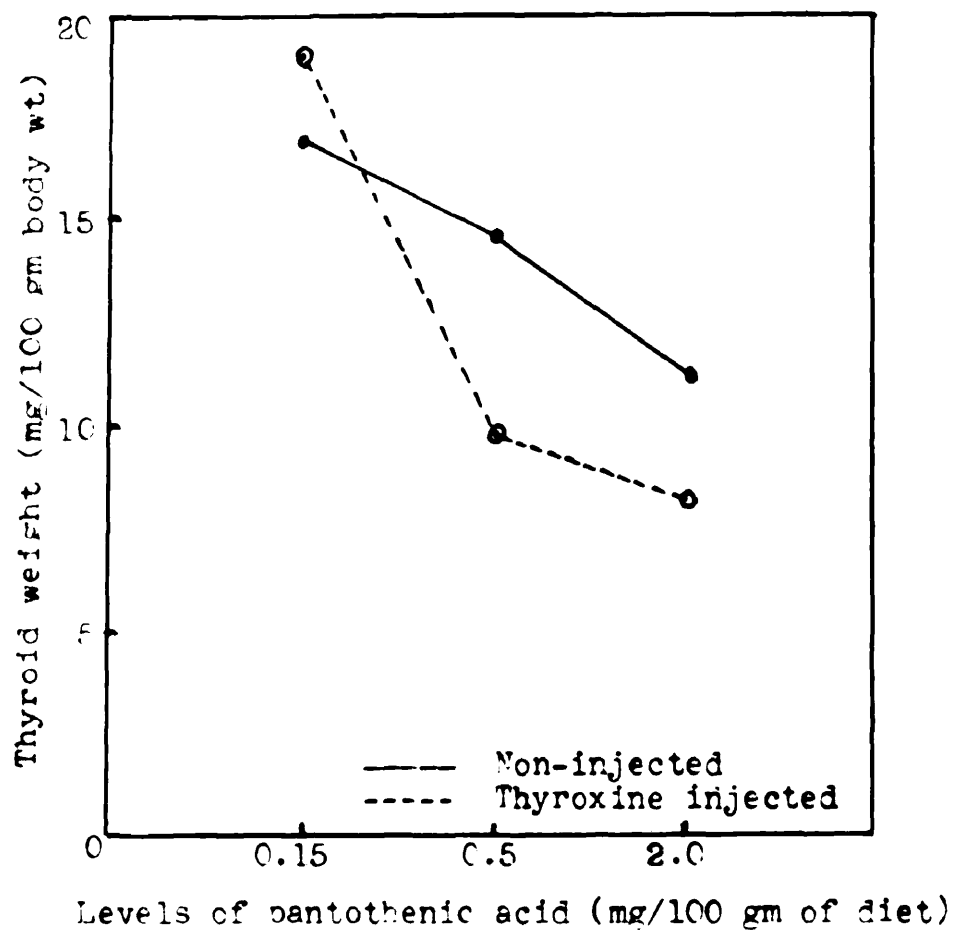


Figure 6. Average thyroid weight as influenced by thyroxine injections and level of pantothenic acid in the diet.

(Combined results of expts. 1, 2, and 3)

- (1) Mortality. The combined results of all experiments with pantothenic acid and thyroxine, given in table 17, show that the mortality of chicks was 38.4% for the non-injected groups including a total of 177 chicks as compared to 47.2% for the 182 chicks of the injected groups. From the data presented in the tables the injection of thyroxine in chicks, receiving different levels of pantothenic acid in diet, does not appear to effect the mortality rate in any consistant manner, although the overall mortality was grester for all groups of chicks injected than for those non-injected with thyroxine. This difference in mortality is not considered significant.
- (2) Body weight. In all the experiments the mortality of the chicks receiving no or 0.15 mg. of added pantothenic acid per 100 grams of diet was so high that the number of surviving chicks does not permit any conclusions concerning the effect of thyroxine injection on body weight of chicks receiving low levels of pantothenic acid. In higher levels of pantothenic acid, no consistant effect was observed.
- (3) Thyroid glands. The data given in tables 12, 15, 16, 17 and figures 5 and 6, show that there is a difference in the thyroid weights of uninjected chicks fed different levels of pantothenic acid. In general, as the level of pantothenic acid in diet was reduced, the thyroid size, expressed as milligram per 100 grams body weight, increased. It is believed that any exception to this trend (group 1

in table 16) can be attributed to the small number of chicks involved. As the level of pantothenic acid was increased, the thyroid weights were correspondingly decreased. The results of the groups in all three experiments receiving the 0.15 mg. level of added pantothenic acid and those receiving the 2.0 mg. level were combined (groups 3 and 7 in table 17). The differences in thyroid weights between these combined groups were found to be significant at the 2% level when analyzed by using "t" test. This inverse relationship between thyroid weight and level of pantothenic acid in diet also was observed in the chicks which received thyroxine by injection.

- (4) Comparative effects of number of thyroxine injection and length of experimental period. When the number of injections were varied but the same total amount of thyroxine (1.0 mg.) was administered the effects on mortality, body weight and thyroid size (table 15 in text and tables 13 and 14 in appendix) were similar. The effect of pantothenic acid deficiency in thyroxine injected chicks was more pronounced as revealed by the differences in thyroid weights, expressed in milligrams per 100 grams of body weight, when the period of injections was longer than one week. This indicates that a one week experimental period is not adequate to reveal any appreciable difference in the size of the thyroid glands due to the injection of thyroxine. However, when thyroxine was administered during

a two or three week period an effect on the size of the thyroid glands was observed.

The relationship between the pantothenic acid content of the diet and the average thyroid weight of chicks in these experiments is similar to those observed in the study of the relationship of the riboflavin level of the diet to thyroid weight. In both studies, this relationship was also evident in chicks which had been injected with thyroxine.

- (5) Effects of injection of thyroxine on the dermatitis of pantothenic acid deficiency. Severe dermatitis was observed in the mouth, eyes, and on the feet of the chicks fed diets deficient in pantothenic acid. The severity of the symptoms decreased as the level of pantothenic acid in the diet was increased. However, the severity of the dermatitis was noticeably less in the deficient chicks which had been injected with thyroxine. This decrease in the severity of dermatitic symptoms was more obvious when the period of injections was of two or three weeks in length as compared to a period of one week. As long as the same amount of thyroxine was administered during an experimental period of the same length, the number of injections did not appear to influence the effect of thyroxine on the amelioration of the dermatitic lesions of pantothenic acid deficiency.

D. Experiment with vitamin B₁₂ and thyroxine. This experiment was conducted to study the effect of injected thyroxine in chicks fed diets containing inadequate and adequate amounts of vitamin B₁₂. A crude vitamin B₁₂ concentrate obtained from Merck & Co. was used as a source of this vitamin. This material also contained charcoal and soybean flour.

During a preliminary period of two weeks the chicks were fed diet A shown in table 2. At the end of this period the chicks were distributed into uniform groups as described under general experimental procedure.

During the following three weeks experimental period, basal diet B (table 2) was used. Both of these basal diets contained protein of vegetable origin and were very deficient in vitamin B₁₂. The results of this experiment are presented in table 18.

From these results it is evident that no mortality occurred which can be attributed as being due to a lack of this vitamin or to the injection of thyroxine. Better growth was obtained in the groups of chicks receiving vitamin B₁₂ crude concentrate than in those receiving only the basal diet. This was also true when thyroxine was administered. The addition of vitamin B₁₂ crude concentrate, at a level to supply 1.5 gamma of vitamin B₁₂ per 100 grams of diet, was found to be adequate under the conditions of this study. There was a slight depression

Table 18. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of vitamin B₁₂.

(Experiment 1)

Group:	vitamin B ₁₂ : mg/100 gm of feed :	Thyroxine: injected* :	Number : chicks : 5 weeks :	Mortality : during : expt. period : %	Average : weight, : 5 weeks : gm :	Average : thyroid wt. : mg/100 gm : body wt. :
1	0.375	-	11 (12) ^{**}	8.3	333	11.3
2	0.375	+	11 (12)	8.3	319	6.0
3	0.75	-	11 (12)	1 sick	331	11.1
4	0.75	+	12 (12)	0.0	351	4.7
5	1.5	-	11 (12)	8.3	351	10.2
6	1.5	+	12 (12)	0.0	360	4.8
7	3.0	-	12 (12)	0.0	335	10.0
8	3.0	+	12 (12)	0.0	321	5.7
9	0.0	-	10 (12)	16.6	261	11.3
10	0.0	+	11 (12)	8.3	257	6.5

* Six injections of 0.2 mg each per 100 grams of body weight in course of 3 weeks.

** Figures in the parentheses indicate total number of chicks at the start of experimental period.

of growth in both the injected and non-injected groups of chicks fed diets containing 3.0 gamma of vitamin B₁₂ supplied by this crude concentrate.

Again, the thyroid size was found to be slightly larger, when expressed as milligrams per 100 grams of body weight, in the chicks receiving no or low levels of added vitamin B₁₂ in the diet, as compared to those receiving adequate amounts of this vitamin. However, these differences were quite small. The injection of thyroxine noticeably depressed the thyroid size in chicks irrespective of their vitamin B₁₂ intake.

DISCUSSION

A. Experiments with riboflavin and thyroxine. In the experiments involving a study of the effect of the dietary level of riboflavin and thyroxine injection, presented above, more than twice the mortality occurred in the thyroxine treated groups of chicks as compared to that of the chicks in the non-injected groups. This difference in mortality rate may be due either to a thyrotoxic effect, following thyroxine injection, in the riboflavin deficient chicks, or to an increased requirement for riboflavin as a result of the thyroxine injections. The findings of the present study would suggest that the latter is more probable, since a greater increase in mortality was observed as a result of thyroxine injections in the chicks receiving the lower levels of riboflavin. If this is true, this observation is quite comparable to that of Handler and Pollis Jr. (1948) who reported that rats deficient in cystine and vitamin choline were more susceptible to hyperthyroidism. Moreover, Raab (1941) demonstrated that the administration of thyroxine produced heart failure resulting in death when adrenalin or substances having adrenalin activity were subsequently injected. Furthermore, Hertz (1946) stated that deficiencies of B-complex vitamins resulted in a disturbance of the normal hormonal mechanism.

The growth response observed in these experiments, with chicks receiving low levels of riboflavin, was slightly greater in the groups of chicks injected with thyroxine than in those not receiving thyroxine. This increased growth response was greatest when the chicks were given six injections during a three week experimental period.

In this connection Allardyce et al. (1947) reported that the basal metabolic rate of male and female rats fed a diet deficient in riboflavin dropped below the original level and a loss in weight resulted when desiccated thyroid was continuously fed. They further indicated that, although rats were able to overcome the action of desiccated thyroid without vitamin supplementation, the addition of several vitamins, including riboflavin, facilitated a rapid recovery.

In addition Bethell et al. (1947) assumed that the increased metabolic rate, which results from the administration of thyrotoxic material, may necessitate an increased dietary intake of members of the B-complex vitamins. This assumption was also confirmed by other workers mentioned under the review of literature.

Since the differences in growth, as a result of thyroxine injection of chicks receiving low levels of riboflavin were small, and since only a few chicks survived, these differences cannot be interpreted as having any real

significance. It is considered likely that these small differences in the average weights of the chicks in these groups may be attributed to a greater mortality of the smaller chicks. As a result the average body weights of the surviving chicks would be higher in the groups of chicks receiving thyroxine.

The results of the present experiment show that enlargement of the thyroid glands resulted from a dietary deficiency of riboflavin. In previous studies, Haque, Billie, Shaffner and Briggs (1949) indicated that a deficiency of riboflavin produced a goiterogenic effect in New Hampshire chicks. These results have been confirmed by the present study. It was observed in these experiments that as the deficiency of riboflavin became greater the thyroid size was correspondingly increased. The same was true even in the injected groups of chicks although there also was a decrease in the thyroid size regardless of the dietary level of riboflavin, when thyroxine injections were administered. The cause of enlarged thyroids in the non-injected chicks may possibly be due to a partial cessation of thyroxine production by the thyroid glands. The thyrotropic hormone of the pituitary gland stimulates the thyroid glands to secrete thyroxine when the thyroxine production is reduced. In the deficiency of riboflavin the thyroid glands may not be able to form thyroxine in normal amounts; as a result the pituitary gland would

continue to secrete the thyrotropic hormone which would give rise to an increase in thyroid growth.

Haque, Lillie, Shaffner and Briggs (1949) also indicated that riboflavin may be required by the chicks for the formation of thyroxine. However, if a deficiency of riboflavin results in a decreased ability of the thyroid glands to produce thyroxine, it would appear that the injection of thyroxine would overcome this effect and result in normal thyroid size. Since that was not found to be true in this study, riboflavin appears to function in some other way. It is possible that riboflavin is required, possibly in an enzyme system, for preformed thyroxine to exert its action on the pituitary gland in the maintenance of the normal hormonal balance. If such is true a deficiency of riboflavin would be expected to result in a hyperfunction and an increase in size of the thyroid glands.

Another possible explanation is that a deficiency of riboflavin limits the growth rate of the body to a greater extent than that of the thyroid glands. Since the thyroid size is expressed in terms of milligrams per 100 grams of body weight, any factor which would limit the growth of the body to a greater extent than that of the thyroid glands, would result in an increased relative size of the thyroid.

The depigmentation of the wing feathers in the

deficiency of riboflavin indicates the necessity of riboflavin in the formation of feather pigments in the chicks. This effect does not appear to be due to a decrease in the production of thyroxine. Had it been so, then the chicks injected with thyroxine would not have shown this condition. This observation appears to be related with the report of Haque, Lillie, Shaffner and Briggs (1948) who suggested that perhaps the melanin producing cells were affected directly by folic acid deficiency. This also may be true in the case of a riboflavin deficiency.

- B. Experiments with folic acid and thyroxine. In these experiments, the injection of thyroxine in chicks, receiving diets containing inadequate amounts of folic acid resulted in a mortality rate which was more than twice as great as that observed in chicks receiving the same amount of folic acid but not injected with thyroxine. It is obvious however, that, even in the absence of the thyroxine injection, the low levels of folic acid were inadequate and resulted in an appreciable mortality. The increased mortality, as a result of the thyroxine injections in the chicks fed a ration deficient in folic acid, suggests that the administration of thyroxine enhanced the degree of the folic acid deficiency. If this is true, no explanation can be given for the increased mortality observed in the groups of chicks fed a ration containing 1000 gamma of

folic acid as a result of thyroxine injection.

On the other hand, the thyroxine injection itself may have been the cause of part of the mortality, since increased mortality was observed in certain of the groups receiving adequate levels of this vitamin when thyroxine was administered. Reference is made to table 10, which shows that mortality occurred in chicks fed diets containing both low as well as high levels of folic acid when injected with thyroxine. This would suggest that thyrotoxicity was the cause for part of the mortality.

Another possible cause for the increased mortality, observed in this study, is that the thyroxine injections produced a deficiency of vitamin B₁₂ or an unidentified factor(s). Robblee et al. (1948) reported that condensed fish solubles and liver extract contained a substance, the requirement for which was increased by the administration of desiccated thyroid or iodinated casein to chicks. Later, Nichol et al. (1949) found vitamin B₁₂ to be as effective as fish solubles or liver extract in this connection. However, this explanation is not considered likely, since chicks from dams receiving a normal ration were used in all experiments and the experimental ration included 18% crude casein, which contains an appreciable amount of vitamin B₁₂.

The injection of thyroxine in chicks receiving different levels of folic acid in the diet produced variable

effects on the body weight. For the most part the body weight was slightly depressed when thyroxine was injected in chicks receiving inadequate amounts of folic acid. However, in one experiment, this was not found to be true. For this reason no significance can be attached to the small differences in growth response obtained in these experiments as a result of thyroxine injection. However, the experiment, in which thyroxine injection did not depress growth, was conducted during warm weather and involved an experimental period of only one week. Under these conditions the injection of thyroxine may have resulted in a stimulation of feed consumption, thereby masking any direct effect on thyroxine on rate of growth.

The slightly larger thyroid size, when expressed as milligrams per 100 grams of body weight, in chicks receiving a diet containing low levels of folic acid, as compared to those receiving diets containing adequate amounts of folic acid, suggests that folic acid may be essential for the formation or function of thyroxine in the chick. However, this relationship was not clearly demonstrated as was done in the studies with riboflavin and pantothenic acid.

Hertz (1945) showed that folic acid was required both qualitatively and quantitatively for hormonal response resulting in new tissue formation. It is quite possible that folic acid may be concerned in a similar way in the

present study.

From the results it appears that larger amounts of thyroxine are needed to depress to an equal extent the thyroid glands of chicks receiving a diet deficient in folic acid than are required in chicks receiving a complete diet. Smaller amounts of thyroxine were needed to depress the thyroids when the folic acid level in the diet was adequate. This further indicates that thyroxine secretion, or function, in chicks is affected when there is insufficient folic acid in their diet. This action might be expected in view of the work of Hertz (1946) who offered the deficiency of certain vitamins as the cause for a disturbance of the normal hormonal mechanism.

- c. Experiments with pantothenic acid and thyroxine. As shown in the results obtained from the experiments with pantothenic acid and thyroxine in chicks, the mortality of chicks receiving diets containing inadequate levels of pantothenic acid was slightly increased as a result of the injection of thyroxine. Although the difference in mortality was slight this effect of thyroxine injection in chicks receiving low levels of pantothenic acid is comparable to that obtained in chicks receiving diets containing inadequate levels of folic acid and riboflavin. However, the difference in mortality was considerably greater in the other experiments.

This observation is in agreement with those of Drill

(1938), Betheil et al. (1947), Abelin (1930), Cowgill and Palmeiri (1933), Himwich et al. (1931-32) and Drill et al (1942) who reported that calcium pantothenate was required in larger quantities during experimentally induced thyrotoxicosis. Abelin (1945, 1946) observed similar results. The results of the present study suggest that a functional relationship between pantothenic acid and thyroxine may exist, since the administration of thyroxine caused harmful effects in chicks fed diets deficient in pantothenic acid.

The weights of the thyroid glands of chicks in both thyroxine injected and non-injected groups were quite variable. However, when the thyroid weights are expressed as milligrams per 100 grams of body weight, the average thyroid weights of the chicks receiving low levels of pantothenic acid were found to be significantly greater than those of chicks receiving adequate amounts of pantothenic acid. This suggests that thyroxine secretion is suppressed when the level of pantothenic acid in the chick diet is inadequate, or that the normal hormonal control of the thyroid glands is interfered with when an inadequate amount of pantothenic acid is present. This observation is in agreement with those of Haque, Lillie, Shaffner and Briggs (1948).

In this experiment, it has been found that an experimental period of one week was not as satisfactory as

longer periods in studying the effect of thyroxine injection and thyroid size. The differences in thyroid weight were greatest when the period of injection was three weeks in length. This indicates that in the deficiency of pantothenic acid, the action of thyroxine on the thyroid glands of the chicks is very mild. Several vitamins appear to be concerned in the normal functions of the thyroid glands in chicks, but the effect of thyroxine injections appears to be less marked in pantothenic acid deficiency than in either folic acid or riboflavin deficiency.

The marked decrease in the deficiency symptoms (dermatitis) of pantothenic acid in chicks as a result of thyroxine injections was first observed in previous work (Haque et al. 1948). This finding has been confirmed in the present study. The protective effect of thyroxine on the manifestation of the dermatitic lesions in pantothenic acid deficiency in chicks was more pronounced when the injections were administered during a period of three weeks. This anti-dermatitic effect of thyroxine further indicates that there is a relationship between this vitamin and the thyroid hormone.

D. Experiment with vitamin B₁₂ and thyroxine. In this study, much more rapid growth was obtained in the chicks which received vitamin B₁₂ in their diets, than in those chicks receiving only the basal diet. This was also true

when thyroxine was injected. These results are in agreement with those of Nichol et al. (1949) who found vitamin B₁₂ to be highly active in stimulating the growth of the hyperthyroid chicks. Robblee et al. (1948) also reported that fish solubles or liver extracts, both of which are good sources of vitamin B₁₂, exerted a similar effect.

In this study a level of crude vitamin B₁₂ concentrate, which supplied 1.5 gamma of vitamin B₁₂ per 100 grams of diet, was found to be adequate for optimum growth. This is in agreement with the finding of Nichol et al. (1949).

Robblee et al. (1948) further observed that the feeding of iodinated casein to chicks, receiving a diet low in vitamin B₁₂, resulted in a depression of growth. On the other hand, when adequate amounts of vitamin B₁₂ containing supplements were added, a greater growth response was obtained in the hyperthyroid chicks than in those not receiving iodinated casein. The results obtained in the present study do not confirm those findings of Robblee et al. (1948), since the injection of thyroxine did not materially effect the growth response of chicks receiving the different levels of vitamin B₁₂ crude concentrate.

When vitamin B₁₂ was not added to the chick diet, the average thyroid weight was found to be 11.2 mg. per 100 grams of body weight. However, it was found to be only 10.2 mg. per 100 grams of body weight in chicks which were

fed an adequate amount of vitamin B₁₂. In this study, as the level of vitamin B₁₂ was increased there was a gradual decrease in the weight of the thyroid glands, as expressed in milligrams per 100 grams of body weight. This suggests the necessity of vitamin B₁₂ also for the normal function of the thyroid glands of the chicks. Further studies are required to elucidate this interaction between vitamin B₁₂ and thyroxine, and their effects on the thyroid glands.

E. General. Under the conditions of these experiments, involving a study of the effects of thyroxine injections in chicks, receiving adequate diets, except for single deficiencies of riboflavin, folic acid, pantothenic acid or vitamin B₁₂, the results, in general, are quite similar for each of these vitamins studied. In this connection, the thyroid weights, when expressed in milligram per 100 grams of body weight, were increased as the dietary level of each vitamin studied was decreased. This was most pronounced in the case of riboflavin and pantothenic acid. Similarly, the injection of thyroxine in chicks, receiving diets deficient in pantothenic acid, folic acid or riboflavin, resulted in an increased mortality rate. This was not observed, however, in the experiment involving vitamin B₁₂.

Although the effect of thyroxine injection on body weight was less marked, in general, a decrease in body weight was observed in chicks, receiving the diets containing

low levels of either folic acid, riboflavin or pantothenic acid. Since the effects are quite similar in each of the vitamin deficiencies, it appears probable that the mechanisms involved in producing these effects are comparable in nature.

In the experiments involving riboflavin, folic acid, and pantothenic acid, in which experimental periods of different lengths were used, it was found that the three weeks experimental period was much more satisfactory in studying the effects of thyroxine injections in vitamin deficient chicks, than was either a one or two weeks experimental period.

SUMMARY

1. In New Hampshire chicks fed an adequate diet, except for single deficiencies of riboflavin, folic acid, or pantothenic acid, the injection of thyroxine resulted in greater mortality. Increased mortality was not observed, however, when thyroxine injections were given to chicks receiving a diet deficient in vitamin B₁₂.
2. In chicks fed diets containing low or suboptimal levels of riboflavin, the injection of thyroxine slightly increased the growth response over the controls. However, the injections of thyroxine in chicks receiving diets deficient in folic acid had no consistent effect on growth, although it appeared to slightly depress the rate of growth in most instances. The mortality of chicks receiving low levels of pantothenic acid was too great at 4 weeks of age to permit a study of the effect of thyroxine injection on body weight. Injection of thyroxine in chicks receiving a diet deficient in vitamin B₁₂ did not noticeably effect the rate of growth.
3. The average weight of the thyroid glands when expressed as mg. per 100 grams of body weight of chicks, receiving diets deficient in riboflavin, folic acid, pantothenic acid, or vitamin B₁₂, was found to be greater than that of chicks receiving adequate levels of each of these vitamins. In general, there was an inverse relationship

between the average weight of the thyroid glands and level of each of these vitamins in the diet. This goiterogenic effect was greatest in the chicks which received diets deficient in riboflavin, or pantothenic acid.

4. In the experiments involving riboflavin, folic acid, and pantothenic acid, where experimental period of different lengths were used, it was found that a three week experimental period was much more satisfactory, in studying the effects of thyroxine injections in vitamin deficient chicks, than either a one week or two week experimental period. This was particularly true in studying deficiencies in thyroid weight and body weight.
5. The injection of different amounts of thyroxine (from .25 to 1.0 mg. per 100 grams body weight) in deficient chicks did not appear to influence the effect on mortality, body weight, and thyroid size.
6. New Hampshire chicks fed a diet deficient in riboflavin developed white streaks of depigmentation in the primary and secondary wing feathers. These streaks were more obvious in chicks which had been injected with thyroxine.
7. Injection of thyroxine resulted in a marked decrease in the hemoglobin content of the blood of chicks, receiving a diet low in folic acid.
8. The severity of the dermatitis of pantothenic acid deficiency was noticeably less in the deficient chicks

which had been injected with thyroxine.

9. Under the conditions of this study 1.5 gamma of vitamin B₁₂ per 100 grams of ration was found to be the optimum level for the New Hampshire chicks.

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FOOT NOTE

1. Thyroxine was supplied by E. R. Squibb and Sons, New York.

APPENDIX

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Table 13. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of pantothenic acid.

(Experiment B)

Group	Added pantothenic acid, mg/100 gm of feed	Thyroxine injected*	Number chicks 4 weeks	Mortality during 4th week %	Average weight, gm 4 weeks	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	0 (1)**	100	0	0.0
2	0.0	+	0 (2)	100	0	0.0
3	0.15	-	0 (2)	100	0	0.0
4	0.15	+	1 (3)	66.6	124	10.5
5	0.5	-	5 (5)	0.0	249	12.8
6	0.5	+	5 (5)	0.0	236	8.5
7	2.0	-	7 (7)	0.0	228	9.5
8	2.0	+	5 (7)	28.5	244	10.9

* The chicks were injected with a single injection of 1 mg of thyroxine per 100 gm of body weight on 21st day of age.

** Figures in the parentheses indicate total number of chicks at the beginning of the experimental period.

Table 14. Effect of thyroxine injection on mortality, body weight and thyroid weight in chicks receiving different amounts of pantothenic acid.

(Experiment B)

Group	Added pantothenic acid, mg/100 gm of feed	Thyroxine injected*	Number chicks 4 weeks	Mortality during 4 week period, %	Average weight, gm	Average thyroid wt. mg/100 gm body wt.
1	0.0	-	0 (7)**	100.0	0	0.0
2	0.0	+	0 (7)	100.0	0	0.0
3	0.15	-	1 (6)	83.3	100	18.0
4	0.15	+	0 (6)	100.0	0	0.0
5	0.5	-	6 (6)	16.6	235	12.8
6	0.5	+	6 (6)	0.0	238	9.7
7	2.0	-	7 (7)	0.0	204	14.6
8	2.0	+	7 (7)	0.0	179	9.7

* The chicks were injected with 2 injections of a level of 0.5 mg per 100 grams of body weight each at the age of 14 and 21 days.

** The figures in the parentheses indicate total number of chicks at the beginning of the experimental period.