ABSTRACT OF THESIS

THE EFFECTS OF EARLY THYROID REMOVAL AND THE FEEDING OF DESICCATED THYROID AND THYROACTIVE PROTEINS ON THE GROWING CHICK

Submitted by

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In partial fulfillment of the requirements

for the Degree of Master of Science

Colorado Agricultural and

Mechanical College

Fort Collins, Colorado

April, 1945

ABSTRACT

Thyroidectomized and non-thyroidectomized Single Comb White Leghorn and White Plymouth Rock chicks were fed basal diet, or basal diet supplemented with thyroactive substances, for the first six or eight weeks of age. Observations were made on feather development, body weight, efficiency of gain, growth of shanks, dermatosis, perosis, and mortality. In some experiments observations were also made on thyroid weight and histology, liver storage of several vitamins, and fat content of the carcass.

A technique for removing the thyroids from day-old chicks was described and some of its advantages were discussed.

Thyroid removal from chicks one to three days old resulted in lower body weight and shorter shank length than normal, and inhibited or markedly retarded, feather development. Thyroid removal from chicks one to three days old also caused typical vitamin deficiency symptoms in the experiments carried out during the winter months.

The liver content of vitamin A, riboflavin, pantothenic acid, nicotinic acid, and total dry matter

was either unaffected or very slightly lower than normal, in thyroidectomized chicks over the first six weeks of age.

Addition of yeast and sardine oil to the diet of thyroidectomized chicks failed to counteract the effects of thyroidectomy.

Addition of massive doses of potassium iodide to the diet of dams, or thyroidectomized chicks failed to counteract the subsequent effects of thyroidectomy in chicks one to three days old.

Addition of small doses of thyroactive iodinated casein, or of desiccated thyroid, counteracted completely the effects of thyroidectomy on all characteristics studied except body weight. The effectiveness was dependent upon the thyroxine content and not upon the iodine content.

Iodinated casein and desiccated thyroid, when added on an equal thyroxine activity basis, did not consistently cause the same response when different experiments were compared or when the products were compared with each other in the same experiment.

Progressive increase in the amount of desiccated thyroid in the diet of growing thyroidectomized and control chicks caused progressive decrease in the measurements of body weight, efficiency of gain, adipose storage in the carcass, and liver storage of vitamin A.

In nearly every instance, the decrease was preceded by an increase with very small additions to the diet.

Both thyroidectomized and non-thyroidectomized chicks receiving very small amounts of desiccated thyroid in the diet stored more vitamin A, riboflavin, nicotinic acid, and pantothenic acid than did the corresponding chicks receiving the basal diet only. In most cases, the initial increase in liver storage resulting from small additions of desiccated thyroid was followed by rapid decrease with successively greater additions.

Feather condition in thyroidectomized Single Comb White Leghorn chicks was restored to normal by as little as 0.0156 per cent desiccated thyroid in the diet. As little as 0.05 per cent of an iodinated casein product in the diet of normal White Plymouth Rock chicks resulted in excellent feather condition. According to the estimate of the producers this was equivalent to 0.25 per cent or more of desiccated thyroid, but according to the assay employed in this investigation, this amount was only equal to about 0.02 per cent of desiccated thyroid in the diet.

A method of assay for thyroxine potency of thyroactive substances was proposed and discussed. The method was based upon the response of the chick thyroid to thyroactive substances included in the diet. The activity of the gland was determined by the evaluation

of width and length of the whole gland and diameter of the follicles. The activity was compared with the activity resulting from the feeding of known amounts of standardized desiccated thyroid.

On the basis of estimated thyroxine production by the chick thyroid, the iodine requirement of growing Single Comb White Leghorn chicks was estimated as approximately 1250 parts per billion in the diet for the first six weeks of age.

The maximum non-lethal dose of desiccated thyroid that could be administered in the diet of growing chicks was approximately 0.50 per cent. The maximum non-toxic dose was between 0.0156 and 0.03125 per cent for Single Comb White Leghorn chicks.

Between 0.002 and 0.02 per cent of desiccated thyroid in the diet was suitable for optimum feather development without simultaneously resulting in sub-normal fat storage, shank length, and body weight in White Plymouth Rock chicks.

Completely thyroidectomized day-old chicks grew to maturity and produced fertile eggs. They had received no thyroid supplementation in the diet, and they showed no regenerated thyroid tissue upon post-mortem examination.

In the absence of thyroid hormone, secondary sexual characteristics did not develop.

Thyroactive substances were found to have an apparent beneficial effect on mature and growing birds suffering from severe symptoms of range paralysis, a virus disease of poultry.

T H E S I S

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COLORADO STATE COLLEGE

OF

AGRICULTURE AND MECHANIC ARTS

January 29 1945

I HEREBY RECOMMEND THAT THE THESIS PREPARED UNDER MY SUPERVISION BY DONALD VICTOR ZANDER

ENTITLED THE EFFECTS OF EARLY THYROID REMOVAL AND THE FEEDING OF DESICCATED THYROID AND THYROACTIVE PROTEINS ON THE GROWING CHICK

BE ACCEPTED AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

MAJORING IN POULTRY NUTRITION

CREDITS 6

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ACKNOWLEDGMENTS

The author is especially indebted to Dr. H. S. Wilgus, Jr., head of the poultry department, who, until his entrance into the Army of the United States, made successful completion of this problem possible through his patient and dependable guidance, constructive criticisms, and dependable and enthusiastic assistance.

The author is also greatly indebted to Dr. F. X. Gassner of the physiology department for his guidance and criticisms of the histological studies involved, for taking the photographs used, and for certain equipment needed.

The author is further indebted to Dr. R. Jensen, now of the pathology and bacteriology section, for instruction in the technique of thyroidectomy, and for performing many of the operations; to Dr. Dudley P. Glick of the pathology and bacteriology section, for laboratory facilities used in carrying out the microbiological assays; to Mr. L. W. Charkey, of the chemistry department (now on leave at Cornell University) for his guidance in the determination of vitamin A and carotene; to Miss Verna Mace, of the physiology depart-

ment, and Dr. F. H. Kratzer, of the poultry department, for assistance in the preparation of charts and plates; to Rowen Frandson, veterinary student, for assistance in obtaining the autopsy records and tissue samples; to Mr. A. M. Vance, poultry plant foreman, for his cooperation and assistance; to Dr. L. W. Washburn for the use of certain special apparatus; and to Dr. W. E. Pyke, of the home economics section, for use of the cold storage unit.

All of the above-mentioned individuals are members of the Colorado Agricultural and Mechanical College staff.

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INTRODUCTION

Following an observation in earlier iodine studies at this station that growth, development, and performance of birds seemed unimpaired despite the extreme goiters produced on certain types of diets (Wilgus 1941) (Wilgus et. al. 1941), a series of experiments was begun in January, 1942, to study the effects of thyroid removal and of thyroid feeding on growth and general characteristics of the chick. It was hoped that through replacement therapy some indication of iodine requirements might be obtained also. Typical vitamin deficiency symptoms occurred in thyroidectomized chicks in the first exploratory experiment; consequently, one experiment was broadened to include the effects on liver vitamin storage. In the final stages of the work, during routine examination of thyroids from birds receiving thyroactive materials, an inverse relationship was noted between dosage and degree of thyroid activity. This relationship was investigated in detail with a view to its potentialities in estimating iodine requirements, and of assaying thyroactive substances of unknown potency.

HISTORY AND REVIEW OF LITERATURE

So vast is the literature pertaining to the thyroid gland that several books and comprehensive reviews have been written on the subject (Harington, 1933; Levine and Remington, 1933; Crotti, 1938; Elmer, 1938; Salter, 1940; and others). The reader should consult one of these for a discussion of the functions of the gland, its interrelationships with other glands, and typical symptoms associated with thyroid removal.

Experimental thyroidectomy has been practiced on such common animals as the rat, mouse, goat, sheep, cow, rabbit, guinea pig, pigeon, and chicken, as well as several of the lower forms of animal life (Crawford and Hartley, 1925; Graham, 1934; Meyer and Wertz, 1938; Emmens and Parkes, 1940; Preheim, 1940; Reineke and Turner, 1941; Brody and Frakenbach, 1942; Karnofsky, 1942; and others). The symptoms are quite similar in all animals. Quite often it is necessary to wait for a long period of time for the effects of thyroid removal to become evident. Considerable experimental work is reported with respect to thyroidectomy in the chicken. In addition to characteristic symptoms as described for mammals, removal of the gland results in marked retar-

dation of feather growth and egg production (Greenwood and Blyth, 1928-29; Winchester, 1939; Taylor and Burmester, 1940). The literature lacks any report on the estimation of minimum thyroxine administration necessary to counteract the effects of thyroidectomy in chicks. Fishbone and Cunningham (1938) found 40 micrograms daily injection of thyroxine were necessary in one experiment to maintain normal heart rate in thyroidectomized rats.

Greenwood and Blyth (1928-29) carried on extensive studies of the influence of the thyroid and of thyroid removal on feather development. They performed operations on growing birds at five weeks of age and older and did not observe any deleterious effects on growth, development, or sexual maturation. Upon examination, however, most of the birds showed some degree of regeneration of thyroid tissue microscopically. Taylor and Burmester (1940) performed thyroidectomy (parathyroid removal was uncertain) on mature laying chickens and noted an abrupt cessation of egg production which could be partially restored by thyroxine injections. As reported by other workers for mammals, adipose storage increased. In similar experiments, Winchester (1939) obtained essentially the same results as those reported by the California workers. He further observed a drop in basal metabolism in the thyroidectomized group.

Barbule formation is markedly suppressed in thyroidectomized birds, resulting in frayed feathers (Parkes and Selye, 1938; Taylor and Burmester, 1940; Emmens and Parkes, 1940). Ewald and Rockwell (cited by Taylor and Burmester) did not demonstrate any abnormal effects in pigeons lacking thyroid glands, but since the initiation of this problem, Marvin and Smith (1943) demonstrated a depressed basal heat production in thyroidectomized pigeons. They described a thyroidectomy technique in which the thyroids were removed in two stages: the first thyroid was removed at two months of age and the other one, four days later. No reference could be found to thyroidectomy performed on day-old chicks or birds.

Young offspring of iodine deficient dams exhibit clinical symptoms of thyroid insufficiency, if they live at all. This suggests that function of the thyroid begins early in life, and work with embryos supports this view (Gilmore et. al., 1940; Rankin, 1941; Gorbman and Evans, 1943). Carpenter (1942) observed colloid and follicular differentiation very early in embryonic chick thyroids cultured in vitro. This information suggests that for experimental purposes the thyroids should be removed at the earliest possible age in order to keep the initial blood and tissue level of thyroxine at a minimum.

In the majority of cases the thyroid and parathyroid are situated some distance apart in the day-old chick. In older birds they are more closely associated, and in adult birds the two glands are intimately attached, though accessory parathyroids may occasionally be found separated from the thyroid. Obviously, there is less danger of injury to the parathyroid glands during thyroid removal in day-old chicks than in more mature birds.

The feeding of whole or dried thyroid gland, both experimentally and clinically, has been practiced for a long time. Since the structure of thyroxine became known, iodinated proteins with thyroxine activity have been produced (Block, 1940; Reineke et. al., 1942-43; and others). Proof of the thyroxine activity has been established by the tadpole assay and by prevention of cretinism in thyroidectomized goats (Reineke and Turner, 1941), and also by measurements of the oxygen consumption in guinea pigs (Koger et. al., 1943). Since these thyroactive substances can be produced in large quantities, they have recently been more extensively investigated in regard to their practical applications.

In general, the effect of thyroid feeding is comparable to increased thyroid activity, and large doses result in hyperthyroidism which may be severe enough to cause death. For this reason, considerable

caution must be exercised when thyroid substances are fed. A characteristic result of hyperthyroidism is loss of weight, the greatest contributing factor being lack of adipose storage. Smith and McLean (1938) have shown that thyroid feeding may lead to premature cessation or retardation of growth of long bones by inhibiting endochondral ossification. This may be preceded by a period of stimulation of growth.

As pointed out by Koger and Turner (1943), large doses were employed in most thyroid feeding experiments. These two workers noted the effects following mild feeding of thyroactive substances to growing animals of four species, including rats, mice, guinea pigs, and rabbits. Great variations in response were noted between individuals, sexes, and species. With the exception of mice and female guinea pigs, body weight and growth were unaffected, or were depressed with increased age and the onset of warm weather. Treated mice stored more protein and attained greater body weight per unit of feed consumed than the controls, while the latter were more efficient in storage of fat and energy. "Male rats were less tolerant to thyroactive preparations than females." The growth rate of male guinea pigs was slightly accelerated when low doses were given for a short period of time, but the same dosage later caused toxic symptoms with increase in age and arrival of warm

weather. In line with these findings, it has been shown that the iodine requirement varies with the season of the year (Levine and Remington, 1933; Elmer, 1938), and thyroid activity is stimulated by cold (Ring, 1942).

There is a definite relationship between thyroactive substances and feather development, as has been clearly shown (Hutt, 1927-30; Cole and Hutt, 1928; Greenwood and Blyth, 1928-29; Parkes and Selye, 1928; Radi and Warren, 1938; Greenwood and Chu, 1939; Emmens and Parkes, 1940; Chu, 1940; Parker, 1943; Irwin et. al., 1943; Turner et. al., 1944). Desiccated thyroid fed orally, or thyroxin given by injection, may cause depigmentation, stimulated feather growth, early moulting, and rapid replacement of feathers. These effects are presumably due to speeded-up cell division (Hutt, 1927). Growth, body weight, and metabolism of chickens are likewise affected by artificially induced hyperthyroidism (Hutt, 1930; Asmundson and Pinsky, 1935; Asmundson et. al., 1936; Sciaky, 1938; Winchester, 1939; Parker, 1943; Irwin et. al., 1943; Turner et. al., 1944). Hyperthyroid birds show an increased metabolic rate and heat production, and suffer loss of flesh which may be severe enough to cause death.

Since completion of the experiments of the present investigation, several reports have appeared in which thyroactive proteins were fed to chickens

(Parker, 1943; Irwin et. al., 1943; and Turner et. al., 1944). Comparable amounts of dietary thyroactive protein, calculated on the basis of biological activity, did not produce comparable results in all three investigations. In general, small amounts increased growth, whereas large amounts depressed growth and efficiency of gain, resulted in lower thyroid weight, retarded adipose deposition, and increased mortality. Feather condition was improved in accordance with the amount of thyroactive protein fed. Thyroids from chicks receiving high levels of iodocasein were invaded with non-thyroid tissue (Turner et. al.).

In preliminary work carried out at this station (unpublished data of H. S. Wilgus, Jr.), thyroactive protein substances were effective in producing excellent feathering, but the toxic effects needed further investigation. The maximum non-toxic dose needed to be determined.

Typical vitamin deficiencies, occurring in thyroidectomized chicks in the exploratory experiments, made investigation of the relation of thyroid function to vitamin metabolism of prime importance. The subject has been thoroughly reviewed by Drill (1943). He presents evidence demonstrating that the requirements for vitamins A, B_1, B_6, C, and pantothenic acid are increased during hyperthyroidism, and, if these increased

requirements are not met, that a relative deficiency of the vitamins will ensue. In the rat, there is a sex difference in response in vitamin metabolism during experimental hyperthyroidism. There is also a difference in degree of hyperthyroidism produced by the commonly used doses of thyroxine or thyroid gland. All of the evidence presented indicated that, in the absence of the thyroid gland, carotene is not metabolized to vitamin A. Schneider and Widmann (cited by Drill) obtained an increase in liver carotene in thyroidectomized guinea pigs injected with thyroxine. This seems to indicate that in the absence of the thyroid gland, carotene probably would not be converted to vitamin A even though thyroxine was injected. Clinical hyperthyroidism lowers serum vitamin A and carotene, but the effects on hepatic storage are controversial. "In amphibia a definite antagonism between thyroxin and vitamin A has been demonstrated." Hyperthyroidism causes a marked reduction in hepatic storage of vitamins B_1 and C.

Inasmuch as the liver is the greatest vitamin storehouse in the body, it is logical that any interference with vitamin metabolism, either in availability or utilization by the tissues, would be reflected in the liver reserves. The liver stores of vitamins would probably be high if the manifestations seen following thyroid removal were due to an interference with vitamin

utilization by the tissues. On the other hand, if the removal of the thyroids resulted in interference with absorption, storage, or chemical conversions to available forms, the liver would probably be low in content of these vitamins.

The thyroid shows great affinity for iodine, so that when the element is administered via the blood stream it rapidly collects in the gland. Recently, Ray and Deysach (1942) have shown that the thyroid also exhibits an affinity for manganese. It is generally accepted that the primary function of the thyroid is to form thyroxine from tyrosine by successive steps of iodination and condensation. The greatest requirement for iodine in the body is for the formation of thyroxine. Information concerning the amount of thyroxine an organism produced would be exceedingly valuable. One could then calculate the amount of iodine theoretically necessary to form the same amount of thyroxine and hence estimate the minimum amount of iodine required by that organism. The apparent inverse relationship between the amount of thyroactive substance fed and the resultant state of activity of the thyroid gland seems to offer an excellent means of estimating the thyroxine output of the thyroid gland. The chick thyroid has been used for assay of the thyrotrophic hormone (Cope, 1938; Bergman and Turner, 1939; Smeltzer, 1939; and Rawson

and Salter, 1940), but no one has used chick thyroid response for assay of thyroxine activity. Although it is common knowledge that the activity of the thyroid is suppressed by thyroxine administration, Koger and Turner (1943), and Turner et. al. (1944), noticed the same effect with synthetic thyroactive substances, no one has shown any direct relationship between activity and dosage. Since completion of this investigation, Adams and Jensen (1944) have reported experiments in which three groups of mice were treated with 0.64, 1.32, and 2.64 mg. thyroxine respectively over 21 to 23 days. They found no difference in absolute thyroid weight between treated mice and controls, but measurements of the height of follicle epithelium showed an average decrease in thyroid activity of 43.43 per cent for the three groups of treated mice. Although they attached no significance to the fact, their data showed a gradual decrease in epithelium height with increased dosage of thyroxine. They also showed that the suppression of activity of the thyroid gland by thyroid administration was due to a decreased thyrotrophic potency of the pituitary gland. Stein (1940) proposed follicle volume as an index of activity of the thyroid follicle.

The General Experimental Plan and Objectives

Thyroids were to be removed from large numbers of chicks immediately after hatching. Groups of these chicks were to be fed various thyroactive substances in the diet over a period of from six to eight weeks. Each diet was to be fed to control chicks with intact thyroids as well as to thyroidectomized chicks. Some thyroidectomized chicks were to be raised to maturity if possible. A histological examination was to be made of the thyroid area in thyroidectomized chicks to check on the completeness of the operation.

The effects of thyroidectomy and of the feeding of thyroactive substances was to be ascertained by observations on such general characteristics as body weight, efficiency of gain, shank length, feather development, body fat storage, and mortality. Incidental observations were also to be noted and evaluated. Since the material would be available the livers of chicks in one experiment were to be assayed for total dry matter, vitamin A, riboflavin, nicotinic acid, and pantothenic acid. The thyroid glands from control chicks receiving various amounts of thyroactive substances were to be carefully studied to ascertain the effects of such substances upon the histology of these glands.

The data obtained from the observations and

analyses were to serve as a basis for arriving at certain conclusions, and making certain deductions, which are enumerated below.

1. Whether the effects of early thyroid removal in the chicken can be completely overcome by feeding thyroactive substances in the diet.

2. The maximum non-toxic level of thyroxine that can be administered to chicks via their diet.

3. The minimum amount of thyroactive substance necessary in the diet to produce optimum feathering in growing chicks, especially of a slow feathering breed, without simultaneously retarding growth and fat storage.

4. The effect of thyroidectomy and thyroid feeding on the hepatic storage of vitamin A, riboflavin, pantothenic acid, and nicotinic acid.

5. The thyroxine out-put of the chick thyroid, and by this means to estimate the iodine requirement of the growing chick.

METHODS AND MATERIALS

In the first two experiments, which were purely exploratory, removal of the thyroids by means of cautery was found to be superior to removal by other surgical means. Thyroidectomy by electric cautery technique was adopted for all of this work, and the following procedure was carried out for all succeeding experiments.

The sex of the chicks was determined (by the author) when they were hatched, to facilitate equal distribution of males and females in the experimental pens. The time required did not permit completion of all the operations the first day after hatching but necessitated two, and sometimes three, days.

The materials and instruments used for these operations consisted of one scalpel, two pairs of blunt forceps, two pairs of fine pointed forceps, one histotribe, one wound spreader, cotton swabs, a satisfactory source of light which would keep the operating site well lighted, and an electric cauterizing needle. Appropriate rheostats were interposed in the circuit and a convenient switch attached. The cauterizing apparatus was then suspended on a tension spring over the table.

A small operating table was made from a six-inch board ten inches long, nailed to two V-shaped blocks to elevate one side about an inch and a half. Two strips of rubber about an inch wide, cut from an old inner tube, were slipped over the ends of the board. This served to hold the chick steady and in a proper position during the operation.

The chicks were anesthetized with a nembutal solution (0.1 gr. per cc.). The dosage used was approximately 0.05 cc. for each 10 grams of body weight. The nembutal was administered into the breast muscle, or directly into the brachial vein. Both methods were satisfactory, but the intravenous injection produced quicker anesthesia. When anesthesia was complete, the down was plucked from the area of operation. This was about an inch wide and extended from the antero-ventral tip of the breast forward for half the distance of the ventral surface of the neck.

The chick was placed on the operating table with the ventral surface uppermost. The wings were held under the rubber strips and the head and neck allowed to hang over the elevated edge of the table. An incision about one inch long was made through the integument, beginning about one-fourth inch anterior to the cranial extremity of the sternum and proceeding anteriorly beyond the crop along the median line. The connective tissue was parted by blunt dissection until

the left thyroid became visible. The spreader was useful to retract the tissues during this procedure. The interclavicular air sac covered the glands completely in most subjects, and in some instances it was punctured in exposing the thyroids. This undoubtedly allowed air to escape into the area beneath the integument causing so-called "windpuff." Death from starvation resulted when this condition was severe enough to prevent proper eating. After freeing the thyroid from the surrounding connective tissue with the fine forceps, the histotribe was clamped around the base of the thyroid between the gland and the associated nerves and vessels. The red-hot cauterizing needle was quickly applied to the gland, destroying it in situ. Two quick applications of a hot needle were more satisfactory than prolonged application of a cooling needle.

After removal of the left thyroid, the crop was freed from the musculature on the right side by blunt dissection. The right thyroid was then removed in a similar manner. After both thyroids were destroyed, the crop was returned to its normal position and the incision closed with suture material. Ordinary sanforized cotton thread proved satisfactory for this purpose. The sutures were removed about two weeks later.

In experiments I-25 and I-26, the control

chicks were operated upon but the thyroids left intact. In this procedure thyroidectomy was duplicated but only to the extent of manipulating the thyroids with forceps and partially freeing them from the surrounding connective tissue. These chicks are referred to as "operated controls." A few chicks were thyroidectomized at two weeks of age. These groups are referred to as "thy. 2 weeks." There were no indications of infections of any kind in the several hundred chicks operated on.

The chicks were kept warm at all times during and after operation. Recovery from anesthesia was usually complete in half an hour.

Post operative mortality was approximately 10 per cent. One contributing cause, a condition termed "gasping," was characterized by continual gasping for breath. The condition became apparent immediately following recovery from anesthesia. It always terminated fatally within a few days due to inability to eat and ultimate exhaustion. The cause of this condition was undetermined.

Another important condition contributing to post operative mortality was crop impaction. This was undoubtedly due to injury of the autonomic nervous system supplying the crop musculature or injury to the musculature itself. The condition was quite common but only in one or two per cent of cases did it persist to

the point of causing permanent damage. In fatal cases the chicks grew thin and died, apparently from starvation.

By using the procedure outlined above, it required about 20 minutes for one person to thyroidectomize three chicks which were previously prepared for operation by an assistant.

Except for instances noted, the chicks used in these experiments were Single Comb White Leghorns from dams receiving diets of various known moderate levels of iodine. The chicks received the low iodine basal diet, described below, until they were started on the experimental diets. The mash for the thyroidectomized chicks was made into a wet-mash for two or three days following the operation. At the start of an experiment, the chicks were weighed and grouped so that the weights of the thyroidectomized groups were comparable, and the weights of the control groups were comparable. Except for errors in day-old sex determination, each group contained the same number of males and the same number of females. The chicks were distributed so that all groups were comparable as to ancestry and diets of dams.

The rearing pens were compartments of a metal battery with contact heating units where the chicks could seek warmth at any time. The batteries were kept

in a room with a minimum temperature of 70 degrees Farenheit. In the summer time the temperature often went up above 90 degrees Farenheit. Feed and water were given ad libitum but care was taken to keep feed wastage at a minimum.

The basal diet used in this study was one which had been employed in previous iodine work and consisted of the following ingredients in the designated proportions.

Ingredient	Per Cent in Diet
Ground yellow corn	33.9
Pulverized oats	20.0
Wheat grey shorts	10.0
Wheat bran	10.0
Alfalfa leaf meal (dehydrated)	2.5
Soybean oil meal	6.0
Meat and bone scrap	11.0
Dried buttermilk	5.0
Pulverized limestone	1.0
Salt (plain)	0.5
Manganese sulphate	0.015
1000-D Delsterol powder	0.1
Total	100.015

This diet contained approximately the following amounts of minerals and vitamins: Iodine, 245 parts per billion; Manganese, 54 parts per million; Calcium, 1.64, phosphorus, 0.97, and choline, 0.1 per cent; Vitamin A, 500, thiamin, 500, riboflavin, 350, pantothenic acid, 1300, and vitamin D, 50-100, units per 100 grams of diet. (Vitamin A in international units, vitamin D in chick units, B vitamins in micrograms).

Test ingredients were added to a small portion of the diet and mixed thoroughly. This portion was then

added to the remainder of the diet and the whole mixed in an automatic feed mixer. In the case of the desiccated thyroid powder, the diet containing the highest concentration was made up first, and each succeeding diet was made up by successively diluting a portion of a more concentrated diet with an equal volume of basal.

The desiccated thyroid used in these experiments was purchased from a commercial firm and bore the USP label. On the basis of the USP standards it contained approximately 0.2 per cent iodine in the form of thyroxine iodine. This should mean that the desiccated thyroid product contained about 0.3 per cent thyroxine.

Two iodinated casein products were tested. The first product, referred to as iodinated casein-B, was estimated to have a slightly higher thyroxine activity than desiccated thyroid.1/ The second product, referred to as iodinated casein-A, was a much more potent product and was estimated to have from five to 10 times the thyroxine activity of desiccated thyroid.2/ The potassium iodide used contained 10 per

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1/ This product was supplied by the Mellon Institute through the courtesy of Dr. F. F. Johnson.

2/ This product was supplied by Cerophyll Laboratories of Kansas City through the courtesy of Dr. W. R. Graham, Jr.

cent calcium stearate as a stabilizing agent.

Each diet was fed to a control group and to a thyroidectomized group. Thyroidectomized and control chicks were maintained in separate cages in experiments I-27 and I-29 and during the last two weeks of experiment I-26. After completion of the experiments, the chicks to be kept under observation were placed on a developing mash which was essentially the same as the experimental basal diet, except that the protein and riboflavin content was lowered slightly.

Residual feed weights, individual chick weights, and incidental observations were recorded each week. Efficiency of gain was calculated by dividing the gain in weight (gm.) by the food consumed (gm.) over the same period.

Shank lengths of all birds of experiment I-29 were measured in sixteenths of an inch, from foot pad to posterior extremity of the flexed hock joint, with a device designed for such measurements by the South Dakota State College Poultry Department.

Feather score was obtained at the end of the experiment. Scoring was patterned after that of Radi and Warren (1938) except that degree of pinniness and degree of body coverage were scored separately. Scoring was limited to the back, following observations in an earlier experiment at this station where feather

condition on the back was found to be an accurate index of general feather condition. Perfect coverage was assigned a score of 0, and absence of any feathers on the back was assigned a coverage score of 30. Absence of any pinfeathers was assigned a score of 0, and if all the feathers which appeared were pinfeathers, a pinniness score of 30 was assigned. Obviously, the pinniness score was automatically 0 when the coverage score was 30. This was an attempt to measure the total number of feathers developed as well as the degree to which these feathers would develop.

The incidence of perosis and dermatosis were noted at the end of each of the last two weeks to check on consistency of scoring. The method of scoring for perosis was similar to that for feathering. The degree of enlargement, or turning of the hock joint, was scored from 0 to 30. No enlargement or turning was assigned a score of 0. Chicks were considered as having dermatosis if fissuring occurred on the bottom of the feet or around the mouth, or if there was marked scaling on the sides and top of the feet.

Rectal determinations of body temperatures were made on the chicks of experiment I-25 on the last two weighing dates.

For fat analyses, four representative birds from each group of experiment I-29 were decapitated,

bled, quickly scalded, and the feathers stripped from them. The thyroids and other tissues were immediately removed and preserved, and the chickens uniformly disemboweled. The lungs were removed but the kidneys were left intact. The feet were removed at the hock. The carcass was then ground in a power-driven food chopper, mixed, and ground again, then re-mixed and stored with plenty of dry ice in the refrigerator at 10 degrees below zero centigrade, pending analysis of moisture and fat.

Moisture content was determined on two 25-gram samples of finely ground, thoroughly mixed carcass material by vacuum drying at 50 degrees centigrade for 24 hours. A 10-gram sample from each moisture-free residue from above was used for duplicate fat determinations. Fat content was determined directly by extraction with petroleum ether on a Bailey-Walker apparatus, with subsequent vacuum drying of the fat residue at 50 degrees centigrade overnight.

Livers, thyroids, and other tissues for endocrine studies were obtained at the end of the experimental periods.

At the termination of experiment I-29, livers from all birds were preserved individually with dry ice and stored in the refrigerator, at 10 degrees below zero centigrade, for five and one-half months until vitamin

assays could be run. Vitamin A was then determined by a modification of the method of Dann and Evelyn (1938), employing saponification with potassium hydroxide. The vitamin A was estimated with an Aminco photometer, using the antimony trichloride reaction. The unused chloroform extracts were pooled by groups and the carotene content determined for the group. The B-vitamins were extracted by the method of Cheldelin et. al. (1942), and assayed by the methods of Snell and Strong (1939), Snell and Wright (1941), and Pennington et. al. (1940). The samples were homogenized in a Waring Blendor with a specially designed cup before digestions with takadiastase and papain enzymes. The filtered aqueous extracts were washed with ethyl ether, before determination of the vitamin content by growth response of Lactobacillus casei. Toluene was used for an overlying protective fluid during preparation and storage of the extracts. The size of the livers limited the samples to five grams for vitamin A determination and three grams for B-vitamin assays. The remainder was used for moisture determination. The latter was determined after vacuum drying at 50 degrees centigrade for 24 hours.

The method of checking for thyroid regeneration in the first two experiments, I-25 and I-26, was considered inadequate, so the details of tissue selection and treatment have been omitted. Most of the chicks showing regeneration were apprehended in experiment I-26

but some may have escaped detection. In the last two experiments, I-27 and I-29, all chicks were killed. The thyroid areas were removed from all thyroidectomized chicks and preserved in Bouin's fixative solution. The thyroids were removed from all control chicks and trimmed free of extraneous tissue. The left gland was preserved in Bouin's fixative solution for histological examination and the right gland was preserved in 95 per cent alcohol for iodine analysis. Iodine analysis was not completed for experiment I-27. After 12 hours in the preservative, the thyroid glands were removed, blotted on blotting paper, weighed on an analytical balance and returned to their respective bottles prior to further treatment. All tissues fixed in Bouin's solution were transferred to 70 per cent alcohol after 24 to 48 hours in the fixative. The tissues for histological study were cleared in dioxane, embedded in paraffin, and sectioned at five micron intervals. Staining was done according to a differential method of Dr. F. X. Gassner of the department of physiology.

A "thyroid area," as referred to in these experiments, was obtained by taking a section of tissue beginning with the bifurcation of the carotid and brachial arteries and extending anteriorly to the posterior lobe of the thymus which was included. This area included the carotid artery, jugular vein, the vagus nerve, and the attached connective tissue to-

gether with all the visible parathyroid tissue.

"Thyroid areas" were sectioned according to the following plan. Beginning with the first tissue cut from the block on the microtome, ribbons of 50 or less sections, each five microns thick, were taken from the block and laid on a pan of warm dilute egg albumen solution. The last few sections of the ribbon were placed on a slide and observed unstained, under the microscope. If thyroid tissue was present, a serial mount was made and sectioning continued until parathyroid tissue was found and mounted for staining. If thyroid tissue was not found, another strip was obtained and examined similarly. This process was continued until several strips were laid side by side on the surface of the albumen solution. The centermost sections were mounted on one slide for staining and reference and the remainder discarded. This resulted in a series of sections separated by a sectioning interval of approximately 200 microns and separated from unstained examined sections by a sectioning interval of approximately 100 microns. This procedure was continued until all of the tissue was traversed. In this manner, only isolated groups of a few thyroid cells could escape detection, and parathyroid tissue could not escape detection. Therefore, two problems were solved in one operation; namely, (1) to prove the absence of thyroid

tissue, and (2) to prove the presence of parathyroid tissue. The data for any birds which showed thyroid regeneration, or which did not have parathyroid tissue demonstrable under the microscope, were deleted from the analyses.

Various measurements of thyroids from control chicks of experiment I-29 were obtained by use of an eyepiece micrometer, and the proper conversion factors applied to convert the values into actual metric units. The largest cross section of the serially sectioned thyroid was selected for measurements. The greatest length (L), and the greatest width (W), of the cross section were obtained. In addition, the average diameters of the eleven largest follicles in the cross section were measured and summated (ΣD). Various treatments of these measurements are discussed as they are presented.

The two exploratory experiments were carried out in December of 1941 and January of 1942. The records for the other experiments are as follows:

Exp. No.	Breed of chick	Date hatched	Exp. started	Exp. ended	Age in days
I-25	W. Pl. Rocks	2/1/42	2/7/42	3/26/42	54
I-26	S.C.W.Leghorn	3/20/42	3/26/42	5/4/42	44
I-27	S.C.W.Leghorn	7/9/42	7/16/42	8/20/42	42
I-29	(W. Pl. Rocks) (S.C.W.Leghorn)	10/16/42	10/22/42	12/3/42	48

Disposal:

I-25 Representative birds from each group killed for tissue samples. The remainder continued on basal for future observations.

I-26 Representative birds from each group killed for tissue samples. The remainder continued for future observations.

I-27 All birds killed for thyroid glands and other tissues.

I-29 All birds killed for thyroid glands and other tissues.

In the first experiment (I-25), desiccated thyroid powder was fed to four groups at the rate of 0.25, 0.50, 1.0, and 2.0 per cent respectively in the diet. Another group received 0.379 per cent iodinated casein-B. As a check against any beneficial effects due to iodine as such in the thyroactive substances, one group was given 0.0727 per cent potassium iodide. The lowest level of desiccated thyroid, the iodinated casein, and the potassium iodide each supplied approximately 500,000 p.p.b. of iodine in the diet. Experiment I-26 was designed to retest the critical diets of the previous experiment, but with S.C.W. Leghorn chicks. Only the lowest level of desiccated thyroid was included in this experiment. The chicks of one group were from dams receiving an extremely high level of iodine (180,000 p.p.b.). In order to check the possibility of deleterious effects due to a deficiency of B-vitamins or vitamin D, one group received 10 per cent dried

yeast and one-half per cent sardine oil (400-D).

Experiment I-27 was designed as a further attempt to ascertain the minimum amount of desiccated thyroid substance necessary to counteract the effects of thyroid removal. Desiccated thyroid powder was fed to four thyroidectomized groups and four control groups at 0.0, 0.0625, 0.125, 0.25, and 0.50 per cent of the diets respectively.

The last experiment involved still lower levels of desiccated thyroid, in the attempt to determine the minimum amount of desiccated thyroid necessary to counteract the effects of thyroid removal, especially on feathering. In addition, a detailed study was made on the effect of graded doses of thyroactive substances on thyroid function and liver vitamin storage. Two iodinated casein products were also tested for their thyroactive potency both with White Plymouth Rocks and with S.C.W. Leghorns.

In each experiment one group of thyroidectomized chicks and one group of control chicks were reared on basal diet for comparison.

The day-old chicks used for comparative thyroid measurements were from a different lot than those used for I-29. They were obtained at a later date and from dams receiving an adequate stock diet.

During the course of this investigation,

desiccated thyroid powder and iodinated casein were administered orally, in small capsules, to chickens suffering from range paralysis. The source of these birds was the department production stock, and most of them were apprehended, while on the range, at about four months of age.

EXPERIMENTAL RESULTS

Symbols Used

The treatments and results are shown in tables 1 to 26, figures 1 to 14, and plates I to VI. Certain symbols, adopted for the sake of brevity in reporting the results, are explained below.

UN	Unoperated control chicks. Thyroid glands intact.
OP	Operated control chicks. Thyroid glands intact.
T, or Thy.	Thyroidectomized chicks. Thyroids removed within the first three days after hatching.
Thy-2-wks.	Thyroidectomized chicks. Thyroids removed at two weeks of age.
Thy. wt.	Thyroid weight.
Regen. or Reg.	Thyroidectomized chickens in which regenerated thyroid tissue was found either by macroscopic or by microscopic observation.
Iod. Cas.	Iodinated casein preparations with thyroxine activity.
Des. Thy.	Commercial desiccated thyroid powder (USP).
KI	Potassium iodide, containing 10% calcium stearate.
S.C.W. Leghorn	Single Comb White Leghorn.
W.P. Rock	White Plymouth Rock.
M, F,	Male, Female.
Surv.	Survivors.
W	Width of the largest cross section of thyroid.
L	Length of the largest cross section of thyroid.
ΣD	Summation of the diameters of the 11 largest follicles.
B. Wt.	Body weight.
Mcg.	Micrograms. B-vitamins are expressed in micrograms.
I.U.	International units. Vitamin A is expressed in international units

Exploratory Experiments

In the first exploratory experiment, the thyroids were excised at two weeks of age. After about two months, the birds appeared normal in every respect. When examined later they all showed regenerated thyroid tissue present. The amount of thyroid tissue varied from tiny nodules to full sized glands.

In the second exploratory experiment, the thyroids were destroyed in situ, by cautery technique, on the day of hatching. Nearly all chicks survived and grew well for several weeks, but at about six weeks of age, a peculiar differentiation became apparent (plate I, numbers 1 and 2). One group appeared to be normal in every respect and developed to maturity without any apparent abnormalities. Later, upon autopsy, these birds showed various degrees of thyroid regeneration, all of which approached normal sized glands. A second group was somewhat intermediate in appearance. The body size and development of the head parts was about half that of the first group. In addition, there was an extreme hyperemic condition of the head parts. Later, upon autopsy, these birds were observed to have small nodules of regenerated thyroid tissue present. In some cases, they occurred on one side only, and in others, on both sides. Still a third group developed conditions indicative of complete thyroidectomy, i.e.,

stunted growth, apparent increased adipose deposition, protruding abdomens, and failure of development of secondary sexual characteristics. Development of body feathers was greatly retarded, but the wing and tail feathers grew considerably. When autopsy was performed later, no thyroid tissue was found by macroscopic examination.

In addition to the above observations, which agree with those of other workers, conditions typical of various avitaminoses were pronounced in the third group. The feathers of several birds had a broken and frayed appearance, characteristic of pantothenic acid deficiency. Others exhibited severe dematosis of the feet and mouth, which might be attributable to a deficiency of pantothenic acid or of biotin. One bird developed curled toe paralysis characteristic of moderate deficiency of riboflavin, but oral administration of synthetic riboflavin in solution in doses of several hundred micrograms every other day for two weeks, failed to correct this condition. A form of complicated perosis in the form of enlarged hocks and slight dislocation of the tendon was prominent. This suggested a possibility of choline, biotin, or manganese deficiency, or a combination of all three, or a disrupted calcium: phosphorus metabolism. Each deficiency symptom was not limited to a single individual but combined symptoms

PLATE I

The effect of thyroidectomy and of thyroid regeneration in Single Comb White Leghorn chickens. Thyroids removed by cautery at one day of age.

1. Exploratory experiments. Males.

   A. No thyroid tissue found on macroscopic post-mortem examination.
   B. Tiny nodules of regenerated thyroid tissue found on post-mortem examination.
   C,D. Complete regeneration of thyroid glands.

2. Exploratory experiments. Females.

   A. No thyroid tissue found on macroscopic post-mortem examination.
   B. Tiny nodules of regenerated thyroid tissue found on post-mortem examination.
   C,D. Complete regeneration of thyroid glands.

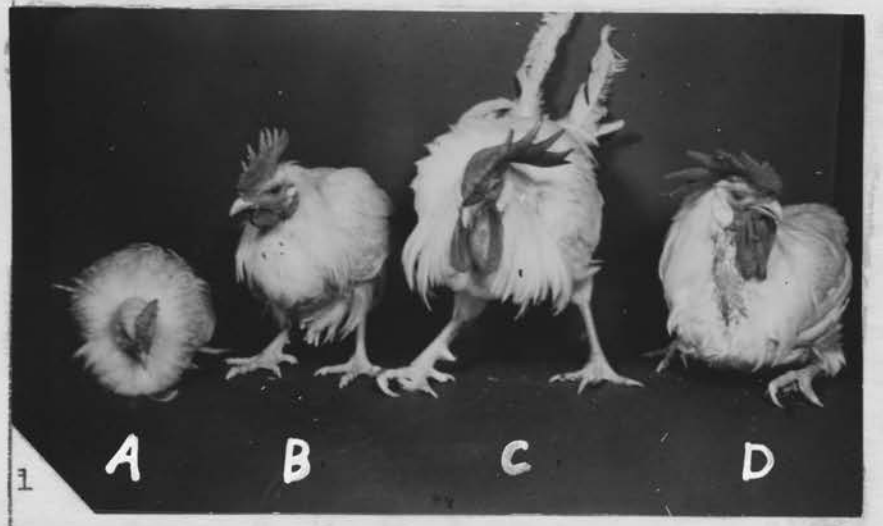
3. Experiment I-27. Females. Six weeks of age.

   A. Completely thyroidectomized chick.
   B. Control chick.

4. Experiment I-27. Males and females. Six weeks of age.

   A,B. Completely thyroidectomized.
   C,D. Partially regenerated thyroid glands.
   E,F. Control chicks.

PLATE I



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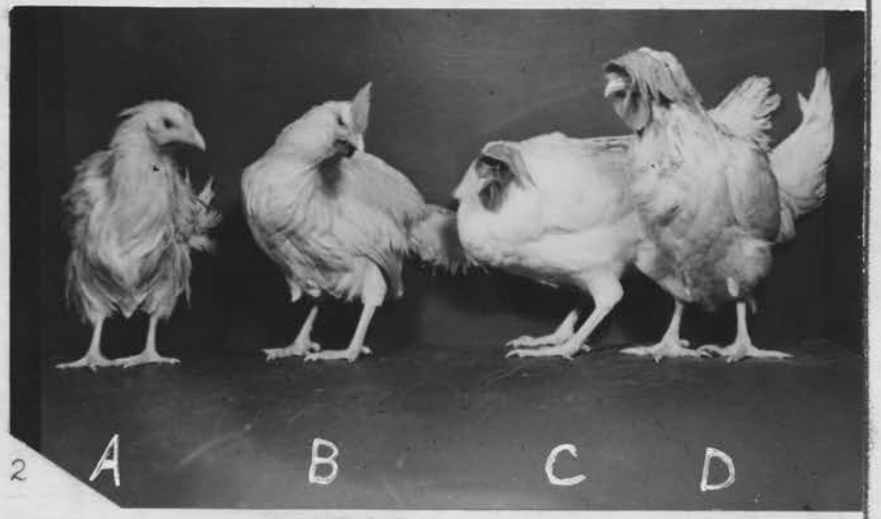

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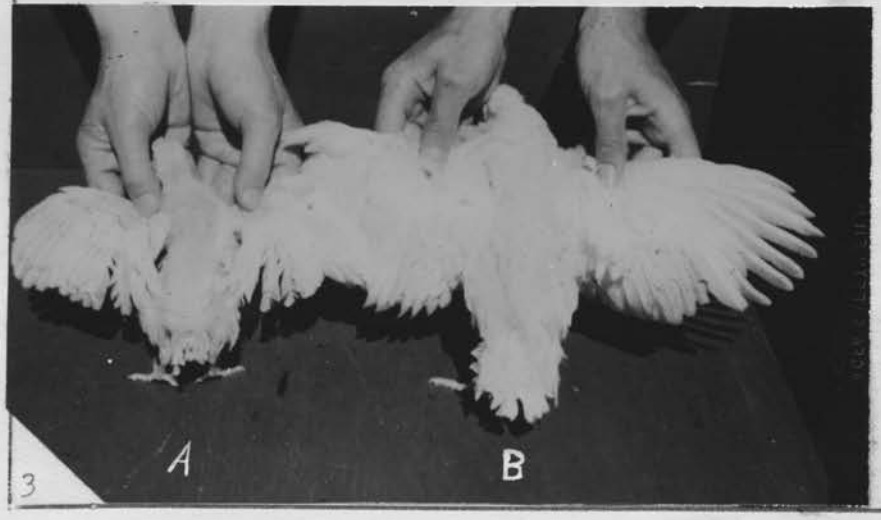

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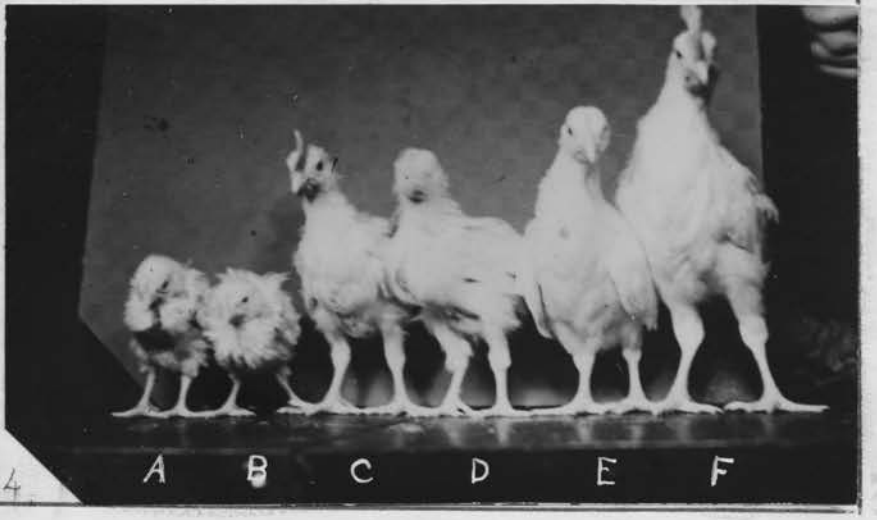

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were evident in several of the birds. The birds were eating well so the deficiencies could not be attributed to low food intake.

Body Weight

The succeeding four experiments were carefully controlled and the data tabulated. The effects of thyroid removal on external characteristics in chicks of experiment I-27 are shown in plate I, numbers 3 and 4. Similar effects were noted in experiments I-25 and I-26. The difference in size and in feather condition is quite noticeable. The size of the chicks with thyroid regeneration was intermediate between that of control and thyroidectomized chicks (Plate I, number 4).

The effects of thyroidectomy and various dietary supplements on body weight are shown in tables 1 to 6, and figures 1 and 2. Comparison of groups 2-B and 3, of table 1, and groups 1-B, 3-B, and 2 of table 2 shows that the operation in itself had no deleterious effects on either Single Comb White Leghorn or White Plymouth Rock chicks. The operated control chicks attained an even greater final weight than the unoperated chicks. Examination of weekly weights showed an initial depressing effect but this was soon overcome when the chicks began eating heartily. Following this observation, the control operation was eliminated in the succeeding experiments.

Thyroid removal definitely retarded growth (as measured by body weight) in unsupplemented groups of all experiments, but did not entirely prevent growth (tables 1, 2, 3, and 4). This agrees with observations of other workers on mammals. Thyroid removal at two weeks of age also had a depressing effect on body weight, but the greater initial weight at the time of thyroidectomy was evident in the greater final weight. The influence of regenerated thyroid tissue was reflected in increased body weight of such chicks over that of thyroidectomized chicks. In all instances, the final weight of chicks with regenerated thyroid tissue was intermediate between that of controls and that of thyroidectomized chicks.

Addition of a large dose of B-vitamins in the form of yeast, and of vitamin D in the form of sardine oil, had no protective action against thyroidectomy effects, nor did these supplements improve the weight of controls (table 2). This may be regarded as evidence that the basal diet used was adequate in respect to these vitamins.

Administration of extremely high levels of iodine to the dams did not protect their offspring from the effects of thyroid removal, nor did it affect the weight of the offspring used as controls (table 2).

Potassium iodide appeared to have a slight

protective action against the effects of thyroidectomy on body weight in experiment I-25 (table 1), but this was not born out in experiment I-26 (table 2). On the other hand, the inhibitory effect on the growth and general characteristics of controls was consistent for both experiments (tables 1 and 2).

Thyroactive iodinated casein consistently protected against the deleterious effects of thyroidectomy in both Single Comb White Leghorn and White Plymouth Rock chicks (tables 1, 2, and 5). Both iodinated casein and potassium iodide, in the amounts used, supplied 500,000 parts per billion of iodine in the diet in experiments I-25 and I-26. Therefore, the failure of potassium iodide to protect the thyroidectomized chicks against the deleterious effects of thyroidectomy may be regarded as evidence that the protective action of thyroactive substances is dependent upon their thyroxine content and not upon their iodine content.

Iodinated casein-B, which was reputed to have approximately the same thyroxine activity as desiccated thyroid powder, did not affect the body weight of controls in experiments I-25 and I-26 (tables 1 and 2), whereas a lower level of desiccated thyroid resulted in lower body weight in both experiments (tables 1 and 2). This indicates that either the iodinated casein did not

have as high a thyroxine content as was thought, or more of the thyroxine activity was lost via the digestive tract than was the case with the desiccated thyroid. In the last experiment, this product appeared to have a slight stimulatory action but the difference was not great.

The highest level of iodinated casein-A, used in the last experiment, resulted in definitely lower body weight in the female Single Comb White Leghorn chicks, but apparently higher weights in males (table 5). Since there was only one male survivor in the group receiving the lower level, this result should not be emphasized. Gradual increase in the percentage of iodinated casein-A in the diet of normal White Plymouth Rock chicks caused, at first, an increase in body weight. This was followed by a gradual decrease in such weights (table 6).

Desiccated thyroid at the level of 0.25 per cent in the diet partially protected against the effects of thyroid removal in experiment I-25 (table 1). Body weight of control chicks receiving desiccated thyroid was lower than the body weight of chicks in the basal group. In the next experiment (I-26), the same amount in the diet of Single Comb White Leghorn chicks (0.25 per cent) restored body weight of thyroidectomized chicks to normal, but the body weight of

Table 1.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON BODY WEIGHT.
Experiment I-25. White Plymouth Rock Chicks

Supplement	None				KI		Iod Cas -B		Des Thy	
Per Cent in Diet					0.0727		0.379		0.25	
Chick Group No.	1	2-A	2-B	3	4-A	4-B	5-A	5-B	6-A	6-B
Operation	Thy 2-Wks	Thy	Op	Un	Thy	Op	Thy	Op	Thy	Op
Number Males (Survivors)	3	3	4	6	2	4	0	4	2	1
Number Females (Survivors)	5	3	2	8	2	2	3	2	2	2
Beginning Weight (Survivors)*	-	51	47	48	46	46	48	46	44	46
Final Weight, Males	437	358	604	554	425	461	-	544	465	540
Final Weight, Females	399	393	602	532	385	445	480	545	368	435
Av. Final Weight (M & F)*	418	376	603	543	405	453	480	545	416	488

*Average for males plus average for females, divided by 2.

Table 2.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON BODY WEIGHT.
Experiment I-26. S.C.W. Leghorn Chicks

Supplement Per Cent in Diet	None						Yeast-10% S.Oil-0.5%		KI 0.0727		Iod Cas-B 0.379		Des Thy 0.25	
Chick Group No.	1-A	1-R	1-B	2	3-A*	3-B*	4-A	4-B	5-A	5-B	6-A	6-B	7-A	7-B
Operation	Thy	Reg	Op	Un	Thy	Op	Thy	Op	Thy	Op	Thy	Op	Thy	Op
No. Males (Surv)	7	4	10	11	1	2	2	3	2	3	1	5	1	3
No. Females (Surv)	5	3	5	4	3	3	3	5	3	5	4	3	3	4
Begin. Wt. (Surv)**	48	48	50	58	50	49	46	48	47	49	44	51	46	46
Final Wt. Males	282	333	408	400	270	428	263	379	213	361	272	404	420	346
Final Wt. Females	256	316	345	346	235	312	206	317	272	325	298	336	310	333
Av. Final Wt.**	269	324	387	371	253	370	234	348	243	343	285	370	365	340

*Chicks from dams receiving high iodine level.
**Average for males plus average for females, divided by 2.

Table 3.--EFFECT OF DIETARY DESICCATED THYROID ON BODY WEIGHT.
Experiment I-27. S.C.W. Leghorn Chicks

Supplement	None			Desiccated Thyroid Powder							
Per Cent in Diet				0.0625		0.125		0.25		0.50	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Number Males (Survivors)	4	1	5	6	5	2	5	2	4	3	1
Number Females (Survivors)	2	2	5	4	4	1	5	3	6	1	5
Beginning Weight (Survivors)	48	41	49	42	52	39	46	44	46	39	46
Final Weight Males	180	285	384	314	344	295	334	323	341	272	255
Final Weight Females	192	281	300	274	310	205	283	302	307	272	291
Average Final Weight (M & F)*	186	283	342	294	327	250	309	313	324	249	273

*Average for males plus average for females, divided by 2.

Figure 1
THE EFFECT OF DESICCATED THYROID FEEDING ON BODY WEIGHT
Experiment I-27
S.C.W. Leghorn Chicks

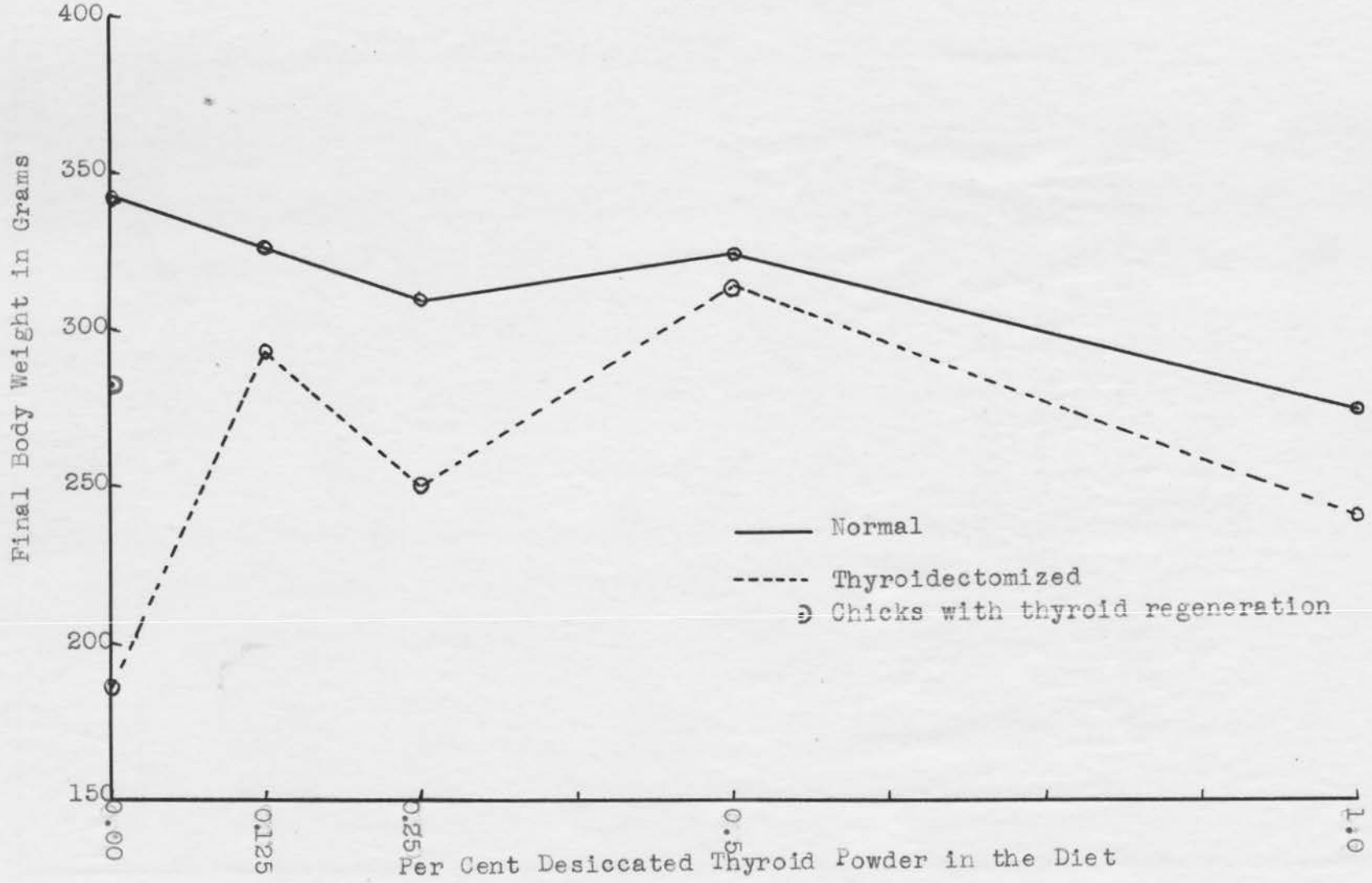


Table 4.--EFFECT OF DIETARY DESICCATED THYROID ON BODY WEIGHT.
Experiment I-29. S.C.W. Leghorn Chicks

Supplement	None			Desiccated Thyroid Powder								
Per Cent in Diet				0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
No. Males (Survivors)	1	2	3	3	3	4	3	3	2	2	2	1	2
No. Females (Survivors)	4	1	4	3	4	2	4	2	4	5	3	2	2
Begin. Wt. (Survivors)*	45	54	49	52	51	46	53	51	56	50	52	54	60
Final Wt. Males	320	315	400	377	443	353	345	308	427	318	415	270	248
Final Wt. Females	259	300	391	373	381	365	388	238	390	265	315	248	260
Average Final Wt. (M&F)*	289	308	396	375	412	359	366	273	409	292	365	259	254

*Average for males plus average for females, divided by 2.

Figure 2.--EFFECT OF DESICCATED THYROID FEEDING ON BODY WEIGHT
Experiment I-29. S.C.W. Leghorn Chicks

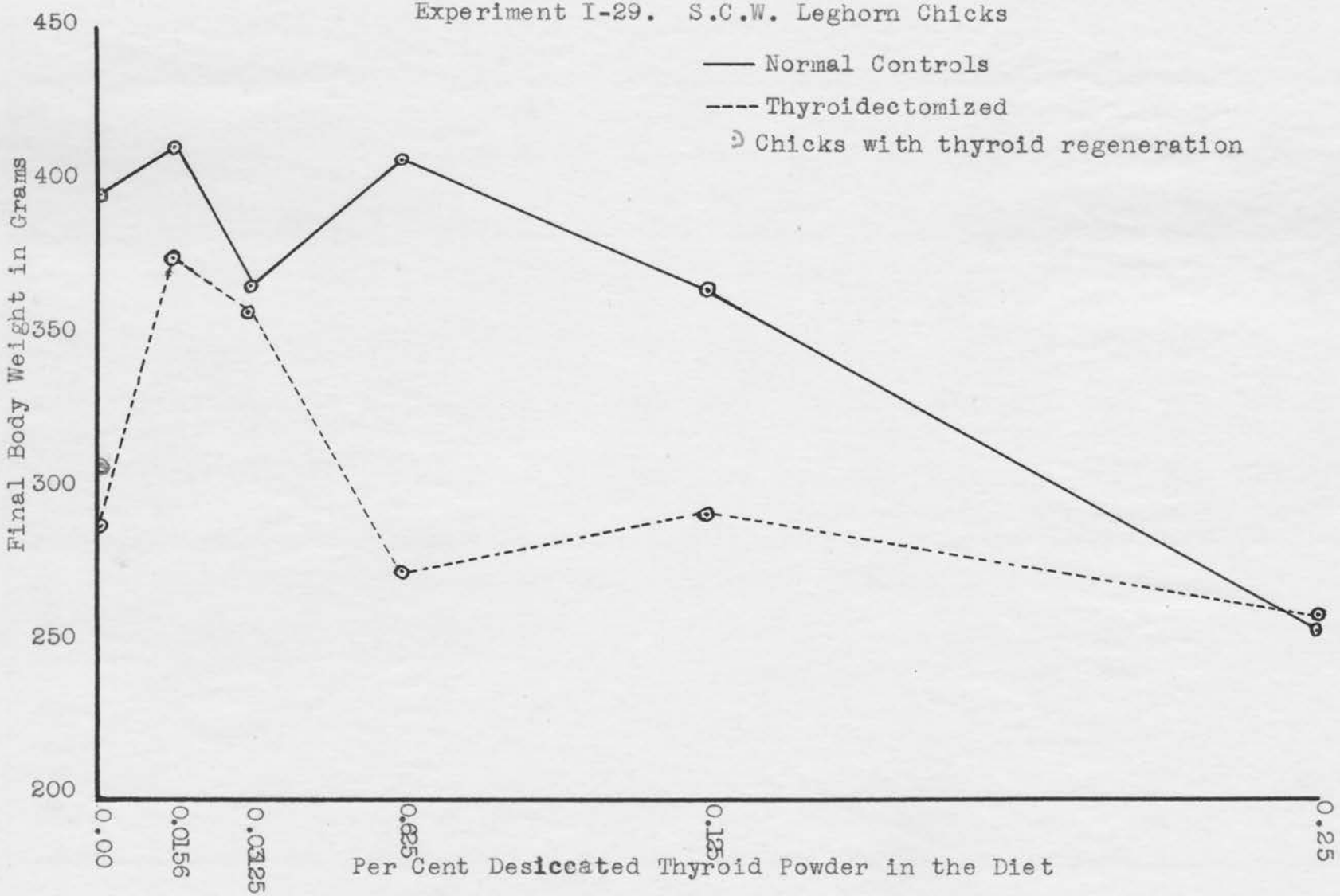


Table 5.--EFFECT OF DIETARY IODINATED CASEIN ON BODY WEIGHT.
Experiment I-29. S.C.W. Leghorn Chicks

Supplement	None		Iod. Cas. - A				Iod. Cas. - B			
Per Cent in Diet			0.008		0.016		0.04		0.08	
Chick Group No.	1-T	2	13	14	15	16	17	18	19	20
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
No. Males (Survivors)	1	3	5	1	2	4	2	4	4	4
No. Females (Survivors)	4	4	1	6	3	3	3	3	2	3
Beginning Weight (Survivors)*	45	49	48	57	52	53	53	56	53	58
Final Weight, Males	320	400	406	400	440	440	343	416	365	422
Final Weight, Females	259	391	345	383	345	308	322	340	310	398
Average Final Weight (M & F)*	289	396	376	392	393	374	333	378	338	410

*Average for males plus average for females, divided by 2.

Table 6.--EFFECT OF DIETARY IODINATED CASEIN ON BODY WEIGHT.
Experiment I-29. White Plymouth Rock Chicks (Normal)

Supplement	None	Iod. Cas. - A		
Per Cent in Diet		0.005	0.05	0.25
Chick Group No.	21	22	23	24
Number Males (Survivors)	5	3	1	3
Number Females (Survivors)	5	8	5	2
Beginning Weight (Survivors)*	52	53	52	53
Final Weight, Males	437	498	350	410
Final Weight, Females	404	416	474	365
Average Final Weight (Males and Females)*	421	457	427	388

*Average for males plus average for females, divided by 2.

control chicks was lower than that of normal chicks receiving the basal diet.

The effects of graded doses of desiccated thyroid on body weight are shown in tables 3 and 4, and figures 1 and 2. Successive increases in desiccated thyroid greater than 0.0625 per cent in the diet resulted in successively lower body weight of control chicks. The average body weight value for the group receiving 0.03125 per cent of desiccated thyroid was low, due to the extremely low weight values for the three surviving males of the group.

Efficiency of Gain

The effects of the feeding of desiccated thyroid and iodinated casein on efficiency of gain are presented in table 7 and figures 3 and 4. In experiment I-27, the unsupplemented thyroidectomized chicks showed a marked reduction in efficiency of gain, but in the next experiment the thyroidectomized group showed slightly more efficiency than the controls. The effect of progressive increases in desiccated thyroid supplementation on efficiency of gain may be readily analyzed by examination of figure 3. The data are conflicting in regard to the actual amounts involved, but in general, a small amount of desiccated thyroid powder in the diet increased efficiency, and larger amounts resulted in progressive decreases in efficiency

Table 7.--EFFECT OF DIETARY THYROACTIVE SUBSTANCES ON EFFICIENCY OF GAIN.
Experiments I-27, I-29. S.C.W. Leghorn Chicks

Experiment I-27. Desiccated Thyroid Feeding

Supplement	None		Desiccated Thyroid Powder							
Per Cent in Diet			0.0625		0.125		0.25		0.50	
Chick Group No.	1-T,R	2	3	4	5	6	7	8	9	10
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Efficiency of Gain	.26	.34	.34	.34	.31	.37	.26	.38	.16	.24

Experiment I-29. Desiccated Thyroid Feeding

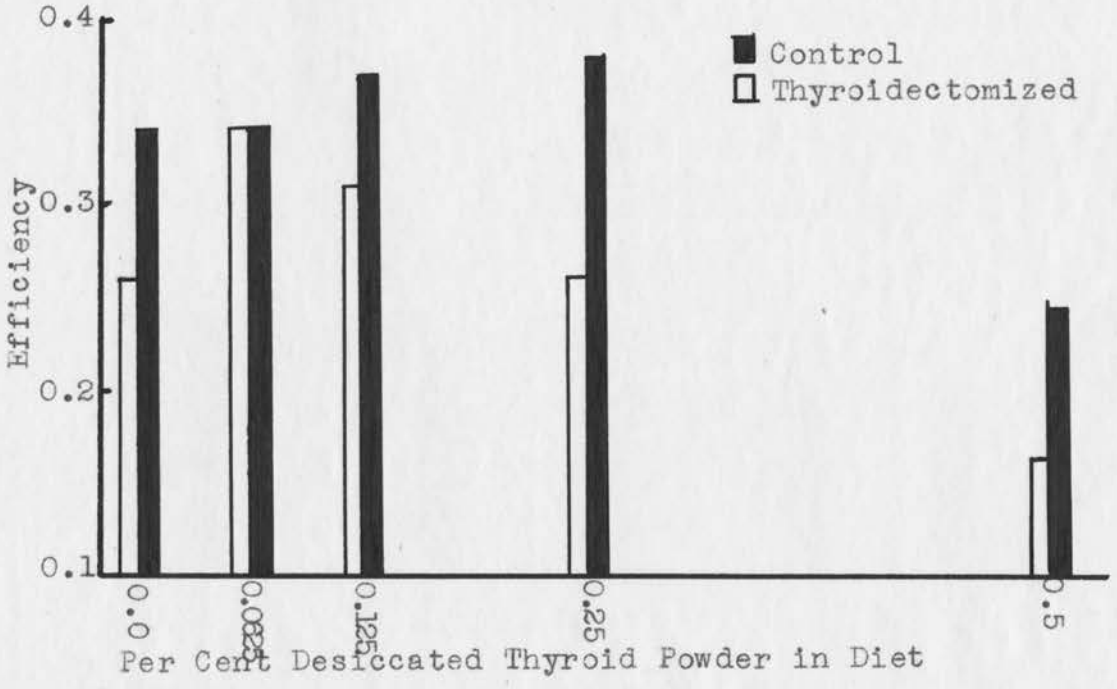
Supplement	None		Desiccated Thyroid Powder									
Per Cent in Diet			0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T,R	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Efficiency of Gain	.34	.33	.34	.35	.36	.33	.33	.33	.31	.28	.24	.23

Iodinated Casein Feeding

Supplement	Iodinated Casein-A				Iodinated Casein-B				None	Iod. - Cas. - A		
Per Cent in Diet	0.008		0.016		0.04		0.08			0.005	0.05	0.25
Chick Group No.	13	14	15	16	17	18	19	20	21*	22*	23*	24*
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Un	Un	Un	Un
Efficiency of Gain	.34	.32	.34	.33	.35	.33	.32	.30	.35	.36	.34	.28

*White Plymouth Rock Chicks

Figure 3.--EFFECT OF DESICCATED THYROID FEEDING ON EFFICIENCY OF GAIN. Experiment I-27. S.C.W. Leghorn Chicks



Experiment I-29

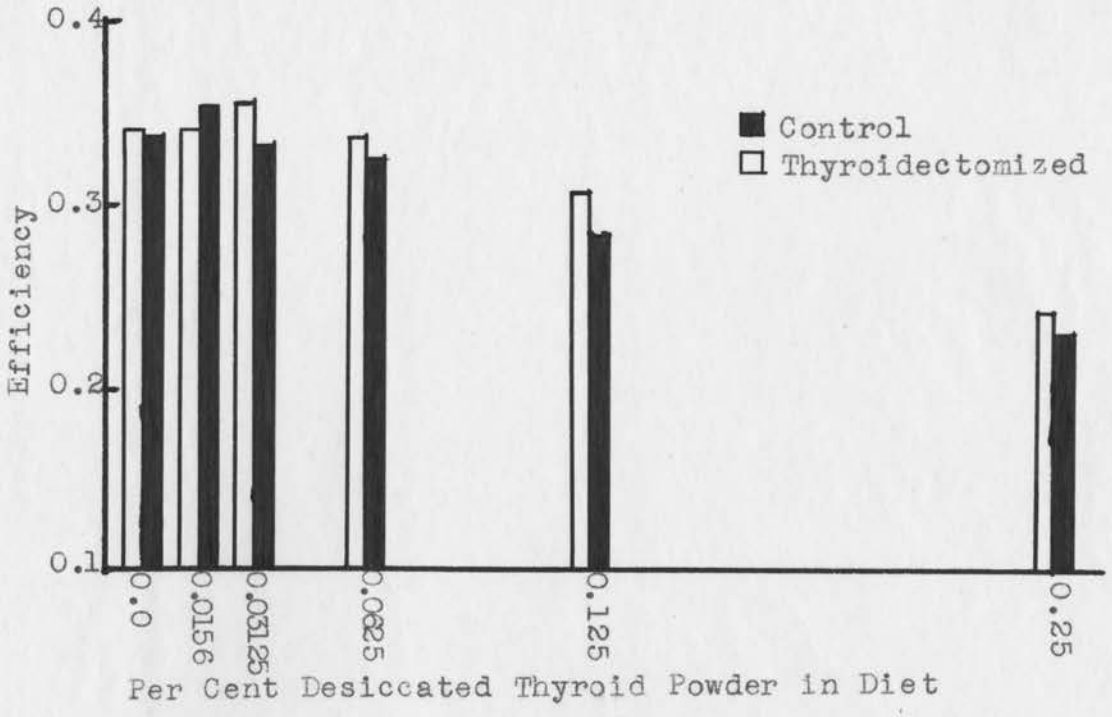
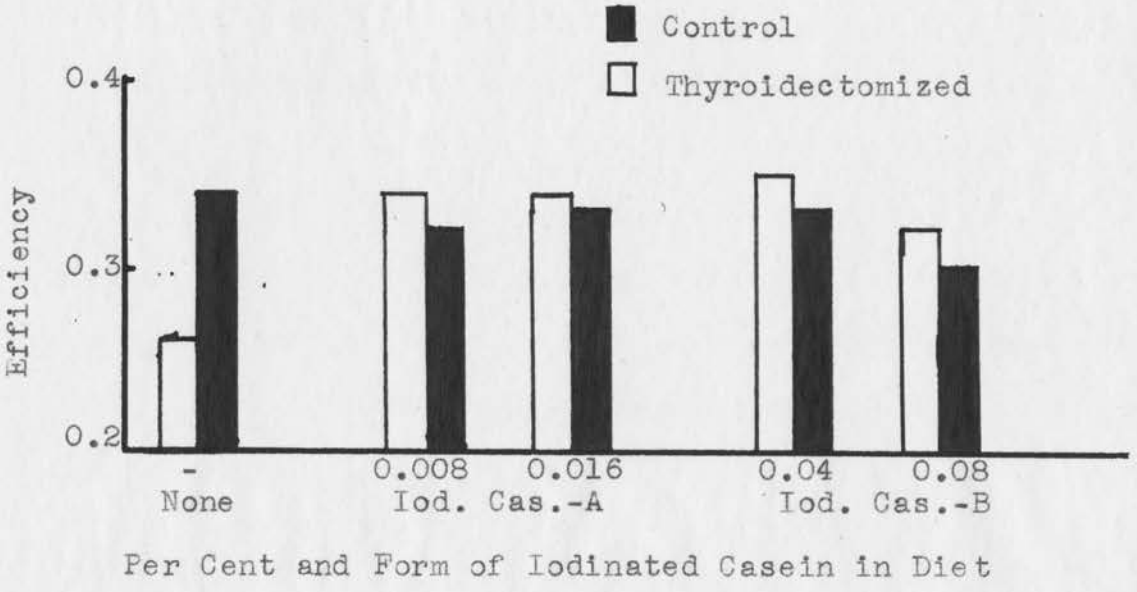
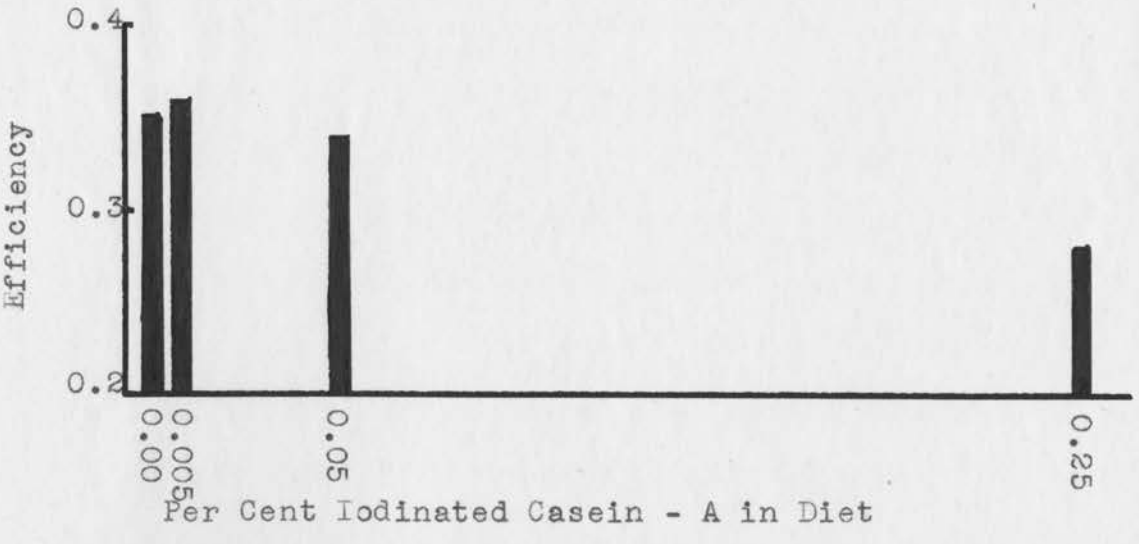


Figure 4.--EFFECT OF IODINATED CASEIN ON EFFICIENCY OF GAIN. Experiment I-29. S.C.W. Leghorn Chicks



Experiment I-29. White Plymouth Rock Chicks



with each successive addition to the diet. Iodinated casein showed little effect on the efficiency of gain in the Single Comb White Leghorn chicks, but the tendency was for the thyroidectomized chicks to be more efficient than the controls. The feeding of high levels of iodinated casein-B resulted in lower efficiency of gain.

The efficiency of gain for normal White Plymouth Rock chicks (figure 4) was increased by the smallest addition of iodinated casein-A, and progressively decreased with each successive addition to the diet.

Feather Condition

The effects of dietary supplements on feather condition are presented in tables 8 to 11. Thyroid removal almost completely inhibited feather growth in White Plymouth Rock chicks over the first eight weeks of age (table 8). Thyroidectomy at two weeks of age caused cessation of feather growth in the pinfeather stage. This is evident from the higher pinniness score. Both iodinated casein and desiccated thyroid improved the feather condition over that of the basal control chicks. On the other hand, potassium iodide inhibited feather development. Daily counts of feathers on the dropping pans showed that the rate of moult was very much higher for the chicks receiving the

higher levels of desiccated thyroid, than for the chicks on any of the other diets.

The data for the Single Comb White Leghorn chicks of experiment I-26 agree with the results of the previous experiment (table 9). Potassium iodide did not prevent the appearance of feathers in thyroidectomized chicks, but inhibited development, causing an unfavorable pinniness condition. Yeast and sardine oil had the same effect.

Paralleling the weight data, the feather condition of thyroidectomized chicks with regenerated thyroid tissue was in each case intermediate between that of the thyroidectomized and control chicks (tables 9 and 10).

The results for the thyroidectomized chicks receiving graded doses of desiccated thyroid are somewhat erratic, but the lowest level of desiccated thyroid used in these experiments (0.0156 per cent) caused an almost perfect feather condition (table 10).

The effect of graded amounts of iodinated casein on feather condition is shown in table 11. The Single Comb White Leghorn chicks showed nearly perfect feather condition. The degree of feathering in the White Plymouth Rock chicks was roughly proportional to the amount of iodinated casein fed. Optimum feather condition was produced by 0.05 per cent of this product in the diet.

Table 8.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON FEATHER CONDITION.
Experiment I-25. White Plymouth Rock Chicks

Supplement	None				KI		Iod Cas-B		Des Thy	
Per Cent in Diet					0.0727		0.379		0.25	
Chick Group No.	1	2-A	2-B	3	4-A	4-B	5-A	5-B	6-A	6-B
Operation	Thy 2 Wks	Thy	Op	Un	Thy	Op	Thy	Op	Thy	Op
Number males	3	3	4	6	2	4	0	4	2	1
Number females	5	3	2	8	2	2	3	2	2	2
Feather Coverage Score										
Males	23	30	21	19	28	24	-	13	8	0
Females	17	23	0	9	25	20	3	3	10	0
Average (Males and Females)	20	26	10	14	26	22	3	8	9	0
Feather Pinniness Score										
Males	27	3	16	8	25	20	0	14	3	0
Females	22	8	0	4	20	18	5	5	8	5
Average (Males and Females)	24	5	8	6	22	19	5	9	5	2

Table 9.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON FEATHER CONDITION.
Experiment I-26. S.C.W. Leghorn Chicks

Supplement	None						Yeast-10% S.Oil-0.5%		KI 0.0727		Iod Cas-B 0.379		Des Thy 0.25	
Per Cent in Diet														
Chick Group No.	1-A	1-R	1-B	2	3-A*	3-B*	4-A	4-B	5-A	5-B	6-A	6-B	7-A	7-B
Operation	Thy	Reg	Op	Un	Thy	Op	Thy	Op	Thy	Op	Thy	Op	Thy	Op
Number Males	7	4	10	11	1	2	2	3	2	3	1	5	1	3
Number Females	5	3	5	4	3	3	3	5	3	5	4	3	3	4
Feather Coverage Score														
Males	23	14	5	0	0	5	3	0	3	0	10	0	0	0
Females	22	13	0	0	5	0	2	2	0	0	1	0	0	0
Av.(M&F)	23	14	3	0	3	2	2	1	1	0	5	0	0	0
Feather Pinniness Score														
Males	25	14	7	0	25	0	30	3	25	2	25	0	0	0
Females	22	13	2	0	22	0	30	2	20	2	4	4	0	0
Av. (M&F)	24	14	4	0	23	0	30	3	23	2	14	2	0	0

Yeast - Dried yeast
S.Oil - Sardine Oil. 400-D
*Chicks from dams receiving high iodine level

Table 10.--EFFECT OF DIETARY DESICCATED THYROID ON FEATHER CONDITION.
Experiments I-27, I-29. S.C.W. Leghorn Chicks

Experiment I-27

Supplement	None			Desiccated Thyroid Powder							
Per Cent in Diet				0.0625		0.125		0.25		0.50	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Number Males	4	1	5	6	5	2	5	2	4	3	1
Number Females	2	2	5	4	4	1	5	3	6	1	5
Feather Coverage Score											
Males	30	5	0	3	0	5	1	13	0	7	5
Females	30	10	0	0	0	10	0	2	5	5	2
Average (Males and Females)	30	7	0	1	0	7	0	7	2	6	3

Experiment I-29

Supplement	None			Desiccated Thyroid Powder									
Per Cent in Diet				0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Number Males	1	2	3	3	3	4	3	3	2	2	2	1	2
Number Females	4	1	4	3	4	2	4	2	4	5	3	2	2
Feather Coverage Score													
Males	15	10	0	5	0	7	2	10	0	7	0	0	8
Females	19	15	4	7	0	5	0	15	1	10	2	10	2
Average (M&F)	17	12	2	6	0	6	1	12	1	8	1	5	5

Table 11.--EFFECT OF DIETARY IODINATED CASEIN ON FEATHER CONDITION.
Experiment I-29. S.C.W. Leghorn and White Plymouth Rock Chicks

S.C.W. Leghorn Chicks

Supplement	Iodinated Casein - A				Iodinated Casein - B			
Per Cent in Diet	0.008		0.016		0.04		0.08	
Chick Group No.	13	14	15	16	17	18	19	20
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Number Males	5	1	2	4	2	4	4	4
Number Females	1	6	3	3	3	3	2	3
Feather Coverage Score								
Males	8	-	5	4	15	1	9	6
Females	5	-	7	7	12	3	5	2
Average (Males and Females)	6	1*	6	6	13	2	7	4

White Plymouth Rock Chicks

Supplement	None	Iod Cas-A		
Per Cent in Diet		0.005	0.05	0.25
Chick Group No.	21	22	23	24
Operation	Un	Un	Un	Un
Number Males	5	3	1	3
Number Females	5	8	5	2
Feather Coverage Score				
Males	30	17	10	22
Females	25	21	8	5
Average (Males and Females)	28	19	9	13

*Not weighted for sex

PLATE II

The effect of dietary thyroactive iodinated casein on feather condition in normal White Plymouth Rock chicks.

Experiment I-29. The chicks were six weeks of age, and had received the experimental diets from hatching time.

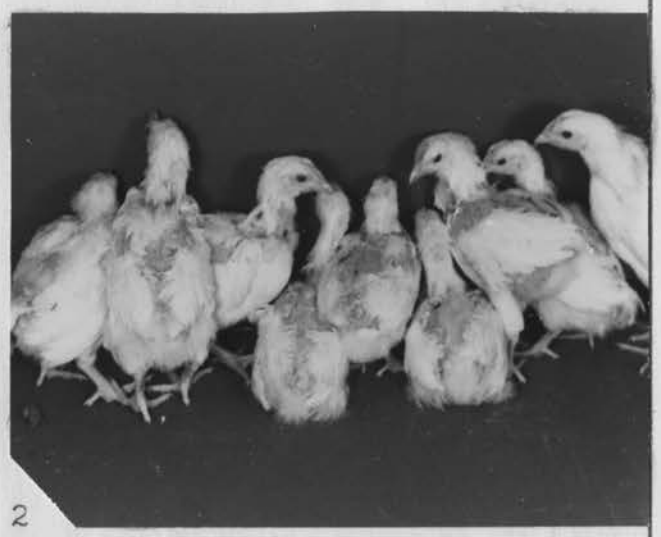
1 and 2. Group 21. Basal. No thyroactive casein in the diet.

3 and 4. Group 22. 0.005 per cent of thyroactive iodinated casein-A in the diet.

5 and 6. Group 23. 0.05 per cent of thyroactive iodinated casein-A in the diet.

PLATE II

1



2

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PLATE III

The effect of thyroidectomy and of dietary desiccated thyroid powder on feather condition in Single Comb White Leghorn chicks.

Experiment I-29. The chicks were six weeks of age and had received the experimental diets from hatching time.

1 and 2. Group 1. Thyroidectomized. Basal diet.

3 and 4. Group 3. Thyroidectomized. 0.0156 per cent desiccated thyroid in the diet.

5 and 6. Group 2. Control chicks. Basal diet.

PLATE III


1


2


3


4


5


6

The effects of thyroidectomy and of desiccated thyroid on feather condition of Leghorn chicks and of iodinated casein-A on feather condition in Plymouth Rock chicks is illustrated in plates II and III.

Perosis and Dermatosis

Table 12 shows the occurrence of perosis and dermatosis. In the first experiment both conditions occurred in thyroidectomized chicks and chicks receiving potassium iodide. Dermatosis did not appear in succeeding experiments.

In experiment I-26 perosis occurred in all thyroidectomized groups, but was not severe in those groups receiving iodinated casein or desiccated thyroid. Yeast and sardine oil, or potassium iodide did not protect the chicks against this condition. Chicks from dams receiving high levels of iodine also showed dermatosis. Perosis did not occur in the last experiment.

Thyroidectomized chicks showing thyroid regeneration were intermediate between thyroidectomized and control chicks in regard to incidence and degree of perosis. This agrees with the data for body weight and feather condition.

Mortality

Mortality in the first experiment was rather high for all groups, and especially high in the groups

Table 12.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON PEROSIS AND DERMATOSIS.
Experiments I-25, I-26, I-27. S.C.W. Leghorn and White Plymouth Rock Chicks

Experiment I-25*

Supplement	None				KI		Iod Cas-B		Des Thy	
Per Cent in Diet					0.0727		0.379		0.25	
Chick Group No.	1	2-A	2-B	3	4-A	4-B	5-A	5-B	6-A	6-B
Operation	Thy 2 Wks	Thy	Op	Un	Thy	Op	Thy	Op	Thy	Op
Survivors (Males and Females)	8	6	6	14	4	6	3	6	4	3
Number Showing Perosis	8	6	0	0	4	1	0	1	0	0
Number Showing Dermatosis	5	2	0	0	3	3	0	0	0	0

Experiment I-26**

Supplement	None						Yeast-10% S.Oil-0.5%		KI 0.0727		Iod Cas-B 0.379		Des Thy 0.25	
Per Cent in Diet														
Chick Group No.	1-A	1-R	1-B	2	3-A	3-B	4-A	4-B	5-A	5-B	6-A	6-B	7-A	7-B
Operation	Thy	Reg	Op	Un	Thy	Op	Thy	Op	Thy	Op	Thy	Op	Thy	Op
Survivors (M&F)	12	7	15	15	4	5	5	8	5	8	5	8	4	7
No. Show. Perosis	12	5	0	2	4	0	5	1	5	2	2	0	1	0
Av.Degree of Per.	10	5	0	1	10	0	14	1	11	1	2	0	1	0

Experiment I-27**

Supplement	None			Desiccated Thyroid Powder							
Per Cent in Diet				0.0625		0.125		0.25		0.50	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Survivors (Males and Females)	6	3	10	10	9	3	10	5	10	4	6
Number Showing Perosis	6	1	0	4	0	2	0	1	0	0	0
Average Degree of Perosis	11	2	0	4	0	5	0	2	0	0	0

*White Plymouth Rock Chicks
**Single Comb White Leghorn Chicks

Table 13.--EFFECT OF VARIOUS DIETARY SUPPLEMENTS ON MORTALITY.
Experiment I-25. White Plymouth Rock Chicks

Supplement	None				KI 0.0727		Iod Cas-B 0.379	
Per Cent in Diet								
Chick Group No.	1	2-A	2-B	3	4-A	4-B	5-A	5-B
Operation	Thy 2 Wks	Thy	Op	Un	Thy	Op	Thy	Op
No. Chicks - Start	9	8	8	16	8	8	8	9
No. Dead - End of 6 weeks	1	2	2	3	3	2	5	3
Per Cent Mortality*	10	25	25	20	40	25	65	35

Supplement	Desiccated Thyroid Powder									
Per Cent in Diet	0.25		0.50		1.00				2.00	
Chick Group No.	6-A	6-B	7-A	7-B	8-A	8-B	8-C**	8-D**	9-A	9-B
Operation	Thy	Op	Thy	Op	Thy	Op	Thy 2 Wks	Op 2 Wks	Thy	Op
No. Chicks - Start	10	9	8	8	8	10	3	3	8	9
No. Died 1-3 days	1	2	1	2	1	7	-	-	4	6
No. Died 3-7 days	0	3	1	3	5	0	-	-	1	3
No. Died 2nd week	1	0	4	0	1	1	-	-	2	0
No. Died 3rd week	3	1	0	0	1	0	1	3	1	0
No. Dead - End of 6 weeks	5	6	6	5	8	9	2	3	8	9
Per Cent Mortality*	50	65	75	65	100	90	65	100	100	100

*Calculated to the nearest 5 per cent
**Thyroidectomized and operated and started on the diet at 2 weeks of age

Table 14.--EFFECT OF DIETARY DESICCATED THYROID ON MORTALITY.
Experiments I-27, I-29. S.C.W. Leghorn Chicks

Experiment I-27

Supplement	None		Desiccated Thyroid Powder							
Per Cent in Diet			0.0625		0.125		0.25		0.50	
Chick Group No.	1-T,R	2	3	4	5	6	7	8	9	10
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
No. at Start	11	10	10	10	8	10	10	10	9	10
No. Survivors	9	10	10	9	8	10	5	10	4	6
Per Cent Mortality*	20	0	0	10	0	0	50	0	55	25

Experiment I-29

Supplement	None		Desiccated Thyroid Powder									
Per Cent in Diet			0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T,R	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
No. at Start	9	7	9	7	10	7	9	7	9	7	9	7
No. Survivors	8	7	8	7	9	7	9	6	7	5	4	4
Per Cent Mortality*	10	0	10	0	10	0	0	15	20	30	55	45

*Calculated to the nearest 5 Per Cent

Table 15.--EFFECT OF DIETARY IODINATED CASEIN ON MORTALITY.
Experiment I-29. S.C.W. Leghorn and White Plymouth Rock Chicks

S.C.W. Leghorn Chicks

Supplement	Iodinated Casein - A				Iodinated Casein - B			
Per Cent in Diet	0.008		0.016		0.04		0.08	
Chick Group No.	13	14	15	16	17	18	19	20
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un
No. at Start	9	7	10	7	9	7	8	7
No. Survivors	7	7	5	7	8	7	8	7
Per Cent Mortality*	20	0	50	0	10	0	0	0

White Plymouth Rock Chicks

Supplement	None		Iodinated Casein - A					
Per Cent in Diet			0.005		0.05		0.25	
Chick Group No.	21		22		23		24	
Operation	Un		Un		Un		Un	
Sex	Male	Female	Male	Female	Male	Female	Male	Female
No. at Start	6	6	5	8	6	6	9	5
No. Survivors	5	5	3	8	1	5	3	2
Per Cent Mortality - Sex*	15	15	40	0	85	15	65	60
Per Cent Mortality - Diet*	8		15		50		65	

*Calculated to the nearest 5 per cent

which received the three highest levels of desiccated thyroid powder (table 13). Chicks receiving the toxic diets died within a few days after starting the experiment. The control chicks were more susceptible than thyroidectomized chicks, and the males more susceptible than the females.

The mortality of experiment I-26 was negligible. One died in each of groups 1-A, 3-A, and 5-A.

In the last two experiments, desiccated thyroid was slightly more toxic to thyroidectomized chicks than to the control chicks (table 14). In experiment I-27, 0.25 per cent desiccated thyroid in the diet was not lethal to control chicks, but in the last experiment, 0.0625 per cent proved lethal. The tendency was for mortality to increase with increased dosage of desiccated thyroid. This was also true with iodinated casein-A (table 15).

Shank Length

Analysis of shank length data for Leghorn chicks shows that average shank length was progressively shorter with progressive increases in amount of desiccated thyroid fed (table 16).

A small amount of iodinated casein-A resulted in a slight increase in shank length over that of controls in White Plymouth Rock chicks. This was followed by progressively shorter shank lengths when the amount

Table 16.--EFFECT OF DIETARY DESICCATED THYROID AND IODINATED CASEIN ON SHANK LENGTH. Experiment I-29. S.C.W. Leghorn and White Plymouth Rock Chicks

S.C.W. Leghorn Chicks							
Supplement		None	Desiccated Thyroid Powder				
Per Cent in Diet			0.0156	0.03125	0.0625	0.125	0.25
Unoperated Chicks							
Chick Group No.		2	4	6	8	10	12
Shank Length*		40.1	41.7	39.5	40.6	39.0	36.7
Body Wt. (Gms)/Shank Length		9.9	9.9	9.3	9.4	9.3	6.9
Thyroidectomized Chicks							
Chick Group No.	1-T	1-R	3	5	7	9	11
Shank Length*	34.0	37.4	39.1	39.4	34.1	34.6	35.9
Body Wt. (Gms)/ Shank Length	8.5	8.2	9.5	9.1	7.7	8.4	7.1

Unoperated White Plymouth Rock Chicks				
Supplement	None	Iodinated Casein - A		
Per Cent in Diet		0.005	0.05	0.25
Chick Group No.	21	22	23	24
Shank Length*	41.8	42.7	42.0	42.1
Body Wt. (Gms)/Shank Length	10.0	10.6	9.8	9.2

* Shank length in 16th of an inch. Reported as averages of males and females.

in the diet was progressively increased.

Body Fat Content

The results of the fat analyses are presented in table 17 and figures 5 and 6. The two lowest levels of desiccated thyroid in the diet in experiment I-29 resulted in increased adipose storage. Progressive increases in desiccated thyroid beyond the second addition to the diet resulted in progressive decreases in fat content of the carcass (figure 5). A similar effect was observed for iodinated casein-A (figure 6). The percentage moisture in the stored carcass samples was quite constant. The lowest value was 28.9 per cent and the highest value was 31.1 per cent. There was no definite trend noticed.

Liver Weight

Analysis of liver weights, either on the dry basis or when related to body weight, failed to show any consistent difference between groups.

Body Temperature

The body temperatures of chicks of experiment I-25 did not differ between groups.

Vitamin Content of Livers

Table 19 and figure 7 show the effect of desiccated thyroid feeding on liver vitamin A storage.

Table 17.--EFFECT OF DIETARY DESICCATED THYROID AND IODINATED CASEIN ON FAT CONTENT OF CARCASS. Experiment I-29. S.C.W. Leghorn and White Plymouth Rock Chicks

S.C.W. Leghorn Chicks

Supplement	None	Desiccated Thyroid Powder				Iodinated Casein - A	
Per Cent in Diet		0.0156	0.03125	0.0625	0.125	0.25	0.008	0.016
Unoperated Chicks								
Chick Group No.	2	4	6	8	10	12	14	16
Per Cent Fat*	22.2	23.5	23.5	21.1	16.7	14.2	24.6	19.2
Thyroidectomized Chicks								
Chick Group No.	1	3	5	7	9	11	13	15
Per Cent Fat*	-**	-**	-**	18.1	16.4	11.4	23.3	25.9

Unoperated White Plymouth Rock Chicks

Supplement	None	Iodinated Casein - A		
Per Cent in Diet		0.005	0.05	0.25
Chick Group No.	21	22	23	24
Per Cent Fat*	19.2	25.3	22.6	10.1

*Per cent fat in moisture-free sample
**Identity of samples lost

Figure 5.--EFFECT OF DESICCATED THYROID FEEDING ON BODY FAT STORAGE
Experiment I-29. S.C.W. Leghorn Chicks

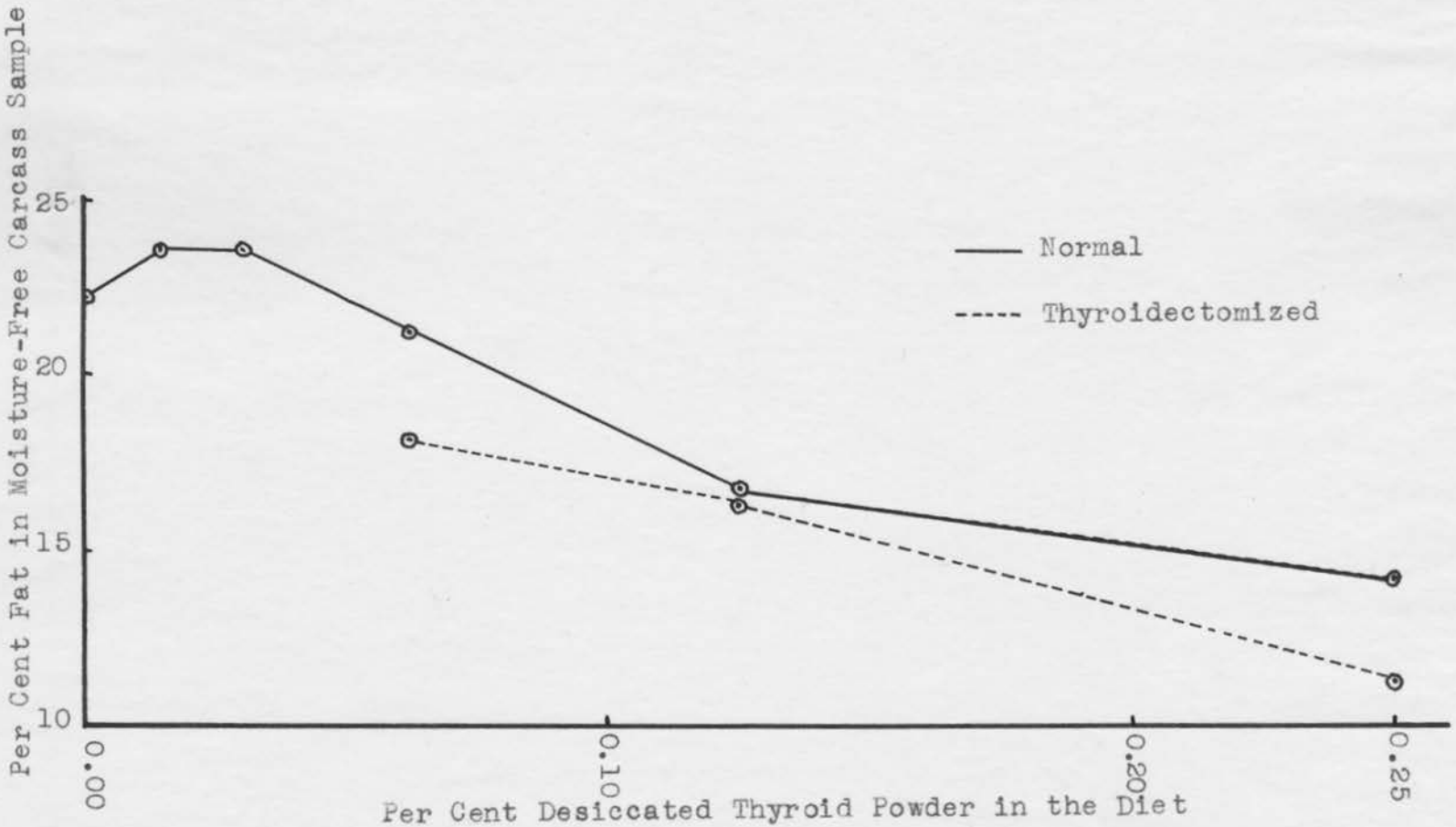


Figure 6.--EFFECT OF IODINATED CASEIN FEEDING ON BODY FAT STORAGE
Experiment I-29. Normal White Plymouth Rocks

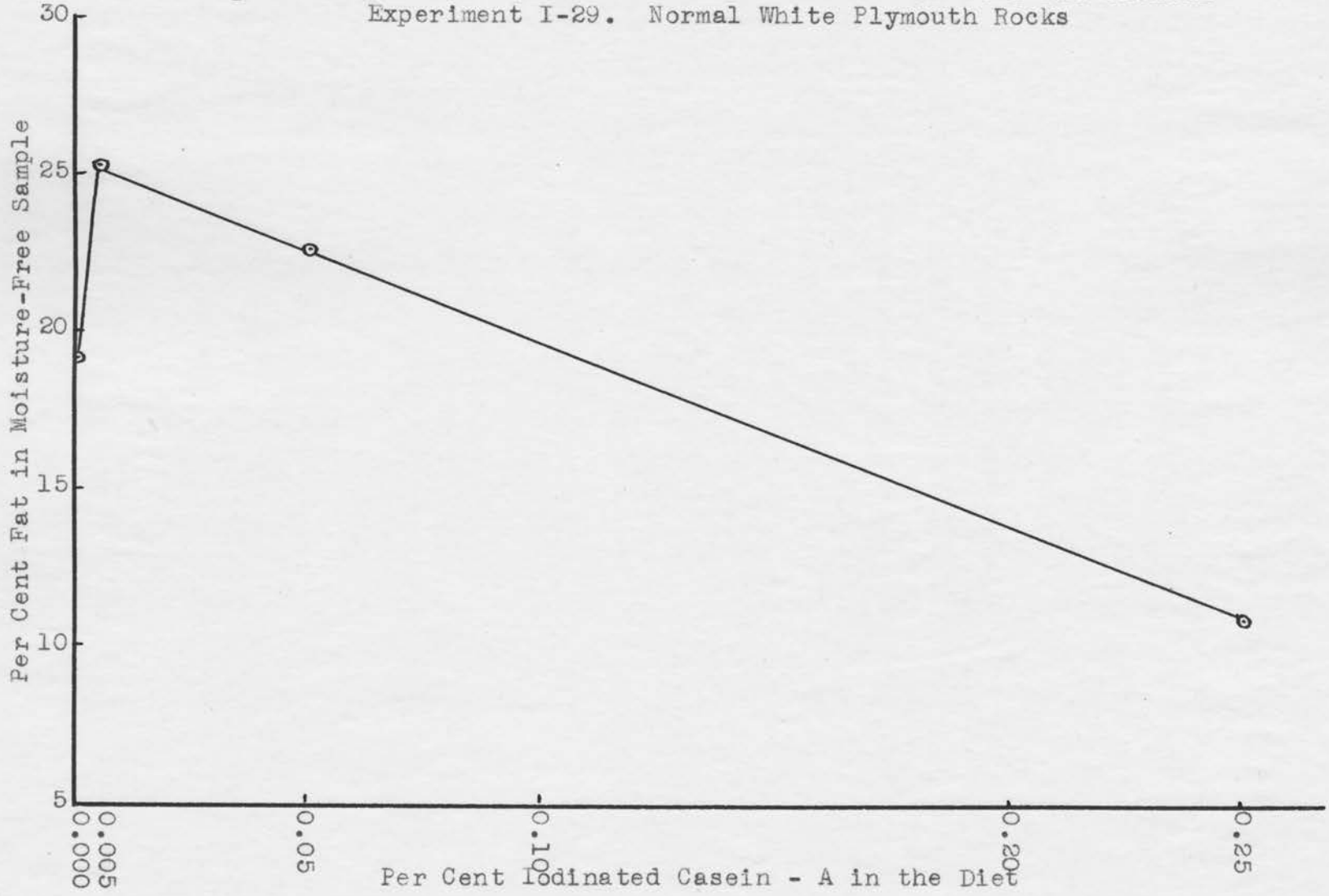


Table 18.--EFFECT OF DIETARY DESICCATED THYROID AND IODINATED CASEIN ON LIVER WEIGHT.
Experiment I-29. S.C.W. Leghorn Chicks

Desiccated Thyroid Feeding												
Supplement	None		Desiccated Thyroid Powder									
Per Cent in Diet			0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Survivors, Male	1	3	3	3	4	3	3	2	2	2	1	2
Survivors, Females	4	4	3	4	2	4	2	4	5	3	2	2
Liver wt., Dry, Males	3.6	4.3	2.7	3.5	3.0	3.4	3.3	3.4	2.9	3.2	3.0	3.3
Liver wt., Dry, Females	2.7	3.8	2.7	2.8	3.2	3.4	2.8	3.8	2.7	3.2	2.8	3.3
Av. Liver wt. (M & F)	3.2	4.1	2.7	3.2	3.1	3.4	3.1	3.6	2.8	3.2	2.8	3.3
Liver Wt.(mg)/Body Wt.(Gm)M.	11.2	10.7	7.4	7.9	8.5	9.9	10.7	8.0	9.1	7.7	11.1	13.3
Liver Wt.(mg)/Body Wt.(Gm)F.	10.4	9.7	7.3	7.4	8.8	8.8	11.7	9.7	10.2	10.1	10.1	12.7
Av.Liver Wt./Body Wt.(M&F)	10.8	10.2	7.4	7.7	8.7	9.4	11.2	8.9	9.7	8.9	10.6	13.0

Iodinated Casein Feeding								
Supplement	Iodinated Casein - A				Iodinated Casein - B			
Per Cent in Diet	0.008		0.016		0.04		0.08	
Chick Group No.	13	14	15	16	17	18	19	20
Operation	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Survivors, Males	5	1	2	4	2	4	4	4
Survivors, Females	1	6	3	3	3	3	2	3
Liver Wt., Dry, Males	3.7	4.3	4.2	4.3	4.2	4.0	4.2	3.9
Liver Wt., Dry, Females	3.4	3.9	3.4	3.1	3.9	4.1	3.5	3.8
Av. Liver Wt. (Males and Females)	3.6	4.1	3.8	3.7	4.1	4.1	3.9	3.9
Liver Wt.(mg)/Body Wt. (Gm) Males	9.1	10.7	9.6	9.8	12.2	9.6	11.5	9.3
Liver Wt.(mg)/Body Wt. (Gm) Females	9.9	10.2	9.9	10.1	12.1	12.0	11.3	9.6
Av. Liver Wt./Body Wt. (Males and Females)	9.5	10.5	9.8	10.0	12.2	10.8	11.4	9.5

In the control groups there was an initial rise in liver vitamin content with small additions of desiccated thyroid to the diet. The response of the females was slightly greater. The maximum storage was attained sooner by females than by males, but also declined more rapidly and reached a lower level with progressive additions of desiccated thyroid to the diet. In males, the liver storage of vitamin A changed slowly, and was less extreme. When related to body weight, the relationships were not changed except that the data for the thyroidectomized males more nearly resembled that for the females. The average carotene content did not differ significantly between any of the groups.

The storage of riboflavin in the liver resembles that for liver vitamin A (table 20, and figure 8). The females responded to less desiccated thyroid. The initial rise in liver riboflavin storage was greater and the decline with progressive increases in dietary desiccated thyroid was more rapid and reached a lower minimum storage level than in the males. When related to body weight, the curves for liver riboflavin storage rise steadily, indicating that retardation of growth (as measured by body weight) is relatively greater than inhibition of liver riboflavin storage.

The data for liver pantothenic acid and nicotinic acid storage are presented in table 21 and

Table 19.--EFFECT OF DIETARY DESICCATED THYROID ON VITAMIN A CONTENT OF THE LIVER. Experiment I-29. S.C.W. Leghorn Chicks

Unoperated S.C.W. Leghorn Chicks						
Supplement	None	Desiccated Thyroid Powder				
Per Cent in Diet		0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	2	4	6	8	10	12
Survivors, Males	3	3	3	2	2	2
Survivors, Females	4	4	4	4	3	2
International Units Vitamin A per gram Moisture-free Liver Sample						
Males	87	110	62	126	102	97
Females	114	177	102	82	73	77
Average	100	143	82	104	87	87
Total Liver Vitamin A (I.U.) content per 100 grams of Body Weight						
Males	56	81	61	102	78	134
Females	110	124	90	80	73	102
Average	83	102	75	91	75	118

Thyroidectomized S.C.W. Leghorn Chicks							
Supplement	None		Desiccated Thyroid Powder				
Per Cent in Diet			0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	1-T	1-R	3	5	7	9	11
Survivors, Male	1	2	3	4	3	2	1
Survivors, Female	4	1	3	2	2	5	2
International Units Vitamin A per gram Moisture-free Liver Sample							
Males	90	84	86	83	90	82	37
Females	38	87	120	154	52	68	55*
Average	64	85	103	118	71	75	46
Total Liver Vitamin A (I.U.) Content per 100 grams of Body Weight							
Males	102	83	60	67	96	77	41
Females	39	100	83	130	62	68	36
Average	70	91	71	98	79	72	38

*One determination only

Figure 7.--EFFECT OF DESICCATED THYROID FEEDING ON LIVER VITAMIN A STORAGE

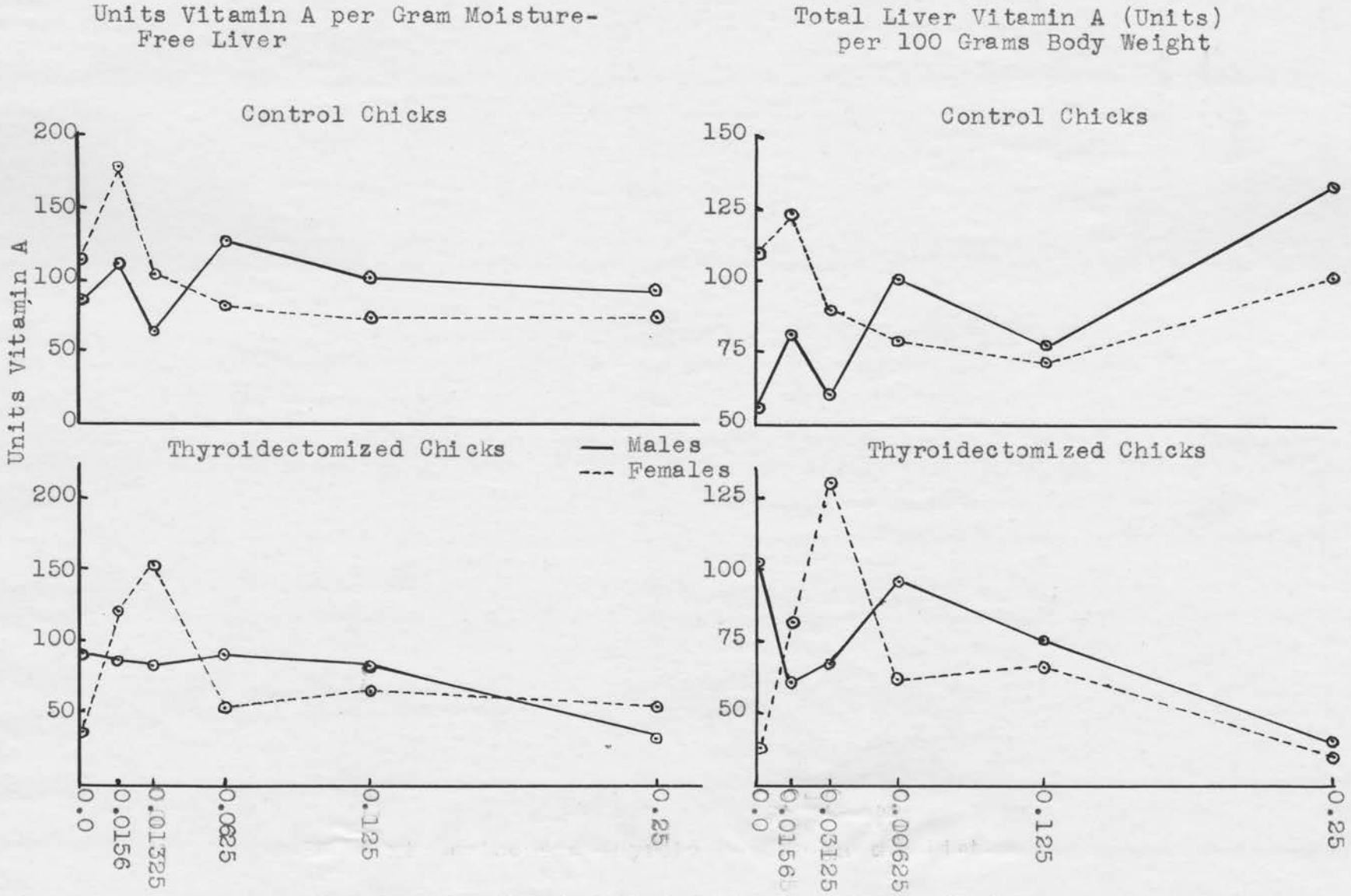


Table 20.--EFFECT OF DIETARY DESICCATED THYROID ON RIBOFLAVIN CONTENT OF THE LIVER. Experiment I-29. S.C.W. Leghorn Chicks

Unoperated S.C.W. Leghorn Chicks						
Supplement	None	Desiccated Thyroid Powder				
Per Cent in Diet		0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	2	4	6	8	10	12
Survivors, Males	3	3	3	2	2	2
Survivors, Females	4	4	4	4	3	2
Micrograms of Riboflavin per gram Moisture-free Liver Sample						
Males	61	84	61*	79	90	78
Females	69	81	86	77	82	73
Average	65	61	67	69	76	96
Total Liver Riboflavin Content (Micrograms) per 100 grams of Body Weight						
Males	65	64	65*	63	69	100
Females	66	58	70	75	83	93
Average	65	61	67	69	76	96

Thyroidectomized S.C.W. Leghorn Chicks							
Supplement	None		Desiccated Thyroid Powder				
Per Cent in Diet			0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	1-T	1-R	3	5	7	9	11
Survivors, Males	1	2	3	4	3	2	2
Survivors, Females	4	1	3	2	2	5	2
Micrograms of Riboflavin per gram Moisture-free Liver Sample							
Males	56	55	74	73	74	81	87
Females	58**	54	85	69	61	65	72***
Average	57	54	79	71	67	73	79
Total Liver Riboflavin Content (Micrograms) per 100 grams of Body Weight							
Males	63	54	53	62	78	77	97
Females	57**	63	62	60	72	66	57***
Average	60	58	57	61	75	71	77

*Three determinations. **Two determinations. ***One determination.

Figure 8.--EFFECT OF DESICCATED THYROID FEEDING ON LIVER RIBOFLAVIN STORAGE

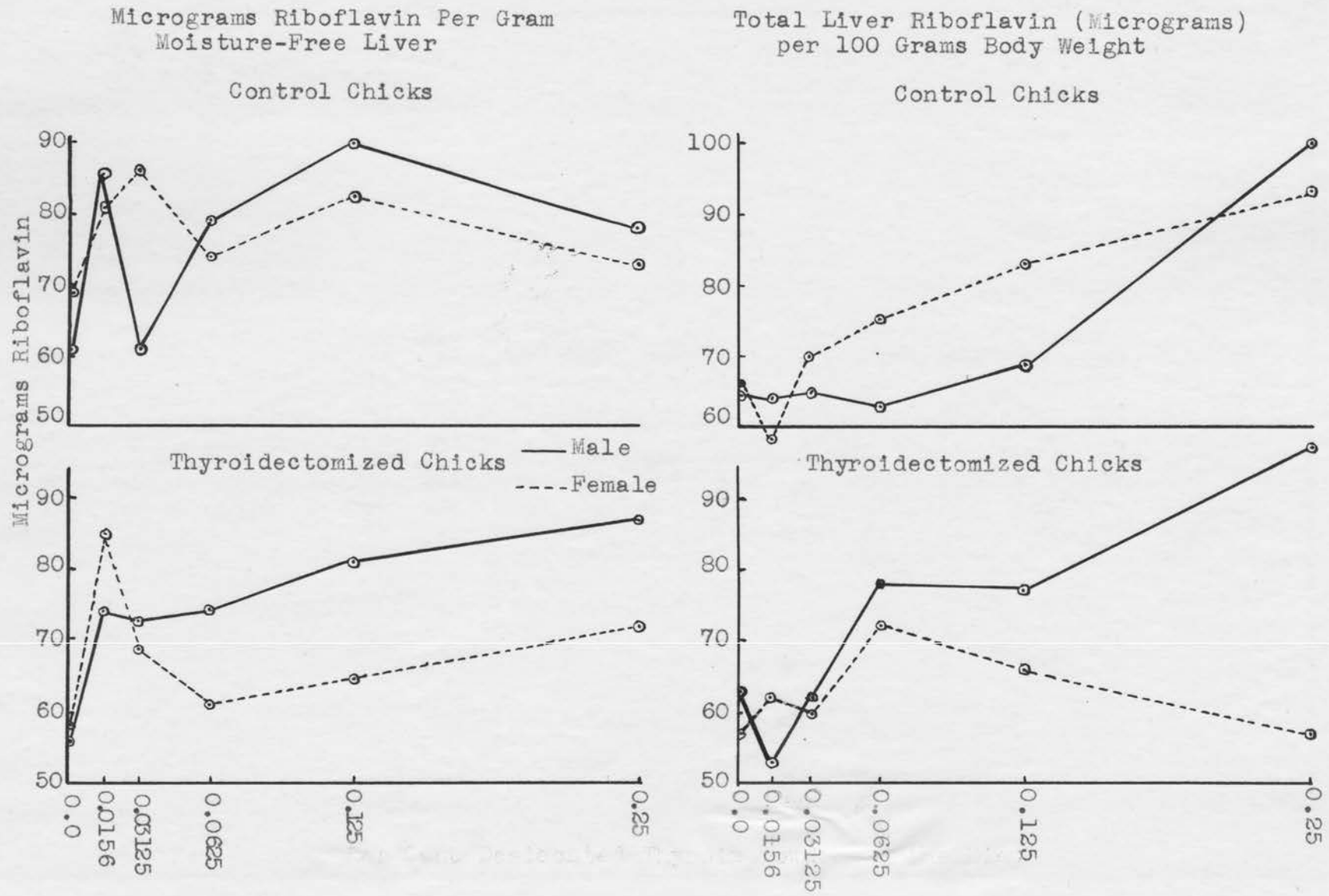


Table 21.--EFFECT OF DIETARY DESICCATED THYROID ON PANTOTHENIC ACID AND NICOTINIC ACID CONTENT OF THE LIVER. Experiment I-29. S.C.W. Leghorn Chicks

Supplement	None			Desiccated Thyroid Powder									
Per Cent in Diet				0.0156		0.03125		0.0625		0.125		0.25	
Chick Group No.	1-T	1-R	2	3	4	5	6	7	8	9	10	11	12
Operation	Thy	Reg	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un	Thy	Un
Survivors, Males	1	2	3	3	3	4	3	3	2	2	2	1	2
Survivors, Females	4	1	4	3	4	2	4	2	4	5	3	2	2
Micrograms of Pantothenic Acid per gram of Moisture-free Liver Sample													
Males	237	395	287	345	382	398	372	335	372	305	314	377	284
Females	256*	352	256	431	502	350	382[x]	311	284	392	275	359[z]	303
Average	246	373	271	388	442	374	377	323	328	348	299	368	293
Micrograms of Nicotinic Acid per gram of Moisture-free Liver Sample													
Males	356	411	465	490	507	447	511	440	455	476	519	502	541
Females	395*	432	506	509	506	422	607[x]	416	443	436	459	478[z]	290
Average	375	421	485	499	506	434	559	428	449	456	489	490	415

*Two determinations
xThree determinations
zOne determination

Figure 9.--EFFECT OF DESICCATED THYROID FEEDING ON LIVER PANOTHENIC ACID AND NICOTINIC ACID STORAGE

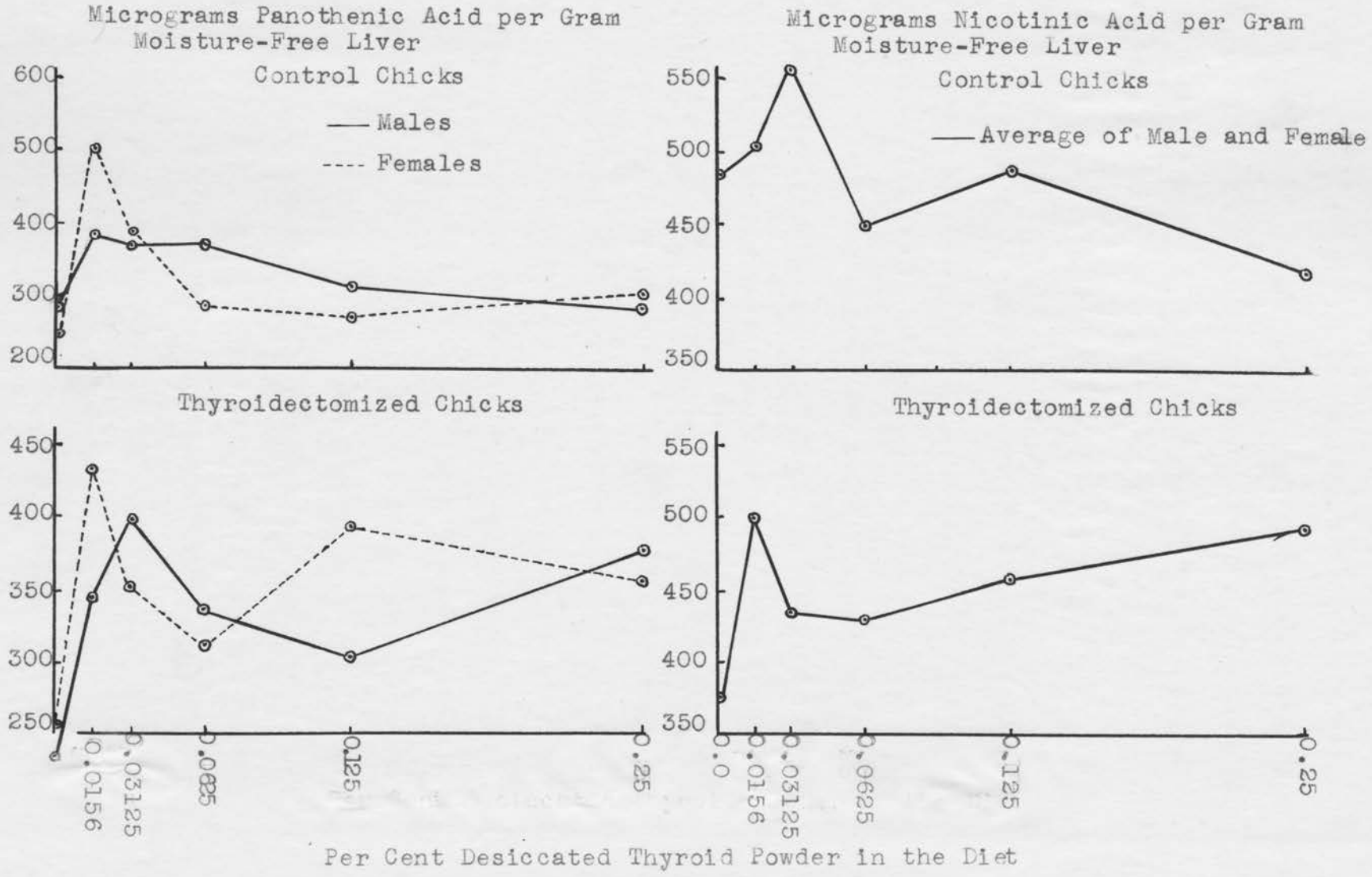


figure 9. By comparing the curves of figures 8 and 9 it appears that the effects of desiccated thyroid feeding on liver storage of nicotinic acid and pantothenic acid were similar to the effects on liver storage of riboflavin.

Thyroid Response

Thyroid response to the feeding of desiccated thyroid and iodinated casein is shown in tables 22 to 26, figures 10 to 14, and plates IV and V.

When thyroid weight in milligrams, and thyroid weight in milligrams per 100 grams of body weight were plotted against dosage of desiccated thyroid given in experiment I-27 the resultant curves rapidly decline for the first two dosage levels (figure 10). Beyond 0.125 per cent in the diet the decline is negligible.

Figures 11 and 12 are presented as a critical analysis of the relative merit of the various thyroid measurements employed in experiment I-29 and summarized in tables 23, 24, and 25. In plotting the curves each series of absolute values was converted to a new series in which the value for the basal group was 10 in each case. In so doing, a given constant was used for each series. This procedure facilitated critical comparison of the various measurements.

Upon examination of these two sets of curves it is apparent that 0.0625 per cent desiccated thyroid

in the diet inhibited thyroid development and activity as much as any greater amount. It is also apparent that 0.0625 per cent desiccated thyroid in the diet inhibited the activity of the thyroid sufficiently so that the various measurements were approximately equal to those of a day-old chick thyroid. Any amount of desiccated thyroid powder less than 0.0625 per cent in the diet was insufficient tomeet the demands of the chick for thyroid substance (presumably for thyroxine) and the gland became sufficiently active to make up the deficiency.

All the thyroid measurements taken showed an almost arithmetical inverse relationship to dosage in the diet up to 0.03125 per cent. The sum of the width and length of the thyroid gland was inversely proportional to the amount of desiccated thyroid in the diet up to 0.0625 per cent (figures 11-13). This relationship is discussed below. The product of width and length of the thyroid gland showed an inverse logarithmic relationship to the percentage of desiccated thyroid in the diet up to 0.0625 per cent (figure 14). Thyroid weight, follicle volume, and summation of follicle diameters were all logarithmically related to the amount of desiccated thyroid in the diet, up to 0.03125 per cent. Thyroid weight was not considered to be reliable as a method of assay for thyroxine acti-

vity since the higher levels of feeding resulted in considerable invasion of the thyroid, and displacement of thyroid follicles, by what appeared to be non-thyroid tissue. This condition is illustrated in plate V, number 6.

The curve of figure 13 relating dosage of desiccated thyroid to the sum of the width and length of the thyroid was plotted directly from table 24. Using the values in table 26, the relative potency of the unknown iodinated casein products can be estimated by extrapolation. Through this procedure the values of Weight plus Length for pens 14 and 16 (4.08 and 3.42 respectively) are found to correspond to approximately 0.021 and approximately 0.042 per cent desiccated thyroid powder along the horizontal axis. Doubling the amount of iodinated casein-A in the diet had exactly the same effect as doubling the amount of desiccated thyroid, and 0.008 per cent iodinated casein-A had thyroxine potency equal to 0.021 per cent desiccated thyroid powder. In relative terms, the iodinated casein-A assayed a little more than 2.5 times the potency of desiccated thyroid. This does not agree with the estimate of potency furnished with the product, i.e. five to ten times that of desiccated thyroid powder.

By a similar process of extrapolation, iodinated casein-B is found to have approximately three

times the potency of desiccated thyroid at the 0.04 per cent level and only 1.7 times the potency of desiccated thyroid at the 0.08 per cent level. However, the greater amount of the product necessitates extrapolation from the lower portion of the curve which may not be within the most sensitive range. Furthermore, the value for the response of the thyroid is derived from males only.

Plates IV and V are included to illustrate the inhibition of activity of the thyroid gland by feeding thyroactive substances. The relationship between the amount of thyroactive substance in the diet and the degree of activity of the thyroid gland is readily apparent. Feeding desiccated thyroid at 0.0625 per cent or more in the diet prevents activity of the gland, but does not depress it below the state of activity of the thyroid of a day-old chick.

Long Time Effects of Thyroidectomy

Thyroidectomized chicks from experiments I-25 and I-26 grew to maturity and produced eggs. Several chicks were hatched from eggs produced by one of these females. Plate VI shows the characteristics of completely thyroidectomized adult chickens from which the glands were removed at one to three days of age. The feathers were extremely long and narrow, and there was complete lack of development of secondary sexual

Table 22.--EFFECT OF DIETARY DESICCATED THYROID ON THYROID WEIGHT.
Experiment I-27. S.C.W. Leghorn Chicks

	S.C.W. Leghorn Chicks				
Supplement	None	Desiccated Thyroid Powder			
Per Cent in Diet		0.0625	0.125	0.25	0.50
Chick Group No.	2	4	6	8	10
Survivors, Males	3	4	4	4	1
Survivors, Females	3	3	4	4	3
	Thyroid Weight in Milligrams*				
Males	44.2	17.3	13.5	13.9	6.2
Females	35.9	21.1	12.1	20.3	9.7
Average	40.0	19.2	12.8	17.1	7.9
	Milligrams Thyroid Weight per Kilogram of Body Weight				
Males	107	52	42	39	21
Females	110	68	42	71	33
Average	109	60	42	55	27

*Average for left thyroids plus average for right thyroids

Figure 10.--EFFECT OF DESICCATED THYROID FEEDING ON THYROID WEIGHT
Experiment I-27

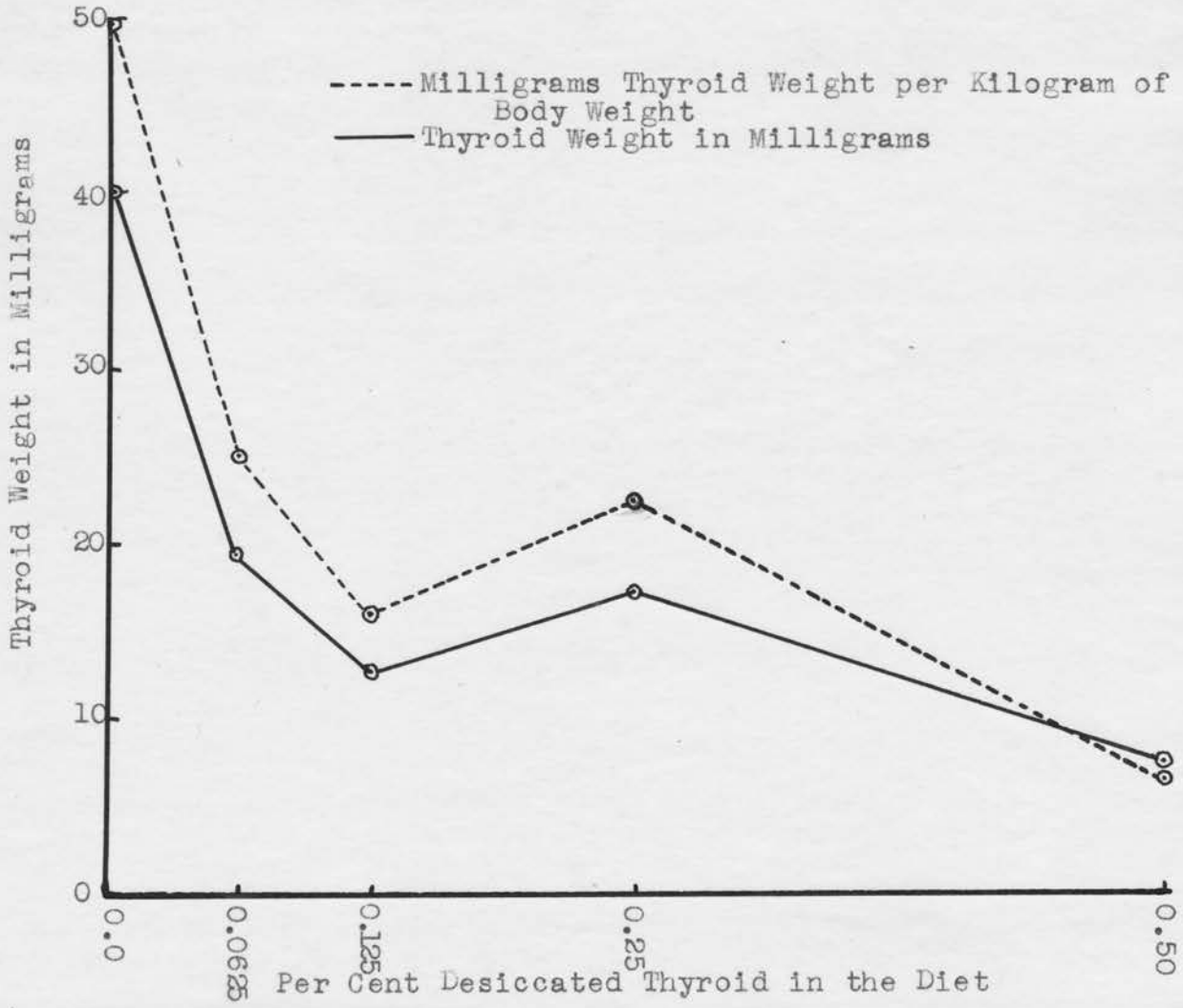


Table 23.--THYROID WEIGHT AND IODINE ANALYSIS.
Experiment I-29

		Desiccated Thyroid Feeding					
Supplement		None	Desiccated Thyroid Powder				
Per Cent in Diet	Day-old		0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	Chicks	2	4	6	8	10	12
Thyroid Weight							
No. Thy.Incl.,Male	15	2	1	2	1	1	1
No. Thy.Incl.,Fem.	9	2	4	2	1	1	1
Average Thyroid Weight (milligrams)*							
Males	1.2	11.6	6.5	3.3	2.6	2.8	2.8
Females	1.2	10.6	8.6	4.1	3.3	2.5	2.6
Average*	2.4	22.2	15.1	7.4	5.9	5.3	5.4
Milligrams Thyroid Weight per Kilogram Body Weight**							
Males	37.5	60.0	31.0	19.9	13.0	13.6	19.6
Females	24.5	58.8	44.4	19.5	15.2	15.6	22.6
Average**	62.0	59.4	37.5	19.7	14.1	14.6	21.1
Thyroid Iodine							
No. Thy.Incl.,Male		3	3	3	2	2	2
No. Thy.Incl.,Fem.		4	4	4	4	3	1
Iodine Content of Thyroids (micrograms)***							
Males		11.2	4.1	1.4	0.9	1.6	1.5
Females		6.0	5.3	2.0	1.4	1.2	2.9
Average***		17.2	9.4	3.4	2.3	2.8	4.4

*Left Thyroid only. The average weights for males and for the females were added together and the resultant value taken as an estimate of the average weight of two glands.
**Calculated from weight of left thyroid only for the two sexes. Average values for males and for females added and the resultant value taken as an estimate for the average of two glands.
***Right thyroid only. The averages for the two sexes added together and the resultant value taken as an estimate for the value of two glands.

Table 24.--THYROID HISTOLOGICAL ANALYSES*
Experiment I-29

		Desiccated Thyroid Feeding					
Supplement		None	Desiccated Thyroid Powder				
Per Cent in Diet	Day-old		0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	Chicks	2	4	6	8	10	12
No.Thy.Incl.,Male	15	2	2	2	2	2	2
No.Thy.Incl.,Fem.	9	3	4	3	3	1	1
Width plus Length of Thyroid (millimeters)							
W + L, Male	2.42	4.80	3.80	3.40	2.37	3.77	3.80
W + L, Female	2.58	4.70	4.62	4.10	3.17	3.00	3.00
W + L, Average	2.50	4.75	4.21	3.75	2.77	3.38	3.40
Width times Length of Thyroid (millimeters)							
W x L, Male	1.45	5.25	3.24	2.54	1.08	2.27	2.76
W x L, Female	1.64	5.19	4.90	3.58	2.51	2.21	2.00
W x L, Average	1.54	5.22	4.07	3.06	1.79	2.24	2.38
Summation of the Diameters of Eleven Largest Follicles (microns)							
ΣD, Male	526	811	626	592	474	405	532
ΣD, Female	479	1063	832	557	504	517	396
ΣD, Average	502	937	729	574	489	461	464
Thyroid Volume (x 1000 cubic microns)**							
Volume, Male	57	210	97	81	42	26	59
Volume, Female	43	474	227	68	50	54	29
Volume, Average	50	342	162	74	46	40	44

*Measurements obtained from largest cross sectional area of serially sectioned thyroids.

**Calculated from sex averages of follicle diameter according to the formula:

$$\left(\frac{\Sigma D}{11}\right)^3 \cdot \frac{\pi}{6}$$

Figure 11.--EFFECT OF DIETARY DESICCATED THYROID ON VARIOUS THYROID MEASUREMENTS
Experiment I-29. S. C. W. Leghorn Chicks.

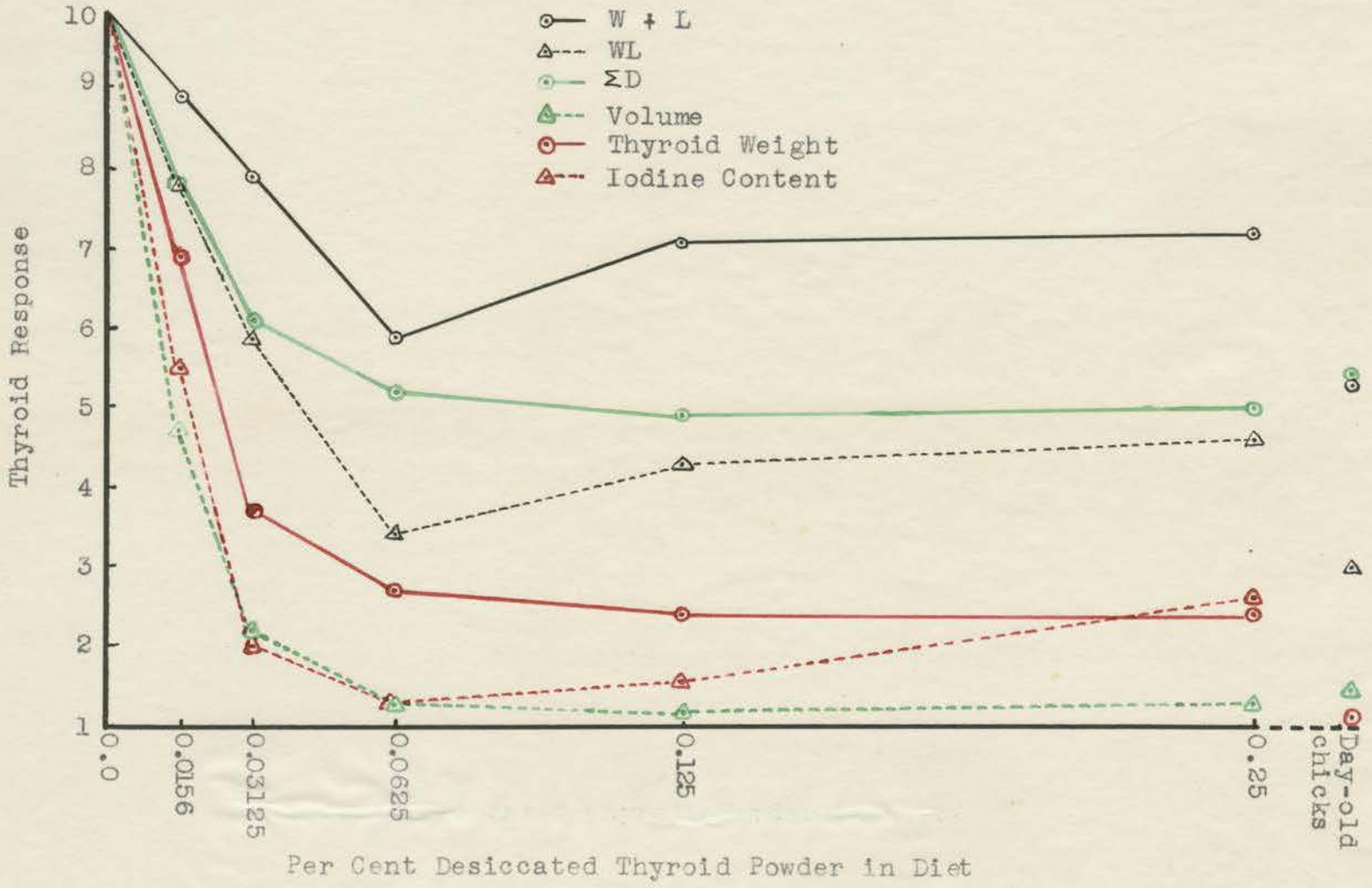


PLATE IV

The effect of dietary iodinated casein on the histology of the thyroid gland of White Plymouth Rock chicks.

Experiment I-29. The chicks were six weeks old and had received the experimental diets from hatching time.
Magnification -- 300 diameters.

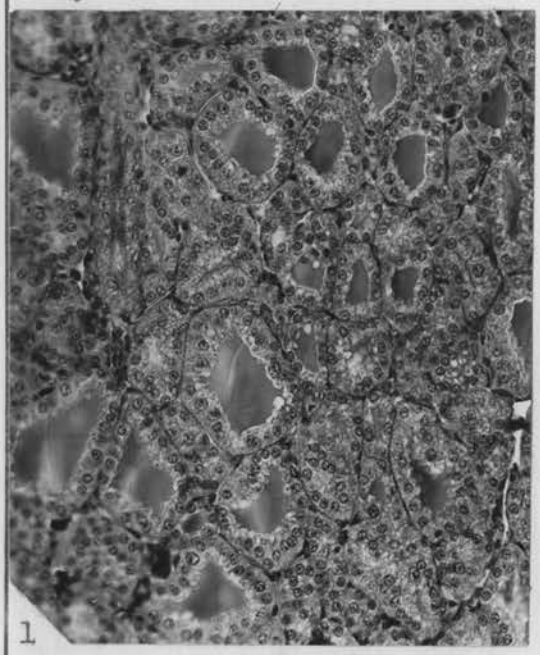
1. Group 21. Basal diet.
   Epithelium tall and active.
   Maximum follicle diameter.

2. Group 22. 0.005 per cent iodinated casein-A in the diet.
   Increased colloid storage.
   Epithelium low; decreased activity.
   Some reduction in follicle diameters.

3. Group 23. 0.05 per cent iodinated casein-A in the diet.
   Colloid storage about equal to that in a day-old-chick thyroid.
   Epithelium very low; inactive.
   Greatly reduced follicle diameter.
   Follicle diameter approximately equal to that of a day-old-chick thyroid.

4. Group 24. 0.025 per cent iodinated casein-A in the diet.
   Colloid storage about equal to that in a day-old-chick thyroid.
   Epithelium very low; inactive.
   Greatly reduced follicle diameter.
   Follicle diameter approximately equal to that of a day-old-chick thyroid.

PLATE IV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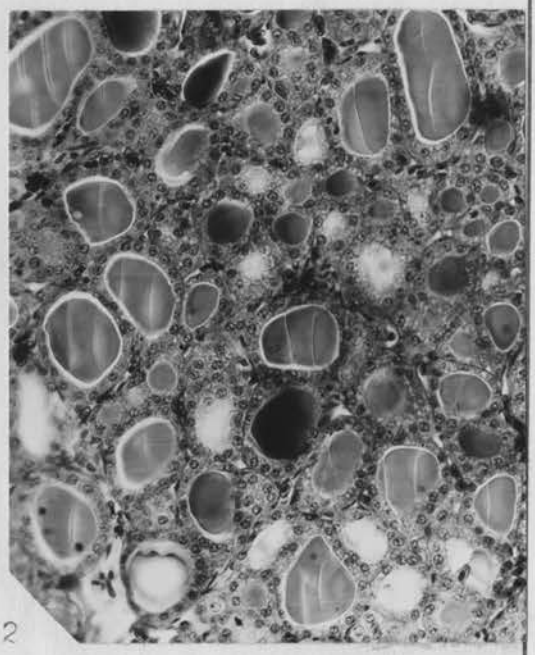
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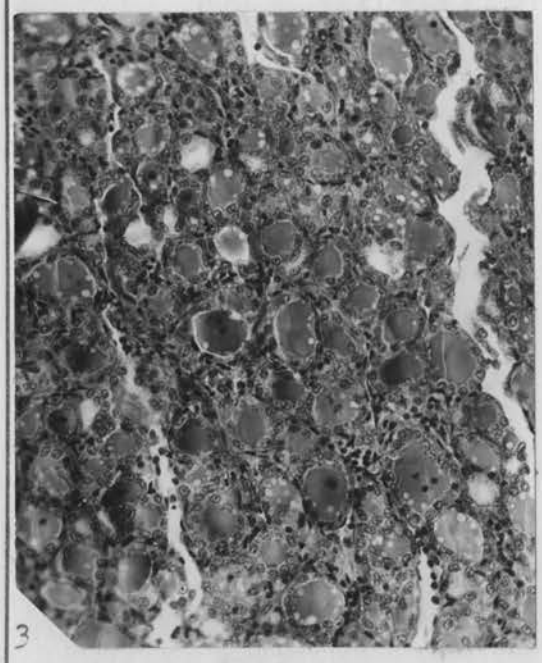
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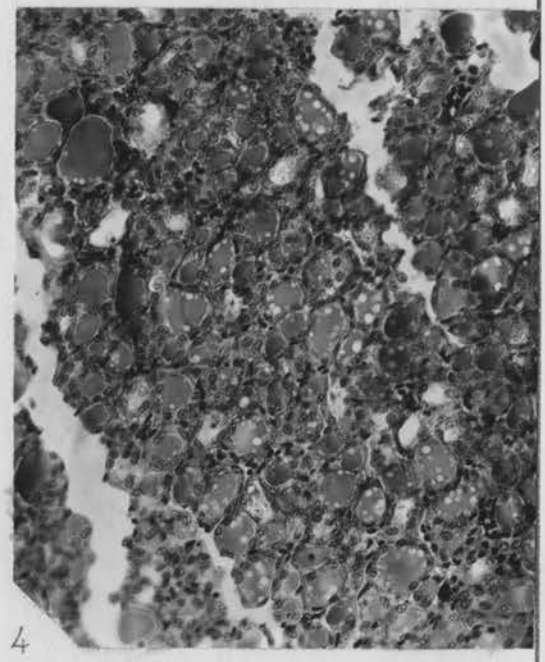
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PLATE V

The effect of desiccated thyroid powder on the histology of the thyroid gland of Single Comb White Leghorn chicks.

Experiment I-29. The chicks were six weeks old and had received the experimental diets from hatching time.

1. Group 2. Basal diet.
   Magnification -- 300 diameters.
   Maximum follicle diameters.
   Maximum height of epithelium and glandular activity.
   Moderate colloid storage.

2. Group 4. 0.0156 per cent desiccated thyroid powder in the diet.
   Magnification -- 300 diameters.
   Reduced follicle diameters.
   Reduced height of epithelium and glandular activity.

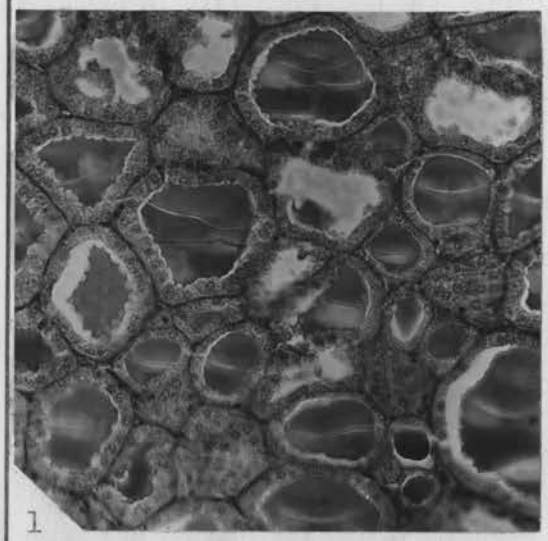
3. Group 6. 0.03125 per cent desiccated thyroid powder in the diet.
   Magnification -- 300 diameters.
   Further reduction in follicle diameters.
   Further reduction in height of epithelium and glandular activity.

4. Group 8. 0.0625 per cent desiccated thyroid powder in the diet.
   Magnification -- 300 diameters.
   Very little additional reduction in follicle diameters. Follicle diameters approximately equal to those of the day-old chick.
   Very little additional reduction in height of epithelium and glandular activity.

5. Day-old chick.
   Magnification -- 300 diameters.

6. Group 22. 0.005 per cent iodinated casein-A in the diet.
   Magnification -- 80 diameters.
   Non-thyroid tissue (appearing dark) dispersed throughout the gland.

PLATE V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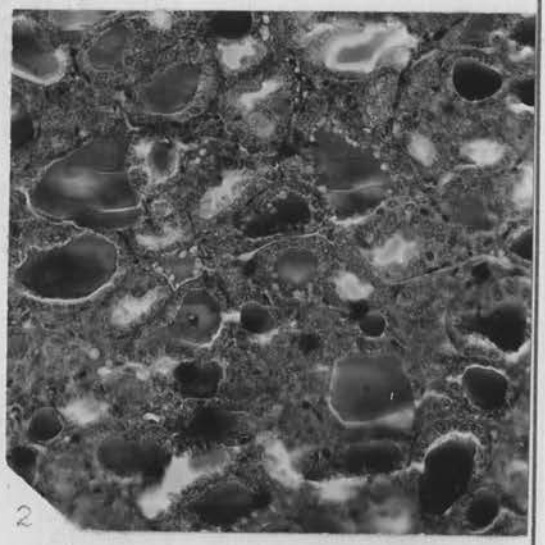
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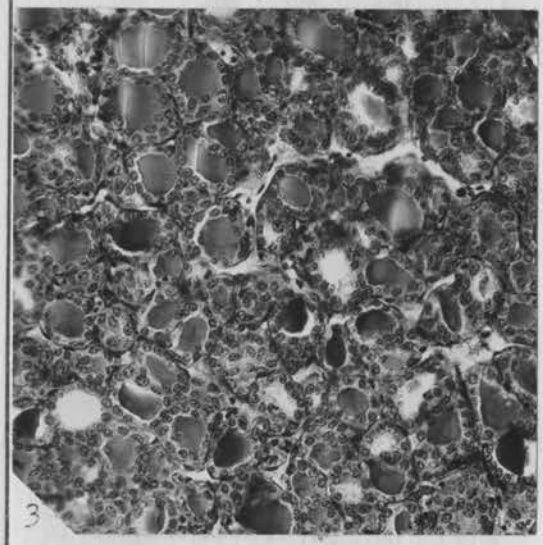
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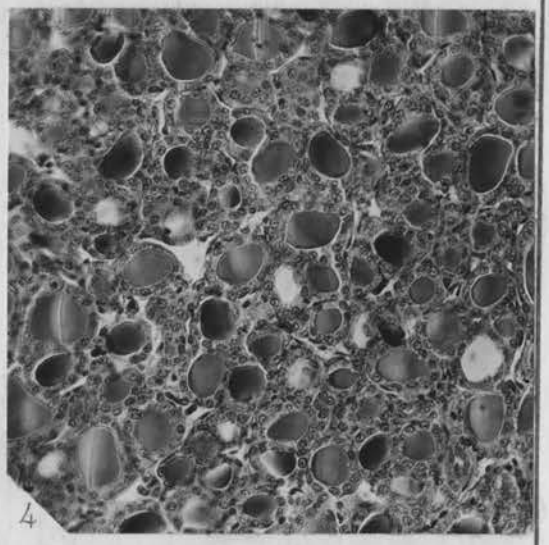
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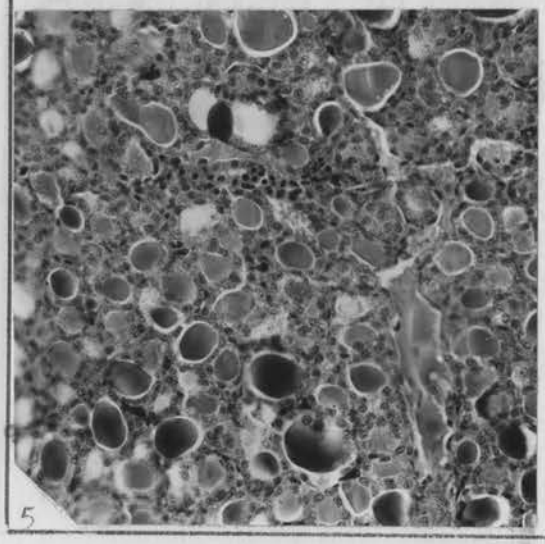
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Table 25.--THE RELATIONSHIP BETWEEN BODY WEIGHT AND THYROID HISTOLOGICAL ANALYSES. Experiment I-29

		Desiccated Thyroid Feeding					
Supplement		None	Desiccated Thyroid Powder				
Per Cent in Diet	Day-old		0.0156	0.03125	0.0625	0.125	0.25
Chick Group No.	Chicks	2	4	6	8	10	12
No.Thy.Incl.,Male	15	2	2	2	2	2	2
No.Thy.Incl.,Fem.	9	3	4	3	3	1	1
	Width x Length (microns) / Body Weight (Grams)						
WL/B. Wt., Males	42.5	13.6	7.5	7.6	2.6	5.5	11.2
WL/B. Wt., Females	47.5	14.5	12.7	9.5	6.1	6.9	8.7
WL/B. Wt., Average	45.0	14.1	10.1	8.6	4.4	6.2	10.0
	Summation Diameters (microns) / Body Weight (Grams)						
ΣD/B. Wt.,Males	1.48	2.10	1.39	1.80	1.11	0.97	2.13
ΣD/B. Wt.,Females	1.56	2.94	2.16	1.44	1.31	1.61	1.72
ΣD/B. Wt.,Average	1.52	2.52	1.77	1.62	1.21	1.29	1.92
	Follicle Volume (cubic microns) / Body Weight (Grams)						
Vol./B.Wt.,Males	1580	525	229	235	98	63	238
Vol./B.Wt.,Females	1300	1210	596	175	128	171	111
Vol./B.Wt.,Average	1440	868	408	205	113	117	175

For method of obtaining measurements see table 24.

Figure 12.--EFFECT OF DIETARY DESICCATED THYROID ON RELATIONSHIP OF VARIOUS THYROID MEASUREMENTS TO BODY WEIGHT

Experiment I-29. S. C. W. Leghorn Chicks.

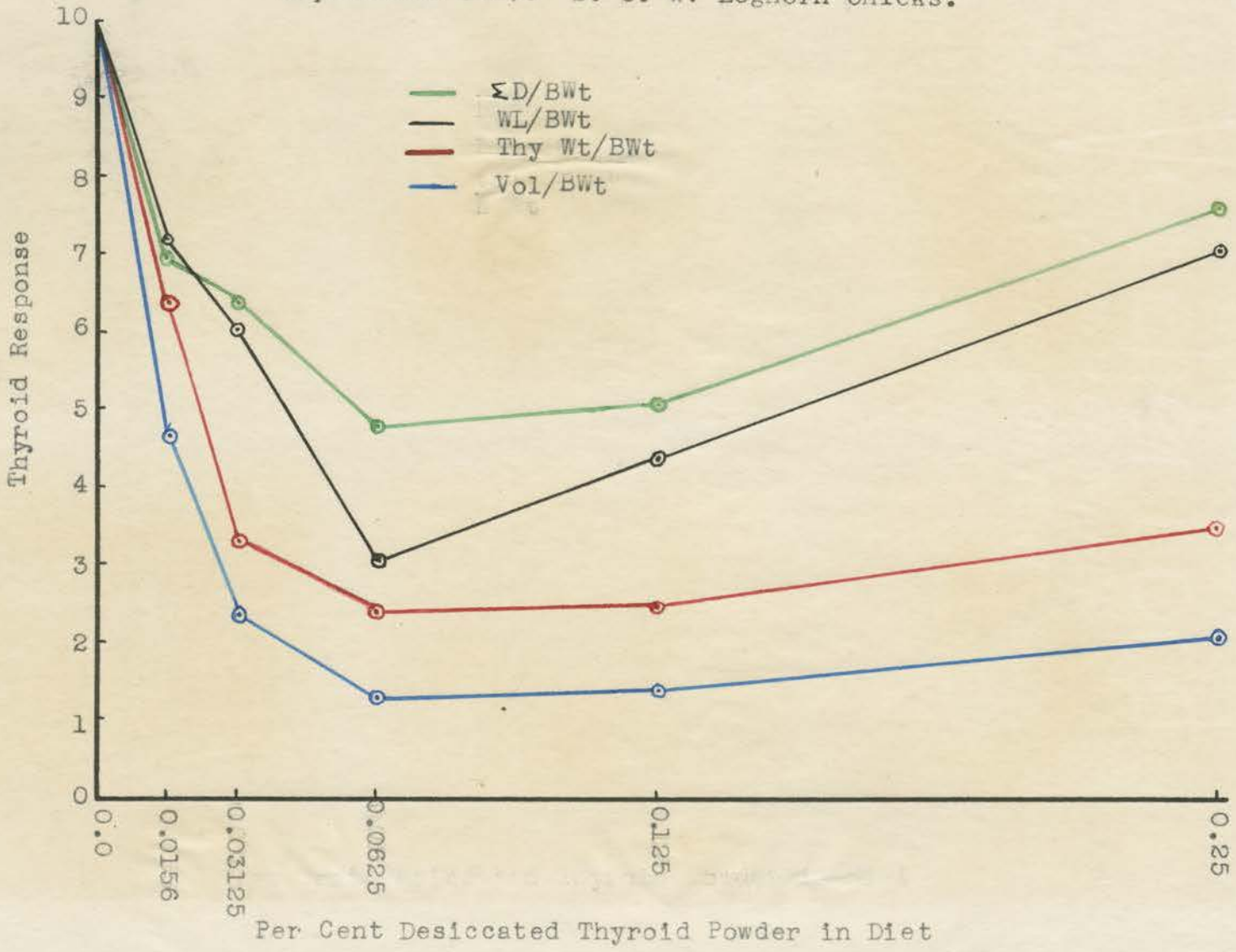


Figure 13.--EFFECT OF DESICCATED THYROID FEEDING ON SUM OF WIDTH AND LENGTH OF LARGEST CROSS SECTION OF THYROID. Arithmetic Relationship. Experiment I-29

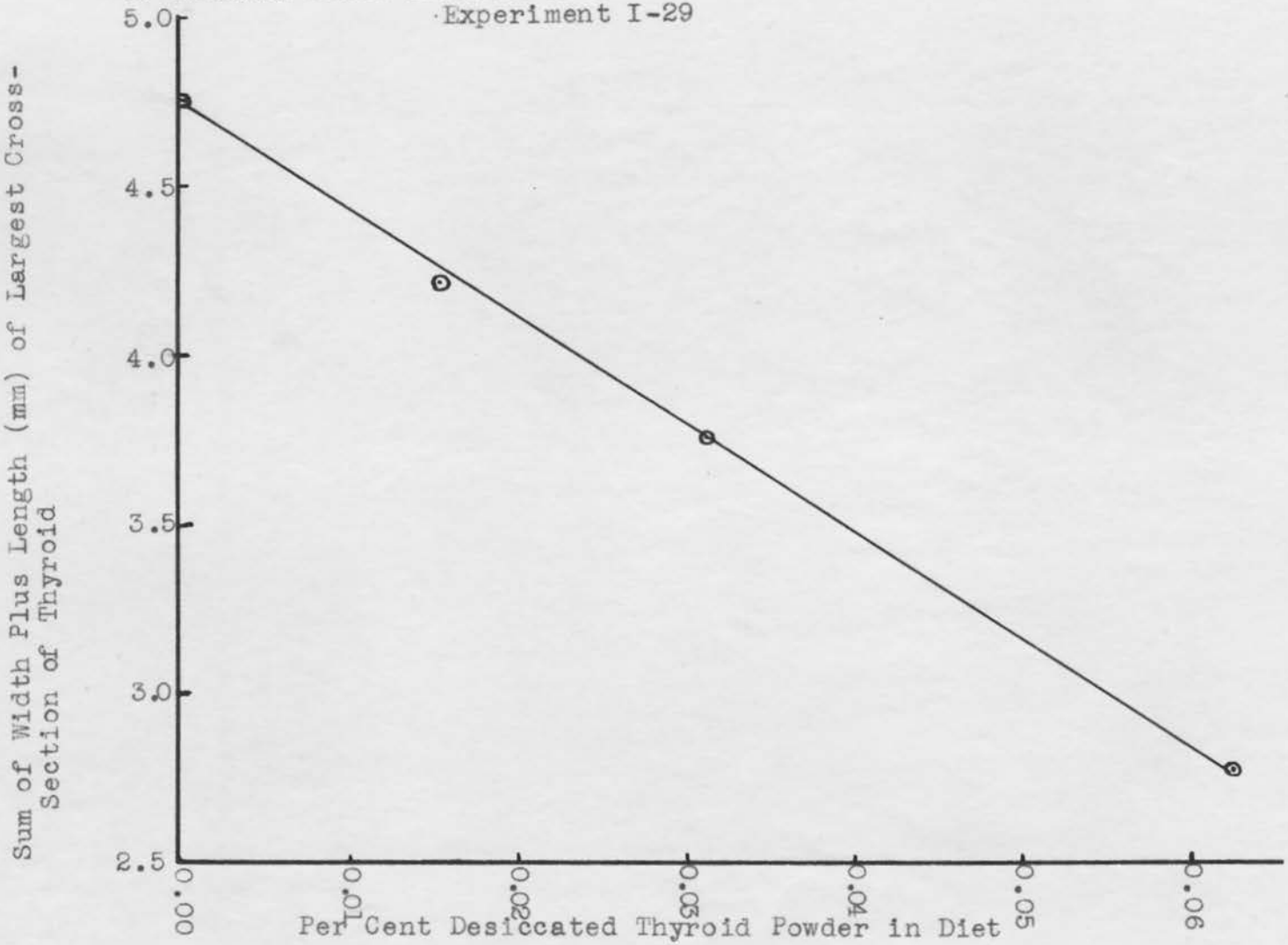


Figure 14.--EFFECT OF DESICCATED THYROID FEEDING ON PRODUCT OF WIDTH AND LENGTH OF LARGEST CROSS SECTION OF THYROID. Logarithmic Relationship. Experiment 1-29

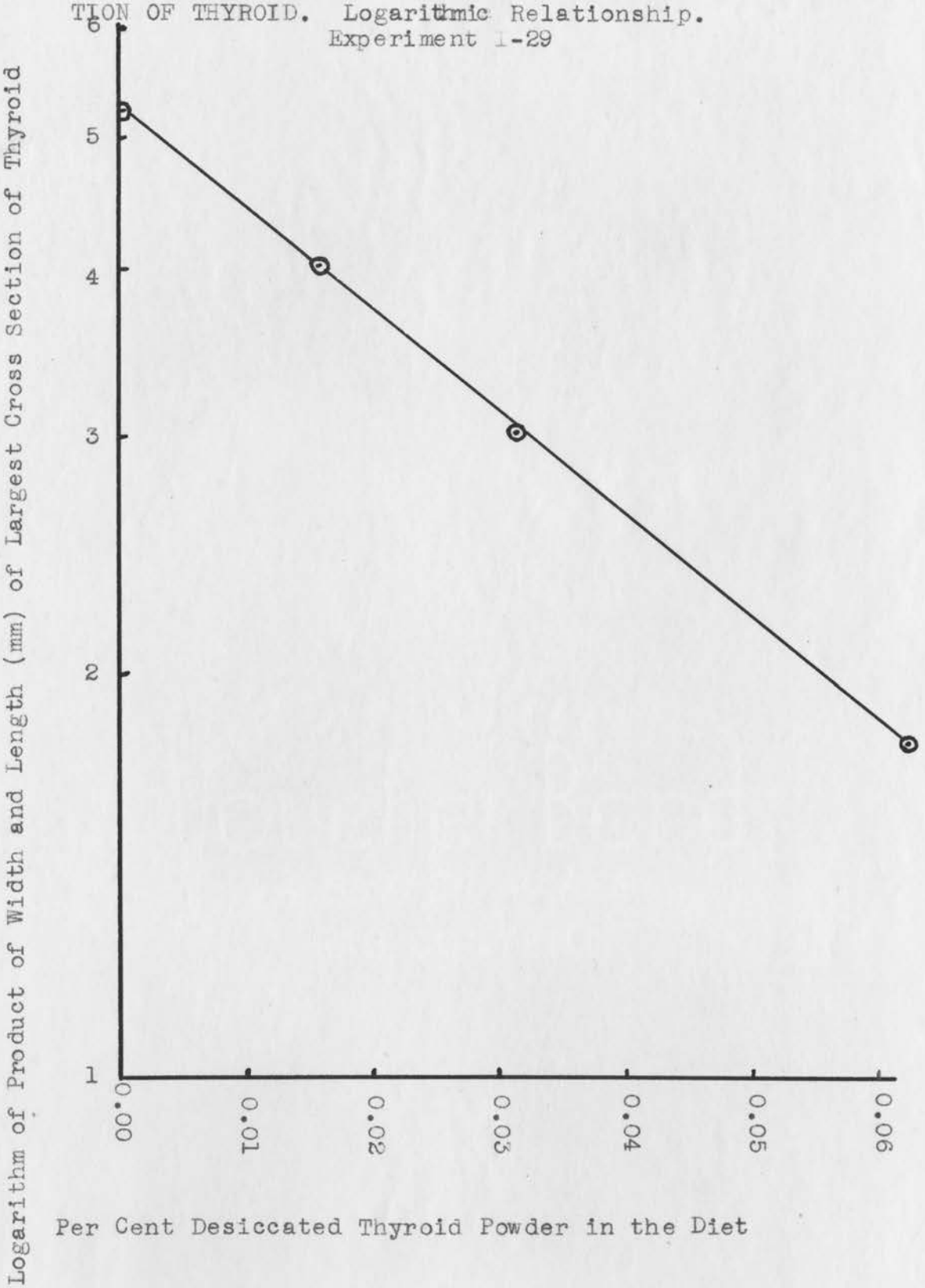


Table 26.--THYROID HISTOLOGICAL ANALYSES*
Experiment I-29

	Iodinated Casein Feeding			
Supplement	Iodinated Casein - A		Iodinated Casein - B	
Per Cent in Diet	0.008	0.016	0.04	0.08
Chick Group No.	14	16	18	20
No. Thyroids Incl., Male	1	1	4	3
No. Thyroids Incl., Female	5	2	3	0
Width plus Length of Thyroid (millimeters)				
W + L, Male	4.45	3.60	4.07	3.37
W + L, Female	3.72	3.25	4.57	-
W + L, Average	4.08	3.42	4.32	3.37
Width times Length of Thyroid (Sq. mm.)				
W x L, Male	4.27	3.08	3.63	2.60
W x L, Female	3.22	2.04	5.09	-
W x L, Average	3.74	2.56	4.36	2.60
Summation of the Diameters of Eleven Largest Follicles (microns)				
ΣD, Male	615	595	832	597
ΣD, Female	616	452	843	-
ΣD, Average	615	523	833	597

*Measurements obtained from largest cross sectional area of the serially sectioned thyroid.

PLATE VI

I. The effect of complete thyroidectomy at one to three days of age on subsequent mature characteristics.

II. Typical condition of bird suffering from fowl paralysis.

1. Experiment I-25. White Plymouth Rocks.

A. Completely thyroidectomized at one day of age.
Long, narrow, silky feathers.
Low hanging abdomen.
Secondary sex characteristics undeveloped.
The chicken was laying eggs at the time the photograph was taken.

B. Control chicken.
Larger in size.
Secondary sex characteristics developed.
The chicken was laying eggs at the time the photograph was taken.

2. Experiment I-26. Single Comb White Leghorns.

A. Control chicken.
Larger in size.
Secondary sex characteristics well developed.
The chicken was laying eggs at the time the photograph was taken.

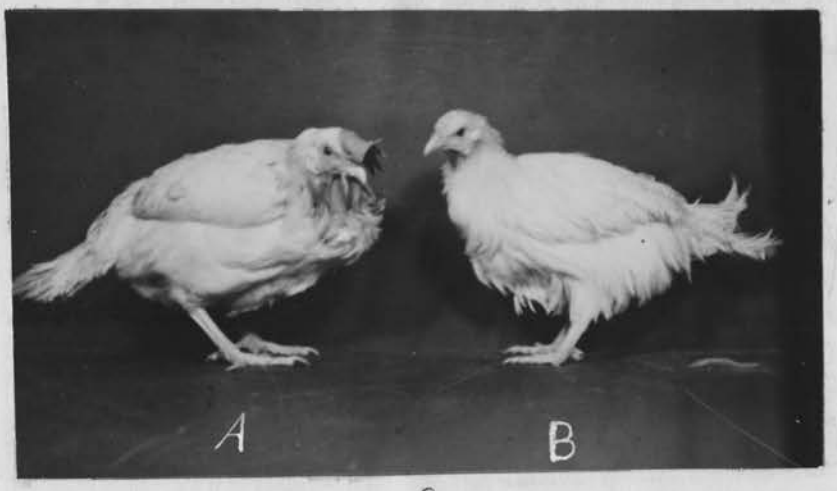
B. Completely thyroidectomized at one day of age.
Smaller in size.
Long, narrow, silky feathers.
Secondary sex characteristics undeveloped.
The chicken was laying eggs at the time the photograph was taken.

3. Single Comb White Leghorns.

Typical fowl paralysis condition. Note that one leg is thrust forward. The other leg was drawn up under the bird. The chicken was unable to use either leg.

PLATE VI

1



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characteristics, even though the ovaries were active. Macroscopic examination was considered adequate to demonstrate the absence of thyroid tissue in these birds, because of the long period of thyroidectomy. Such a long period of thyroidectomy should have been sufficient stimulus to cause thyroid regeneration of considerable magnitude in any chicken with thyroid tissue remaining.

The Effect of Thyroactive Substances on Fowl Paralysis

During the course of these studies, a number of chickens, suffering from range paralysis were treated with thyroactive substances administered in small capsules. Various stages of paralysis were observed, from slight limping to complete disability. One or both legs were paralyzed and in some cases, the wings also. A typical bird used for these studies is shown in table VI (figure 3). The diseased chickens were usually received several at a time. The procedure was to destroy and examine a representative paralyzed bird from each lot received, to maintain a less severaly affected bird in the cage without treatment, and to treat the remainder with desiccated thyroid or iodinated casein.

The 28 birds for which the records were complete were classified into three groups. Eight were killed for autopsy records, five controls received no treatment, and 15 were treated. Two treated birds

showed no recovery whatever. Three treated birds showed partial recovery, but never appeared normal, and eventually died. Four recovered temporarily and appeared normal, but eventually developed paralysis again and died. Five appeared to recover completely. Treated chickens were considered as partially recovered if they improved sufficiently to get up and stand on their feet again. Temporarily recovered birds improved to the point where they appeared normal, and administration of thyroactive substances was discontinued. After a period of several weeks these birds again developed paralysis without recovery. Both partially and temporarily recovered chickens failed to respond to treatment the second time. Circumstances made it necessary to discontinue observation of the apparently completely recovered birds after several months.

DISCUSSION

The results of this study largely confirm those obtained by other workers in this field. The failure of Greenwood and Blyth (1928-29) to observe any deleterious effects on growth and development probably means that their chicks were incompletely thyroidectomized. All through the present investigation thyroid tissue, even in very small amounts, tended to restore to normal all the characteristics studied.

When the results obtained by feeding potassium iodide are compared with the results obtained by feeding thyroactive casein it is apparent that the thyroidless chick is able to synthesize little, if any, thyroxine from elemental iodine.

Thyroidectomy greatly retarded growth as measured by both body weight and shank length. The data on efficiency of gain are conflicting. The analyses of fat were not obtained for unsupplemented thyroidectomized chicks, but examination by handling the chicks seemed to indicate, as also reported by other workers, that fat deposition was increased. At six weeks of age body weight of thyroidectomized chicks was not restored to normal by any of the amounts of desic-

cated thyroid fed in the last two experiments. A very small addition of desiccated thyroid to the diet of thyroidectomized chicks resulted in a marked improvement of growth, but increased thyroid feeding resulted in lower body weights than the maximum attained by chicks receiving the most nearly optimum amount. This fact serves as an argument in favor of the view that administration of dried thyroid gland to thyroidectomized chicks does not entirely replace the thyroid function when compared with the intact chick.

The lower body weight of unsupplemented thyroidectomized chicks was undoubtedly due to arrested skeletal development (shortened shank length) with coincidental reduction in total body protein, because the thyroidectomized chicks were well fleshed and appeared fat on handling. On the other hand, the lower body weight which resulted from feeding thyroactive substances to the thyroidectomized as well as to the control chicks, was due largely to less adipose storage. Retarded skeletal development was a contributing factor in lower body weight only when large doses of desiccated thyroid were given. This is evidenced by the fact that the decline in adipose storage paralleled the decline in body weight, whereas, only the extremely high additions of thyroactive substances had a marked inhibitory action on skeletal development as measured by

shank length. Lower efficiency of gain, which resulted from thyroid feeding, was probably due to less fat storage since the two results were closely parallel. Protein and ash content of the moisture-free carcass were not determined but the sum of these two components must necessarily change inversely with the change in fat content of dried samples.

Growth of control chicks was retarded by 0.125 per cent desiccated thyroid in the diet in both of the last two experiments. The next lower addition in experiment I-29 (0.0625 per cent) did not result in lower body weight of control chicks. Shank length, efficiency of gain, and fat storage were less in chicks receiving 0.0625 and 0.03125 per cent desiccated thyroid in the diet than in control chicks. It seems that the maximum amount that may safely be given is probably somewhere between 0.0156 and 0.03125 per cent in the diet of Single Comb White Leghorn chicks. It is important to note that normal chicks receiving the same amount of desiccated thyroid in the diet (e.g. 0.25 per cent) attained much lower body weight than controls in the last experiment (table 4), but only slightly lower than controls in the previous experiments (tables 1, 2, and 3). This variation may be due to seasonal influences.

Development of feathers was very markedly inhibited by thyroid removal which confirms the work of

others. The inhibition was greater in the slow feathered, heavy breed (White Plymouth Rock), than in the lighter breed (Single Comb White Leghorn). It is significant that the growth of wing and tail feathers was not greatly inhibited in Single Comb White Leghorn chicks. These feathers begin to develop soon after hatching and this may mean that thyroid removal exerts its influence early in the differentiation and development of the feather. Since completely thyroidectomized chicks eventually develop feathers of silky nature (plate VI), and since feeding thyroactive substances speeds up feather development (plate V), (Hutt, 1927), the deleterious effect of thyroid removal must exert itself by slowing up cellular activity in the feather follicle. Further evidence for this lies in the fact that thyroid removal at two weeks of age appears to arrest feather development in the pinfeather stage.

Feeding thyroactive substances markedly improved feather development of normal White Plymouth Rock chicks (plate II) but the feather condition of Single Comb White Leghorn chicks was very good without treatment, so there was little chance for improvement. The least amount of desiccated thyroid fed to thyroidectomized chicks (0.0156 per cent) was sufficient to cause feather development comparable to that of control chicks (plate III).

The minimum amount of desiccated thyroid necessary in the diet of thyroidectomized Single Comb White Leghorn chicks to restore feather condition to normal did not exceed 0.0156 per cent.

When 0.005 per cent iodinated casein-A was added to the diet of normal White Plymouth Rock chicks, the feather condition was greatly improved. Body weight, shank length, fat storage, and efficiency of gain were also increased. Chicks receiving ten times this amount had nearly perfect feather condition but the values for body weight, shank length, fat storage, and efficiency of gain were lower than the values for controls. The optimum amount must lie somewhere between 0.005 and 0.05 per cent. This product had approximately 2.5 times the activity of desiccated thyroid powder, according to the assay employed in this investigation. On this basis the amount of desiccated thyroid in the diet which would cause optimum feather condition, without resulting in body weight, efficiency of gain, skeletal size, or fat storage lower than normal lies somewhere between 0.002 and 0.02 per cent for White Plymouth Rock chicks. This agrees quite well with the estimate for Single Comb White Leghorn chicks, i.e., 0.0156 to 0.03125 per cent. The estimate is much lower than the amount (0.08 per cent) which Irwin et. al. (1943) reported as causing slight increase in body weight of

Barred Rock chicks, or the amounts (0.025 per cent and 0.050 per cent) which Parker (1943) reported as causing greater gains in Rhode Island Red chicks. The maximum value compares favorably with the amount (0.01 per cent) reported by Turner et. al. (1944) as causing body weight lower than control chicks to 12 weeks of age in Barred Rock cockerals. In all three reports referred to above, the products used were reported to contain approximately ten times the thyroxine activity of desiccated thyroid powder. In contrast to the results with iodinated casein-A, iodinated casein-B (potency approximately equal to desiccated thyroid) failed to influence body weight of normal White Plymouth Rock chicks when given at 0.379 per cent in the diet. This value compares favorably with the results of other workers.

The discrepancy between the chick responses to thyroactive substances obtained in this study and those obtained by other workers may have been due to environmental differences, or to inherent differences in the chicks used. A third possibility is that synthetic thyroactive substances may vary in their effectiveness for chicks. In any case, the necessity for caution in feeding thyroactive substances is evident, and the need for a chick assay for thyroactive substances intended for poultry feeding is emphasized.

This work and that of previous workers

indicates that the amount of thyroid hormone necessary for optimum feather condition is not the same as the amount necessary for optimum growth, efficiency of gain, and fat storage. The work also indicates that feather condition, but not body weight, of thyroidectomized chicks can be restored to normal by feeding thyroactive substances in the diet.

Thyroid insufficiency favors the development of mineral and, or, vitamin deficiency symptoms (including perosis and dermatosis). The action may be through interference with vitamin utilization, since the liver storage was not lowered by thyroidectomy, and additions of vitamins to the diet did not prevent the deficiency symptoms, whereas additions of thyroactive substances did.

The incidence and degree of perosis and dermatosis were quite high among the unsupplemented thyroidectomized chicks in the earlier experiments but not so great in later experiments. Vitamin deficiency symptoms failed to develop except in the exploratory experiments and in experiment I-25. A probable explanation for this is the change in season of the year. The later experiments were carried out in warmer weather and the requirement for thyroid hormone may not have been as great during this time. Ring (1942) showed that the activity of the thyroid is stimulated by cold. This contention

is also supported by Koger's and Turner's work (1943), showing that non-toxic doses of thyroactive substances may become toxic with arrival of warm weather. The perosis condition may have been due to disturbed manganese metabolism. The intact thyroid stores large amounts of manganese as well as iodine (Ray and Deysach, 1942).

Body temperature was not affected either by thyroid removal, or by feeding thyroactive substances, at least not sufficiently to be detected by rectal determinations. Completely thyroidectomized chickens which were reared to maturity were unable to survive severe winter weather and a heating unit had to be furnished to keep the mature birds alive. This can be viewed as evidence that the thyroid hormone is an important factor in the production of body heat.

Feeding large doses of desiccated thyroid did not cause observable vitamin deficiency symptoms in this investigation. This was probably because there was an adequate supply of vitamins in the diet to meet any increase in demand that may have resulted from thyroid feeding. On the other hand, large doses seemed to cause a slight decrease in the vitamin A content of the liver.

Contrary to the majority of evidence as quoted by Drill (1943), the present study indicates

that carotene is converted to vitamin A in the absence of the thyroid gland. The unsupplemented, thyroidectomized chicks were able to store about the same amount of vitamin A in the liver as were the normal control chicks receiving the same diet. This is very important because the vitamin A activity of the diet was due almost entirely to carotene; approximately 2.5 International Units of vitamin A per 100 grams of diet were supplied by the dried buttermilk and meat and bone scrap. The carotene content of the liver of thyroidless chicks did not exceed the liver content in control chicks. This was true in the unsupplemented group and in the groups receiving thyroid substance. Schneider and Widman (cited by Drill, 1943), on the other hand, found that thyroxine administration to thyroidectomized guinea pigs increased the hepatic storage of both carotene and vitamin A.

Thyroid hormone seems to have some influence on storage of vitamin A or upon the conversion of carotene to vitamin A because small additions of desiccated thyroid to the diet increased the liver storage of vitamin A in both thyroidectomized and control chicks.

The hepatic storage of the water soluble vitamins was affected by thyroidectomy and mild thyroid feeding in a manner similar to vitamin A, except that the highest dietary levels of desiccated thyroid did

not lower the liver storage greatly. This does not agree with previous evidence as quoted by Drill (1943), that hepatic storage of certain other water soluble vitamins (i.e. B_1 and C) is reduced by hyperthyroidism.

There was a sex difference in response to desiccated thyroid feeding in the present study which confirms the report by Drill (1943). The liver vitamin storage indicated that the females were more sensitive to thyroactive substances. This is evidenced by their quicker response, as shown by the more rapid initial rise in liver vitamin content followed by a more rapid and greater decline. Evidently females are more sensitive than males to small doses of desiccated thyroid but are more able to tolerate excessive amounts of thyroid substance since male chicks were killed sooner than females by toxic dietary levels (experiment I-25). Irwin et. al. (1943), however, found evidence that females were more sensitive to high levels of thyroactive iodocasein.

The close agreement between the various thyroid measurements indicates that 0.0625 per cent desiccated thyroid in the diet is under the conditions of the experiment, a reliable estimate of the amount necessary to prevent activity of the thyroid gland in the developing chick. The lowest level of iodinated casein used by Irwin et. al. (1943) (approximately 0.01 per cent)

caused nearly maximum depression of thyroid weight. On the basis of reported thyroxine activity, that amount was approximately equal to 0.10 per cent desiccated thyroid; almost twice the value reported here. This is fairly good agreement since an amount less than 0.01 per cent might also have caused maximum depression of thyroid weight. Moreover, the biological activity of the product may have been much less for chickens than the activity reported.

The amount of thyroxine released daily by the chick thyroid gland did not exceed the amount obtained through the daily consumption of a diet containing 0.0625 per cent desiccated thyroid. Even this estimate is probably too high since the optimum amount of desiccated thyroid that could be added to the diet was estimated earlier to be between 0.0156 and 0.03125 per cent (page 116). According to the USP standards, 0.0625 per cent desiccated thyroid would supply approximately 1250 parts per billion of iodine to the diet in the form of thyroxine. Theoretically then, the maximum amount of iodine required in the diet of Single Comb White Leghorn chicks the first six weeks of age is not over 1250 parts per billion. This agrees very well with the estimate of 1000 parts per billion of iodine in the diet suggested by Wilgus(1941) as the iodine requirement of growing chickens. The reliability

of the estimate in this investigation depends upon the assumption that the amount of dietary iodine absorbed and converted to thyroxine would be approximately equal to the amount of iodine contained in the thyroxine absorbed from the diet containing 0.0625 per cent desiccated thyroid powder.

The thyroid measurements used appear to offer a means of assaying synthetic thyroactive proteins for thyroxine activity. Further work should be done to establish the most reliable thyroid measure for assay and the most accurate range of thyroxine administration to establish a standard curve of response. Pure thyroxine should be used to determine the loss in activity to be expected by oral administration. The efficacy of reducing the feeding period should be determined and assay of unknown products by injection, instead of oral administration, should also be investigated.

The thyroxine activity of iodinated casein-A as determined in this study is approximately half the potency estimated by the producers. The potency estimated for iodinated casein-B agrees quite well with the estimate of the producer. It is quite possible that the ultimate effect of synthetic products may be different in chickens than in tadpoles or guinea pigs. If this is so, a chick assay procedure would be valuable as a guide to dosage of thyroactive protein since the use of

these substances in poultry rations may be advocated in the future.

The failure of development of secondary sexual characteristics in thyroidectomized females, in which the ovaries were active, agrees with the report by Greenwood and Chu (1939). This observation is significant because it shows that thyroid hormone is necessary for the production of the female sex hormone, as concluded by Greenwood and Chu (1939), or for the female sex hormone to exert its influence on these characteristics.

The apparent beneficial effect of thyroactive substances on fowl paralysis should be thoroughly investigated. Fowl paralysis is one of the hitherto incurable diseases of poultry and is considered one of the most serious from an economic standpoint.

SUMMARY AND CONCLUSIONS

A technique for removing the thyroids from day-old chicks is described and some of the advantages are discussed.

Thyroid removal from chicks one to three days old caused typical vitamin deficiency symptoms in the experiments carried out during the winter months. Thyroidectomy in chicks one to three days old also resulted in lower body weight and shorter shank length than normal, and inhibited or markedly retarded, feather development.

The liver content of vitamin A, riboflavin, pantothenic acid, nicotinic acid, and total dry matter was either unaffected or very slightly lower than normal in unsupplemented thyroidectomized chicks over the first six weeks of age.

Addition of yeast and sardine oil to the diet of thyroidectomized chicks failed to counteract the effects of thyroidectomy.

Addition of massive doses of potassium iodide to the diet of dams, or thyroidectomized chicks failed to counteract the subsequent effects of thyroidectomy in chicks one to three days old.

Addition of small doses of thyroactive iodinated casein, or of desiccated thyroid, counteracted completely the effects of thyroidectomy on all characteristics studied except body weight. The effectiveness was dependent upon the thyroxine content and not upon the iodine content.

Iodinated casein and desiccated thyroid, when added on an equal thyroxine activity basis, did not consistently cause the same response when different experiments were compared or when the products were compared with each other in the same experiment.

Progressive increase in the amount of desiccated thyroid in the diet of growing thyroidectomized and control chicks caused progressive decrease in the measurements of body weight, efficiency of gain, adipose storage in the carcass, and liver storage of vitamin A. In nearly every instance, the decrease was preceded by an increase with very small additions to the diet.

Both thyroidectomized and control chicks receiving very small amounts of desiccated thyroid in the diet stored more vitamin A, riboflavin, nicotinic acid, and pantothenic acid than did respective chicks receiving the basal diet. In most cases the initial increase in liver storage resulting from small additions of desiccated thyroid was followed by rapid decrease with succesively greater additions.

Feather condition in thyroidectomized Single Comb White Leghorn chicks was restored to normal by as little as 0.0156 per cent desiccated thyroid in the diet. As little as 0.05 per cent of an iodinated casein product in the diet of normal White Plymouth Rock chicks resulted in excellent feather condition. According to the estimate of the producers this was equivalent to 0.25 per cent or more of desiccated thyroid, but according to the assay employed in this investigation, this amount was only equal to about 0.02 per cent of desiccated thyroid in the diet.

A method of assay for thyroxine potency of thyroactive substances is proposed and discussed. The method is based upon the response of the chick thyroid to thyroactive substances included in the diet. The activity of the gland is determined by the evaluation of width and length of the whole gland and diameter of the follicles. The activity is compared with the activity resulting from the feeding of known amounts of standardized desiccated thyroid.

On the basis of estimated thyroxine production by the chick thyroid, the iodine requirement of growing Single Comb White Leghorn chicks has been estimated as approximately 1250 parts per billion in the diet for the first six weeks of age.

The maximum non-lethal dose of desiccated

thyroid that could be administered in the diet of growing chicks was approximately 0.50 per cent. The maximum non-toxic dose was between 0.0156 and 0.03125 per cent for Single Comb White Leghorn chicks.

Between 0.002 and 0.02 per cent of desiccated thyroid in the diet was suitable for optimum feather development without simultaneously resulting in sub-normal fat storage, shank length, and body weight in White Plymouth Rock chicks.

Completely thyroidectomized day old chicks grew to maturity and produced fertile eggs. They had received no thyroid supplementation in the diet, and they showed no regenerated thyroid tissue upon post-mortem examination.

In the absence of thyroid hormone, secondary sexual characteristics did not develop.

Thyroactive substances were found to have an apparent beneficial effect on mature and growing birds showing severe symptoms of range paralysis, a virus disease of poultry.

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