THE EFFECT OF HYPO - AND HYPERTHYROIDISM UPON FERTILITY AND HATCHABILITY IN THE DOMESTIC FOWL

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INTRODUCTION

In the production of baby chicks, the foremost object of the poultryman is to secure as many good salable chicks as possible in proportion to the number of eggs produced by each female breeder in his flock. There are many factors of paramount importance in determining the number of chicks obtained from a flock of breeders. Of these, no doubt, the two main factors are fertility and hatchability. Since the cost of hatching eggs represents approximately 65 per cent of the total cost of producing day-old chicks, there is a direct relationship between the fertility and the hatchability of the eggs and the cost of producing baby chicks.

It should be kept in mind that the percentage of fertile eggs is the first factor in determining the number of chicks secured in proportion to the total number of eggs set. Fertility, according to our present knowledge, is not inherited on a genetic basis. However, it is well known, that there are individual differences in the ability of both males and females to produce fertile eggs. The causes of this individual variation in the fertility of the domestic fowl are at present not well understood. Hatchability, on the other hand, is known to be a highly heritable character; but the inheritance of this character is not simple and can not be explained on a simple genetical basis. Several lethal genes are known to have considerable influence upon chick embryonic mortality.

At present little is known regarding the physiological factors affecting fertility and hatchability in the domestic fowl. However, based on the work conducted with smaller experimental animals, such as rats and mice, there is good reason to believe that characters as highly variable as fertility and hatchability may be governed to a considerable extent by a number of physiological factors. Recent experimental work in the field of endocrinology suggests that the thyroid gland through its hormone thyroxine, which regulates many body processes, may play an important role in the reproductive ability of the domestic fowl.

Hypothyroidism has been produced experimentally in many classes of animals, including poultry, by feeding goitrogenic substances such as thiouracil. Thiouracil inhibits the formation of thyroxine by preventing the thyroid gland from utilizing iodine to form thyroxine. By inhibiting the secretion of thyroxine, thiouracil decreases the basal metabolic rate and causes hypertrophy and hyperplasia of the thyroid gland. Mild hyperthyroidism has more recently been produced experimentally in chickens by feeding thyroprotein, a substance having approximately three per cent the thyroidal activity of d,l-thyroxine. Thyroprotein supplements the normal level of natural thyroxine secretion of the thyroid gland, thereby elevating the normal metabolic rate.

During recent years considerable research has been conducted in an endeavor to determine the effects of thiouracil and thyroprotein upon the egg production of laying hens. It has been shown rather conclusively that the fertilizing capacity of the spermatozoa of domestic fowl are somewhat adversely affected by experimental alteration in the thyroid status. However, in the female domestic fowl, little evidence has been presented to indicate the effects of similar treatment upon fertility and hatchability.

With these factors in mind, it seemed of considerable scientific and practical interest to further investigate this field of experimental study in an attempt to determine the effects of hypo - and hyperthyroidism upon fertility and hatchability in the female domestic fowl.

REVIEW OF LITERATURE

The thyroid gland, through its hormone thyroxine, is known to play an important role in animal physiology and has been shown to regulate many body processes. It has been established that the thyroid hormone is necessary for the normal growth and development of young animals and for the maintenance of normal metabolism in animals of all ages. The thyroid hormone is also thought to have a considerable regulatory affect upon the reproductive ability of domestic animals. In recent years considerable work has been conducted to determine the effects of the thyroid hormone upon reproduction, but its exact role still is not well understood.

Since the report of Crew and Huxley (1923), considerable interest has been focused on the influence of the thyroid gland upon the reproductive activity of the domestic fowl. These workers fed chicks a ration containing desiccated thyroid, beginning at three months of age. The treated birds laid an average of 40 eggs more than the controls in their first laying year. However, this difference in egg production was not considered to be entirely due to the treatment; because of the small number of birds used in the experiment. Crew (1925) reported rejuvenation of the fowl by thyroid medication. Desiccated thyroid was fed to five cocks and seven hens, five to eight years of age, for six months from May to October. After molting, the plumage was characteristic of younger fowl, head furnishings became red and turgid and egg production increased.

Cole and Hutt (1927) fed White Leghorn hens desiccated thyroid, containing 0.2 per cent iodine, at a level of 196 milligrams per bird daily for a period of six weeks beginning November 1. This dosage of 59 milligrams per pound of live weight had no effect on body weight or egg production. Zawadowsky et al. (1928) found a significant increase in the egg production of hens fed 0.01 and 0.05 grams of desiccated thyroid daily. Asmundson (1931) fed desiccated thyroid to hens during February and March. The amount fed was equivalent to about one milligram per 1750 grams of body weight, daily. All birds lost weight when given thyroid and egg production ceased in a relatively short time. These results confirm the report that daily doses of 4 and 8 milligrams of desiccated thyroid per kilogram of body weight was lethal to nine month-old chickens of both sexes (Hutt, 1930).

In an attempt to determine the possible relationship between the thyroid and egg production, Winchester (1939) studied the effect of thyroidectomy upon the egg production of hens and found that thyroidectomy caused a decrease in the egg production of a group of White Leghorn hens. However, when these hens were injected with thyroxine, egg production increased to approximately 40 per cent of that of a group of normal fowl. Taylor and Burmester (1940) also studied the effect of thyroidectomy upon egg production. The average egg production of completely thyroidectomized and incompletely thyroidectomized birds was 35.8 and 66.7 per cent, respectively, of that of the controls. Feeding 0.3 grams of desiccated thyroid containing 0.3 per cent iodine per bird per day for a 30-day period to these operated birds had no affect on egg production. Andrews and Schnetzler (1945) reported that there was no apparent effect on the egg production of yearling White Leghorn hens fed thiouracil at a level of 0.2 per cent of the ration.

However, Berg and Bearse (1948) found that the inclusion of 0.1 per cent of thiouracil in the ration resulted in a decrease in the egg production of White Leghorn hens.

It has been suggested that in the selection of breeding stock for a high rate of egg production, birds less subject to seasonal environmental conditions have resulted, and this selection has produced birds whose thyroids maintain a more uniform rate of secretion throughout the year. Consequently, the gonadotropic hormone of the anterior pituitary is increased and the rate of laying maintained at a much higher level. With this theory in mind, Turner et al. (1945a), (1945b), and (1946) fed thyroprotein to laying hens and reported that it was beneficial in maintaining a high level of egg production, especially during the second half of the first laying year and in the second laying year. These results were interpreted to indicate that the seasonal cycle of egg production was due in part to a reduced secretion of the thyroid hormone during the summer months. When the thyroid hormone was maintained at a more uniform level by feeding thyroprotein, egg production was maintained at a more uniform level during this period of lowered normal thyroxine secretion.

Blaxter (1943) also fed thyroprotein to dairy cows and reported a 16 per cent increase in milk production by feeding a dose of 15 grams daily. However, it was not possible to prolong milk production in cows approaching the end of their normal lactation period. Hibbs and Krauss (1947) fed dairy cows thyroprotein at the rate of one gram per 53 pounds of body weight and concluded that a temporary increase of about 20 per cent in milk production will result if the feed intake is adjusted to the increased milk production.

Turner and Kempster (1947) concluded that a mild degree of hyperthyroidism, continually induced by feeding thyroprotein to fowl with advancing age, tends to inhibit the normal rate of senescence as measured by declining egg production. Turner and Kempster (1948) maintained hens on a ration containing thyroprotein continuously for four and five years. During their sixth laying year these hens laid an average of 118.5 eggs in comparison with a similar group of controls, which laid an average of 35.1 eggs. The average of 35.1 eggs laid by the controls was only 29.6 per cent of the average of 118.5 eggs laid by the thyroprotein-fed birds. The thyroprotein-fed birds also laid 26.6 per cent more eggs during their sixth laying year than they laid during the previous year. These results were interpreted as indicating that the normal thyroid hormone secretion rate declines with advancing age and that the thyroid hormone becomes a limiting factor in egg production. The continuous feeding of thyroprotein maintains the circulating hormone at a more uniform level, thus preventing the gradual decline in egg production associated with senescence.

In contrast to the reports of Turner et al., Temperton and Dudley (1947) reported no stimulus to egg production by the addition of thyroprotein to the diets of laying hens. Hutt and Gowe (1948) questioned the marked stimulatory effect of thyroprotein on egg production, as reported by the Turner et al. Hutt and Gowe found that thyroprotein had no effect upon egg production, except to decrease the number laid during the first eight weeks of treatment. Gutteridge and Novikoff (1947) and Berg and Bearse (1948) were also unable to increase egg production by feeding thyroprotein.

The decrease in egg production resulting from thyroidectomy and the inhibition of thyroxine formation by thiouracil indicates that the thyroid gland is closely associated with the processes involved in egg production. On the other hand, the conflicting evidence submitted with respect to the effect of mild hyperthyroidism upon egg production hardly validates the use of thyroprotein in supplementing the bird's normal titer of thyroid hormone.

In female experimental animals the thyroid gland has been shown to have considerable influence upon ovarian function and normal reproduction, but as yet its exact role has not been definitely established. Hyperthyroidism has been shown to have a direct affect on the reproductive processes of both normal and pregnant rats (Weichert, 1930). In the pregnant rat there was a prolongation of the gestation period, while in the normal rat, the dioestrus period persisted as long as desiccated thyroid feeding continued. Fluhman (1934) administered both thyroid extracts and thyroxine to immature rats. The ovarian weight, which is normally increased by gonad-stimulating hormones, was partly inhibited, indicating that the overactive thyroid gland inhibits some phases of ovarian function. This abnormal ovarian function was thought to be the result of variations in the basal metabolism. Evidence was submitted by Kraatz (1939) indicating that hyperthyroidism stimulates the release of the luteinizing hormone from the anterior pituitary of the rat. Thyroid treatment during the summer proved deleterious to reproduction. Ershoff (1945) noted a marked inhibition of ovarian development in rats fed 0.5 and 1.0 per cent desiccated thyroid. No mature follicles or corpora lutea were present in any of these thyroid-fed rats; immature follicles were numerous and many young follicles were observed undergoing atresia. These infantile ovaries were thought to be due to impaired secretion of the anterior pituitary gonadotropins.

In the domestic female fowl, Gutteridge and Novikoff (1947) reported that the hatchability of eggs produced by thyroprotein-fed females was not deleteriously affected. Wheeler and Hoffman (1948b) fed Rhode Island Red pullets 10 grams of thyroprotein per 100 pounds of feed from the day of hatch and found that fertility and hatchability of eggs from the treated females was as good as the control eggs.

Hypothyroidism, induced with thiouracil, in the rat has been shown by Jones et al. (1946) to have no effect on female sterility; but continuation of gestation is interfered with, causing resorption of embryos in 100 per cent of the cases. Andrews and Schnetzler (1945) fed White Leghorn hens on a ration containing 0.2 per cent thiouracil and reported that thiouracil, at the level fed, had no adverse affect upon fertility or hatchability in the adult female fowl.

Since Crew's report on the rejuvenation of the male fowl by thyroid feeding, there has been considerable interest with respect to the influence of the thyroid gland upon the reproductive capacity of males. Cohen (1935) fed desiccated thyroid to male rats before and after puberty and found that these thyroid-fed males bred normally and fathered healthy litters. There was no deleterious effect upon mating reactions or fertility. However, Smelser (1939) reported that injections of thyroxine or the feeding of desiccated thyroid had a deleterious effect upon sperm production in male rats. It was suggested that the amount of male hormone secreted by these hyper-thyroid male rats was reduced, the accessories being of a castrate type.

Reineke et al. (1941) studied the effect of thyroidectomy on the anterior pituitary hormones in young goats and reported that the concentration of gonadotropic hormones was low and that the testes showed a lack of stimulation. Stein and Lisle (1942) also determined the effect of thyroidectomy upon the gonadotropic potency of the pituitaries. The data presented indicated a decrease in the gonado-stimulating potency of the pituitaries of young male rats 21 to 50 days after thyroidectomy. However, hypothyroidism, as induced by thiouracil, was reported by Jones, Delfs, and Foote (1946) to have no effect upon the reproductive capacity of the adult male rats as judged by their ability to sire litters.

Changes in the level of activity of the thyroid gland resulting from high environmental temperature has been suggested by Berliner and Warbritton (1937) as the cause of lowered fertility in rams during the summer. They found that thyroidectomy of rams produced a decrease in semen volume and spermatozoa concentration. Operated rams and rams of lowered fertility administered thyroxine produced semen with an increased number of spermatozoa by August, while untreated rams did not produce normal semen until October of the same year. Further work on the effect of temperature and thyroid involvement in lowered fertility of rams has been conducted by Bogart and Mayer (1946). The evidence submitted by these workers showed that high summer temperatures are a major factor in the production of summer sterility in rams, since thyroxine and thyroactive proteins alleviated the symptoms of summer sterility resulting from impaired spermatogenic activity. Also, it was concluded that the thyroid gland is of major importance in the reproductive physiology of the ram and that changes in environmental temperatures produce variations in the reproductive activity in the ram indirectly through the thyroid gland.

In the domestic fowl, Wheeler and Andrews (1943) investigated the influence of season on semen production. Their results indicated that a significant seasonal variation exists in semen volume, quality, and concentration, the greatest volumes being produced between November and March. More recently, Shaffner and Andrews (1948) studied the effect of hypothyroidism, experimentally induced with thiouracil, upon semen quality in Barred Plymouth Rock males and found that the volume of semen was reduced in comparison with that of the control males. However, neither sperm concentration nor total number of spermatozoa was markedly affected by the feeding of thiouracil but there was a significant reduction in actual male fertility. The effects of mild hyperthyroidism on the semen quality of Barred Plymouth Rock males was also studied by Shaffner (1948). There was no effect on sperm production in a group of six males fed thyroprotein, but the semen quality was reduced as determined by actual fertility tests. These results suggest that either thiouracil or thyroprotein, at the levels fed, decreased the ability of the gonads to produce viable spermatozoa capable of surviving normal lengths of time in the hen's oviduct.

Goiterogenic substances, such as thiourea and thiouracil, fed to females have been shown to produce offspring with enlarged thyroid glands. Hughes (1944) produced cretinism in new born rats by the daily administration of thiouracil and found that the young of thiouracil-treated females showed hyperplasia of the thyroid gland as early as one day of age. Gordon and Charipper (1944) reported that young rats nursed by mothers maintained on a diet containing 0.5 per cent thiourea were found to have hyperemic thyroids. Pregnant rats fed a ration containing thiourea have been reported by Goldsmith et al. (1945) to produce offspring with thyroid hyperplasia. These results were interpreted as indicating that the effects of thiourea were indirectly transmitted through the placenta or the milk. Monroe and Turner (1946) also found a marked enlargement of the thyroid glands of young rats produced by thiouracil-fed mothers and concluded that thiouracil must pass through the mammary gland epithelium into the milk. However, the injections of successively increasing doses d,l-thyroxine into the thiouracil-treated mothers did not check the thyroid enlargement of the young. Since thyroxine was administered in doses as high as 65 per cent above the normal requirements of the lactating rat, it was assumed that the mammary epithelium of the rat is impermeable to thyroxine.

Andrews and Schnetzler (1945) fed thiouracil to hens in a ration at a level of 0.2 per cent and found that chicks with goiterous glands were produced. Evidence was submitted indicating that thiouracil is transmitted through the egg to the developing chick embryo. Hollander and Riddle (1943) and (1946) cited evidence indicating that mild goiter in breeding female pigeons was associated with hyperemic and enlarged thyroid glands of the offspring at hatching time. The hatching of the goiterous squabs tended to be delayed by a day or more and they exhibited various degrees of delibility. Correction of the goiter in the parent by KI therapy prevented both goiter and weakness in the young. Congenital goiter was also observed in baby chicks hatched from hens on a goiterogenic ration deficient in iodine (Gassner and Wilgus, 1940). The thyroid glands of these chicks were 2 to 3 times the average size of the glands of the chicks hatched from hens receiving iodine supplements. Hyperplasia, as determined histologically, was severe.

Changes in the development of frogs, sea urchins, and chicks have been influenced by either thiourea or thiouracil treatment. Gordon et al. (1943) and (1945) subjected frog larvae to the action of thiourea at an early age of development; practically no progress was made in metamorphosis. The inhibitory effect of thiouracil on metamorphosis was overcome completely by the administration of thyroxine. Hughes and Astwood (1944) also inhibited metamorphosis in tadpoles with thiouracil. Cleavage was completely inhibited in sea urchin eggs with high concentrations of thiourea (Bevelander, 1946). Lower concentrations specifically inhibited gastrulation, while the lowest concentrations produced a retardation in the growth of the plutei. Grossowicz (1946) introduced thiourea into the yolk sacs of developing chick embryos and found that the hatching time of the thiourea-treated embryos was delayed up to ten days. In some experiments, with 17-day old eggs, 10 gamma of thyroxine fully neutralized the action of 2 gamma of thiourea when the two drugs were administered simultaneously. It was suggested that the inhibitory effect of thiourea on chick development may be due to the interference with the normal thyroxine metabolism.

The effect of the thyroid hormone or synthetic thyroxine on the metabolic rate and the thyroid size of experimental animals has been well established, but relatively little information is available on the effect of hyperthyroidism of the dam on the rate of embryonic development and thyroid size of her progeny. Greenwood and Chaudhuri

(1928) studied the effect of thyroxine injections on incubating eggs. They attributed the production of dwarf embryos to an increased metabolic rate, suggesting that the active secretion of the thyroid gland may be a major factor in determining growth-rates of chick embryos. Riddle (1930) found overactive thyroid glands in two pigeons (sisters) whose eggs required an extra one to three days for embryonic development, while the eggs of their sisters with normal thyroids and normal metabolism hatched at the normal time. It was suggested that thyroxine normally enters the egg from the mother and that the so-called normal incubation time of the bird embryo is contingent upon an adequate supply of thyroxine within the egg; an inadequate supply of thyroid hormone resulting in a prolongation of the time required for the completion of embryonic development. Wheeler and Hoffman (1948) reported that goiterous chicks were produced by females receiving thyroprotein at a level of 0.02 per cent of the ration. Reduced rate of embryonic development, significantly greater thyroid size at hatching time, and lowered heart rate led Wheeler and Hoffman (1948b) to conclude that the chicks from hyperthyroid hens were themselves hypothyroid.

It has long been known that the activity of the thyroid gland plays an important role in the regulation of metabolic processes. Slight alterations in the rate of thyroid secretion by this gland are accompanied by changes in the rate of normal metabolism, which may affect certain reproductive functions. The seasonal variations in thyroid size in three species of pigeons has been investigated by Riddle and Fisher (1925). The weights of the thyroid glands of these birds, sacrificed during all months of a three-year period, indicated a nearly simultaneous enlargement in the autumn and winter months and a progressive decrease in the size during the months of spring and summer. The season of active reproduction in the pigeon occurred during the period of decline in thyroid size. Riddle (1925) reported that the thyroids and gonads of pigeons 7 to 30 months of age simultaneously undergo seasonal changes in size in the opposite direction. The autumn and winter months of increased thyroid size are periods of decreased gonad size, while the spring and summer months of reduced thyroid size are seasons of gonad enlargement. It was suggested that both thyroids and gonads in birds are intimately related to reproduction. Chaudhuri (1928) found that the iodine content of the thyroid of sexually immature male fowl was lower than that of the thyroid of the sexually mature. The increase in iodine content was coincident with the attainment of sexual maturity, as estimated by the histological picture of the testes. The average iodine content of the thyroid gland at sexual maturity was 0.697 ± 0.033 per cent of the total weight.

In the domestic fowl, thyroid weight changes have been shown by Cruickshank (1929) and Galpin (1938) to be a useful index of thyroidal activity. Cruickshank noted that the iodine content of the thyroid of the chicken varied with the season, being high in the late winter months and low in the summer months. The seasonal variation in the iodine content followed the variation in thyroid weight very closely. Galpin found, similarly, that the thyroid weights of the fowl decreased from the time of the onset of laying until July and then increased in size during the winter months. These findings indicate that there is a direct relationship between iodine content and the weight of the thyroid gland, thyroid weight apparently being indicative of thyroidal activity in the fowl. Winchester (1940) studied the seasonal metabolic rhythms of the domestic fowl and found a parallelism between metabolic rate and egg production. The results of his injection studies on egg production indicated that the laying hen's thyroid gland secretes more than 0.35 milligrams of thyroxine per day.

A method for determining the rate of thyroid hormone secretion of experimental animals was developed by Dempsey and Astwood (1943). They treated rats simultaneously with thiouracil and graded doses of thyroxine. The quantity of thyroxine required to maintain a thyroid of normal weight was considered to be an estimate of the normal thyroid secretion rate of an animal. It was estimated that the thyroid's secretion rate in rats at normal temperature (25°C.) was 5.2 micrograms of l-thyroxine per day. At 1°C. the secretion rate increased to 9.5 micrograms, while at 35°C. it was decreased to 1.7 micrograms. Reineke, Mixner, and Turner (1945) studied the effect of graded doses of thyroxine on the metabolic rate of rats treated with thiouracil. In rats fed sufficient thiouracil to block the formation of the thyroid hormone and kept at a temperature of about 25°C., the metabolic rate was returned to normal by the injection of 4.75 gamma of d,l-thyroxine per day; 4.80 gamma, daily, was required to return the thyroid weights to normal. The seasonal rhythms in thyroid hormone secretion of two-week-old chicks was determined by Reineke and Turner (1946) during one entire year. The maximum secretion in males, equivalent to 2.7 gamma of d,l-thyroxine daily, was observed in the fall months of October and November. The secretion rate declined thereafter to 2.1 gamma during February and finally to 0.9 gamma, where it remained at a low level until August.

In an extensive study of the thyroxine secretion rate of growing chicks, Schultze and Turner (1945) observed that the secretion rate is highest per 100 grams of body weight in young birds and declines gradually with advancing age up to about six months of age. Turner (1948a) estimated the thyroid secretion rate of two-year-old White Leghorn hens and found that the average daily secretion of 1900-gram hens was 13 gamma in January, but by May, when the egg production began to decline, the secretion rate had decreased at least 15 per cent. Also, the thyroid secretion rate of growing and mature mice has been determined by Hurst and Turner (1947).

Young mice, mature female, and mature male mice were estimated to secrete 9.2, 5.59, and 2.6 gamma of d,l-thyroxine per day, respectively. During the summer, with a temperature of 85°F., the daily thyroid secretion rate decreased in the mature males to 0.7 gamma and in the mature females to 5.2 gamma.

The effects of thyroxine on metabolic rate, thyroid size, and physiological processes of animals has been well established. The thyroidal activity of iodinated proteins has been shown by Reineke and Turner (1943a) to be equivalent to approximately three per cent of that of d,l-thyroxine.

Koger and Turner (1943) determined the effects of thyroprotein feeding on the metabolic rate of rats. The CO_2 production of groups of four male rats receiving thyroprotein at levels up to 0.02 per cent was not affected, while feeding a level of 0.04 per cent caused a significant rise in CO_2 production. Whereas, the CO_2 production of female rats was increased by all levels of thyroprotein. Using guinea pigs, Reineke and Turner (1945) similarly determined the effect of thyroprotein upon CO_2 production. The increase in CO_2 output, above normal, varied from 20.8 to 27.6 per cent for the 15 iodinated preparations assayed. Blaxter (1948) reported that iodinated casein markedly accelerated the basal metabolism of sheep. The feeding of 2 grams of iodinated casein per day elevated the metabolic rate 12.4 per cent, while 4 grams daily increased it by 36.9 per cent. In milking cows, the feeding of 25 grams of iodinated casein daily resulted in a 30 per cent increase in heat production (Thorbek et al. 1948).

Many substances have been reported to inhibit the thyroid hormone secretion, decrease basal metabolic rate, and cause hypertrophy and hyperplasia of the thyroid gland. It was suggested by Mackenzie and Mackenzie (1943) that these goiterogenic substances produce their effect by depressing the rate of thyroxine formation. These workers determined the effects of sulfonamides and thioureas on the thyroid gland and basal metabolism of rats, mice, and dogs. The basal metabolic rate of rats fed a ration containing two per cent sulfoguanidine decreased 10 per cent following five to seven days of continued administration and was -20 per cent after 10 to 14 days. Astwood et al. (1943) observed a 30 per cent drop in the B M R of rats given 0.1 per cent thiourea in the drinking water for 27 days. Reineke et al. (1945) noted a depression of 23.7 per cent in the B M R and a two fold increase in the thyroid gland weights of rats receiving 0.1 per cent thiouracil in the drinking water. Meyer and Ransom (1945) determined the effects of thyroidectomy or 0.5 per cent thiouracil in the ration on the metabolic rate of rats and found that they both produced comparable results in reducing the B M R to -41 per cent. Shaffner and Andrews reported a 40 per cent decrease in the resting metabolism of Barred Plymouth Rock males receiving rations containing 0.2 or 0.5 per cent thiouracil for a period of eighteen weeks.

Recently certain compounds, sulfonamides, thiourea, and related substances such as thiouracil, have been found to exert a goiterogenic effect that is not prevented by a relatively high intake of iodine. Mackenzie, Mackenzie, and McCollum (1941) observed that rats receiving a diet containing one to two per cent sulfaguanidine exhibited hypertrophied and hyperemic thyroids, being 3 to 8 times larger than those of the controls. The hyperemic and enlarged thyroid glands were found to exhibit histologically a reduction in colloid and an increase in height of the epithelium. Mackenzie and Mackenzie (1943), in continuing their studies, found that sulfonamides and thioureas had this goiterogenic effect on the thyroid glands of rats, mice, and dogs. In immature rats receiving from 0.5 to 3.0 per cent sulfaguanidine, the thyroid weight at the end of two weeks was proportional to the dietary level and represented an increase of 60 to 350 per cent. Thiourea was approximately eight times more effective than the same level of sulfaguanidine in producing thyroid enlargement. It was suggested that sulfonamides and thioureas exert a depressing influence on the thyroid gland by interfering with the rate of thyroid hormone formation. Astwood et al. (1943) similarly concluded that the goiterogenic action of these substances resides in their interference with the synthesis of the thyroid hormone and as a result the thyroid gland hypertrophies due to the increased secretion of the thyrotropic hormone of the anterior pituitary gland. Astwood (1943) tested the relative effectiveness of 106 chemical compounds in inhibiting the function of the thyroid gland of young rats and found that 2-thiouracil was the most effective goiterogenic substance.

Bauman et al. (1944) administered thiourea to rabbits and noted thyroid hyperplasia. Iodine and thyroxine-iodine analysis of the thyroid glands showed a rapid decrease in both thyroxine and non-thyroxine iodine. This inhibition of thyroxine formation indicated that thiourea had produced a functional thyrostasis of the thyroid cells. Thiouracil administered to rats as a 0.1 per cent solution in the drinking water was found to cause a rapid loss of iodine from the thyroid gland within 24 hours (Bissell and Astwood, 1944). Iodine added to the diet had no effect on the loss or reaccumulation of iodine in the gland, indicating that thiouracil inhibits the production of thyroid hormone by preventing the accumulation of iodine in the gland.

Keston et al. (1944) administered radioactive iodine to young and adult normal and thiourea-treated rats and found that the analysis of the thyroid glands of the thiourea-treated rats, 48 hours after the injections of iodine, revealed no appreciable amount of radioactive iodine in any form, whereas the thyroid glands of normal rats contained considerable quantities of radioactive iodine. Larson et al. (1945) have shown that thiouracil possess the property of inhibiting the collection of radioactive iodine by the thyroid gland within an hour of its time of administration. This effect of thiouracil on reducing the quantity of radioactive iodine collected by the thyroid gland prevents the thyroid gland from utilizing iodine to form the thyroid hormone. Gordon et al. (1946) compared the effects of thiouracil and thyroidectomy upon the metabolism of the rat and found that thiouracil and thyroidectomy depress oxygen consumption to approximately similar levels.

The effects of thiouracil and thiourea on the thyroid glands of chicks were studied by Mixner et al. (1944). They found that 0.1 per cent of these substances in the diet for fourteen days was optimal for the production of maximal enlargement of the thyroid in one to two day-old White Plymouth Rock chicks. Increasing dosages of thyroxine decreased the thyroid enlargement produced by 0.1 per cent thiouracil. The influence of thiouracil on the size of various glands of ten week-old chicks was determined by Glazener and Jull (1946). Greater thyroid enlargement was observed in the males than in the females, indicating a sex difference. In the thiouracil-treated males, the testes were smaller and the comb size reduced, probably indicating a decrease in the production of androgen by the interstitial cells.

The hormone produced by the thyroid gland has been shown by Harington and Salter (1930) to be equivalent to the l-form of thyroxine in biological activity. The isolation of crystalline thyroxine from iodinated protein was first obtained by Ludwig and Mutzenbecker (1936). Harington and Pitt Rivers (1939) confirmed the findings of the two German workers and postulated that the formation of thyroxine in iodinated casein was brought about by either the iodine itself being able to effect the oxidation coupling of two molecules of diiodotyrosine in the biogenesis of thyroxine or the casein itself may contain performed thyronine as one of its constituent amino acids and this may be iodinated to thyroxine.

Lerman and Salter (1939) fed iodinated serum protein to hypothyroid subjects and found that the metabolic rate was raised and the hypothyroid symptoms relieved. Using iodinated casein of high thyroidal activity, Reineke and Turner (1943b) obtained 424 milligrams of crystalline thyroxine from 100 grams of the iodinated material. The biological value of the iodinated protein was found to be equivalent to three per cent d,l-thyroxine or about 1.5 per cent of the l-form. The l-form of thyroxine has been shown by Reineke and Turner (1943a) to produce twice the thyroidal activity of d,l-thyroxine, as estimated by its relative ability to reduce the thyroid weights of thiouracil-treated chicks and rats. Reineke and Turner (1945) determined the thyroxine content of 15 iodinated caseins having thyroidal activity and found that they contained from three to four per cent d,l-thyroxine, as indicated by either chemical analysis or biological assay.

Irwin, Reineke, and Turner (1943) fed thyroprotein with a potency of 3.1 per cent d,l-thyroxine to chicks and found that it had a depressing effect upon the weights of the thyroid glands at twelve to fourteen weeks of age. The thyroid glands of normal chicks at six weeks of age were found to be twice the size of those of a comparable group of chicks fed thyroprotein containing 3.1 per cent d,l-thyroxine (Turner, Irwin, and Reineke, 1944). Koger and Turner (1943) fed male rats a ration containing thyroprotein at various levels from 0.005 to 0.32 per cent and found that there was a tendency for the thyroids of the treated animals to be slightly smaller than those of the controls. Histological examination of the glands showed the thyroids of all treated animals to be inactive and filled with colloid. Wheeler and Hoffman (1948a) observed that thyroprotein treatment resulted in a significant decrease in weight of the testes, adrenals, thyroids, and pituitary glands of the male chickens studied; the thyroid glands of the treated males were 45 per cent smaller in size than those of the controls. Turner (1948b) reported that a mild degree of hyperthyroidism could be maintained in two-year-old hens by feeding thyroprotein at a level of 10 grams per 100 pounds of feed without affecting the glands or organs other than the thyroid gland. The gradual decline in thyroid weight observed in hens during a period of one year was apparently due to the presence of an excess of thyroid hormone, the birds own thyroid finally ceasing to function.

MATERIAL AND PROCEDURE

In the fall of 1947, sixty New Hampshire pullets were selected and placed in individual laying cages. On the basis of subsequent egg production during the pre-treatment period, these females were later divided into three comparable groups. All birds, during this pre-treatment period, received the Maryland Experiment Station mash ad libitum.

On November 13, 1947, treatment was initiated on the following basis: Group A - control, Group B - 10 grams of thyroprotein per 100 pounds of the ration (0.022 per cent), and Group C - 0.3 per cent thiouracil in the ration. Due to decreased egg production, the level of thiouracil was later reduced to 0.1 per cent. Body weight was recorded for each female at the beginning of the experiment and at monthly intervals thereafter. Individual daily egg production and egg weight records were kept. The eggs were weighed to the nearest gram on a Toledo scale.

The semen used to artificially inseminate these females was obtained from a group of twelve New Hampshire males by means of the massage method described by Burrows and Quinn (1937). Each female was inseminated twice weekly with 0.1 cc. of undiluted pooled semen from these males. Starting on the second day following the initiation of insemination, eggs for incubation were saved and set once a week. From the time of collection until setting, each day's eggs were stored in a refrigerator unit held at 55 to 60° F., some eggs being held for as long as seven days while others were held for only one day. At the time of setting, the eggs were pedigreed, so that fertility, hatchability, and embryonic mortality could be determined for each individual female. The eggs of each weekly setting were candled and the fertility determined on the eighteenth day of incubation. At the conclusion of each hatch, hatchability was determined and the remaining eggs broken out to check the date of embryonic mortality.

Day-old chicks produced by these three groups of females were sacrificed and the thyroid glands dissected and weighed to the nearest 0.1 milligram on a precision balance. These observations were first made five weeks subsequent to the initiation of treatment. Two weeks following these preliminary observations, a series of nine weekly hatches of chicks from the same sources were sacrificed and their thyroid glands weighed.

On January 29, 1948, an additional group of five females was injected intramuscularly with 20 micrograms of d,l-thyroxine, daily. After four weeks the daily injected dosage was increased to 40 micrograms and subsequently to 100 micrograms, daily. Day-old chicks produced by these females were also sacrificed and their thyroid glands weighed.

Eggs from normal females were injected with aqueous solutions of thyroxine or thyroprotein by puncturing a small hole in the shell over the air cell and applying the solution by means of a tuberculin syringe to the inner shell membrane. Following the injections, the holes were sealed with paraffin wax and the eggs placed in the incubator. Thyroxine was injected at levels of 6, 12, 20, and 40 micrograms and thyroprotein at levels equivalent to 9, 12, 20, and 40 micrograms of d,l-thyroxine. Similarly, eggs produced by the thyroprotein-fed females were also injected with thyroxine, at levels of 2, 4, 6, 8, and 10 micrograms. Day-old chicks hatched from the injected eggs were sacrificed and the thyroid glands weighed. Approximately 400 eggs were used in these injection studies.

The influence of these exogenous factors on the incubation period was determined by recording, at four-hour intervals, the number of chicks hatched, starting at the twentieth day of incubation and continuing until the completion of each weekly hatch. In order to eliminate the affects of incubation environmental factors, all eggs for each weekly hatch were incubated and hatched in the same machine. These observations were made over a period of sixteen weekly hatches, starting seven weeks after the initiation of treatment.

Smith, Emmens and Parkes (1947) have proposed a method for determining the metabolic rate of small animals by recording their survival time in a closed vessel. These workers found that resistance to anoxia was increased by thyroidectomy and, in contrast, resistance was decreased by the administration of desiccated thyroid or thyroxine. In using this technique in the present experiment, day-old chicks averaging 40 grams were confined in sealed vessels of approximately 500 ml. and maintained at 90° F. The survival time of the chicks under these conditions was recorded in minutes. The terminal symptoms were a progressive increase in the rate and depth of respiration, coma, and brief convolutions. The end point was taken at the time of the last breath, being readily determined within a matter of seconds.

At the end of the first trial, which was terminated twenty-four weeks after the initiation of treatment, all females were maintained on the control ration for an additional six-week post-treatment period. During this period records were obtained on fertility, hatchability, and egg production.

On September 1, 1948, sixty additional New Hampshire pullets were selected and placed in individual laying cages. After a four-week pre-treatment period, these females were divided at random into three groups of twenty birds each. Group D served as controls and groups E and F received 0.022 per cent thyroprotein and 0.1 per cent thiouracil, respectively, in the control ration. Fertility, hatchability, and egg production were obtained on an individual basis for these groups of females. At the beginning of this trial, comb area measurements were made and at monthly intervals thereafter. This measurement consisted of the product of the length at the longest point times the height at the highest point measured in centimeters. Feed consumption was recorded for each group of females during the period of treatment. Body weight was recorded in grams for each female at the beginning of the experiment and once a month thereafter. The females in this series of experiments were maintained on treatment for a period of twelve weeks subsequent to the initiation of treatment.

Since maximum fertility is apparently obtained by inseminating each female twice weekly with 0.1 cc. of semen, during the second trial a somewhat different technique was employed to test the fertility and hatchability of these females. This technique was designed to show differences in fertility and hatchability that might not be otherwise apparent under more optimal conditions of artificial insemination. The technique was conducted as follows: Each female was inseminated once weekly (every Tuesday) with 0.05 cc. of undiluted pooled semen obtained from a group of ten New Hampshire males. Starting on the second day after the first insemination and after each successive weekly insemination, eggs for the following seven days were collected for incubation and labeled according to the day laid. Percentage fertility and hatchability was then determined for seven periods of one day each. This method was used because it was found that the fertility of a group of females tends to decrease slightly towards the end of each seven day period. The fertility and hatchability of eggs are known to decrease rather rapidly after a single mating (Nalbandov and Card, 1943). Therefore, at the end of the 12-week treatment period another method was employed to test the fertility and hatchability of these females. Eggs for incubation were collected over a three-week period following the last insemination and fertility and hatchability then determined for three periods of seven days each. This period was used because it has been shown that fertility tends to decline rapidly in the third week following the last mating.

Since rate of metabolism is known to be influenced to a large extent by the thyroid gland, metabolism tests were made at four-week intervals on the females of the second trial. Resting metabolism was determined by means of the indirect calorimetry method, employing a closed circuit type of respiratory apparatus. All metabolism tests were run at night between 6 p.m. and 12 p.m. after the birds had been fasted for approximately twelve hours. All measurements were recorded on the basis of cubic centimeters of oxygen consumed per kilogram per hour, corrected to standard temperature and pressure.

In order to determine the rate at which the chick's thyroid gland size is affected by feeding its dam thiouracil or thyroprotein,during the second trial, groups of ten chicks were sacrificed at weekly intervals and their thyroid glands weighed. These observations were made beginning with the first hatch after the initiation of treatment and continued for the next ten successive weekly hatches. All thyroid glands in this series were freshly dissected and weighed on a Roller-Smith balance to the nearest 0.1 milligram.

Chicks produced by these three groups of females were reared in electric battery-brooders with raised screen floors and were fed a conventional all-mash ration. In order to ascertain the change occurring in the thyroid weight during the first three weeks of the chick's life, samples of ten chicks, day-old, and 7, 14, and 21 days of age, respectively, were sacrificed, the thyroid glands removed and weighed to the nearest 0.1 milligram.

In order to obtain another measure of the thyroidal activity of the day-old chicks produced by the different groups of females, resting metabolism of groups of 18 of their chicks, maintained at 32° to 35° C., was measured. The method used for these determinations was the same as that employed to estimate the resting metabolism of the females. A series of eight observations were obtained on the chicks produced by the three different groups of females.

ANALYSIS OF FERTILITY AND HATCHABILITY DATA

In calculating the percentage fertility and hatchability of the eggs produced by each group of females, only those females represented in all of the experimental periods were included. Since the ranges in percentages was mostly between 50 and 100, the fertility and hatchability percentages of the eggs produced by each female were converted into angles, angle = $\sin^{-1}\sqrt{\text{percentage}}$. Then, in order to determine the difference among groups, the angles corresponding to the percentages were analysed by means of the analysis of variance method, as recommended by Snedecor (1946).

EXPERIMENTAL RESULTS

PART I

Observations on the Response of Females To Altered Metabolism

Fertility. Tables Ia and Ib present the percentage fertility of the eggs produced by the females in trial I and trial II, respectively.

In both trials, the addition of thyroprotein or thiouracil to the control ration had no affect upon subsequent fertility. In trial I, during the 24-week treatment period, the fertility of group B declined approximately five per cent, but this did not differ significantly from that of either the pre-treatment period or the post-treatment period. In trial II, during the pre-treatment period, the percentage fertility of group D was 77.05, while during the treatment period it increased to 87.04 per cent. This difference was due mainly to the fact that five of the females in this group produced all infertile eggs during the first week following the initiation of insemination.

In trial II, during the 12-week treatment period, each group of females was inseminated every Tuesday at approximately 5 p.m. Since few eggs are fertilized within 24 hours after insemination, the eggs laid on the following day (Wednesday) were assumed to be fertilized by sperm one day old and those laid on the following Tuesday as being fertilized by sperm seven days old. In order to increase the number of eggs included in the seven periods of one day each, the eggs laid following each of the ten successive weekly inseminations were combined and then the percentage fertility was determined for eggs assumed to be

Table 1a. - Fertility of eggs produced by the females in trial I

Period of Treatment	Total No. Set	Number Fertile	Per Cent Fertile
	CONTROL (group A)		
Pre-treatment	140	135	96.43
Treatment	1240	1187	95.72
Post-treatment	248	233	94.95
	THYROPROTEIN (group B)		
Pre-treatment	143	127	88.81
Treatment	1091	910	83.41
Post-treatment	202	177	87.62
	THIOURACIL (group C)		
Pre-treatment	161	144	89.44
Treatment (0.3%)	339	299	88.20
Treatment (0.1%)	1309	1144	87.39
Post-treatment	322	281	84.64

Table 1b. - Fertility of eggs produced by the females in trial II

Period of Treatment	Total No. Set	Number Fertile	Per Cent Fertile
	CONTROL (group D)		
Pre-treatment	366	282	77.05
Treatment	988	860	87.04
	THYROPROTEIN (group E)		
Pre-treatment	389	322	82.77
Treatment	964	781	81.01
	THIOURACIL (group F)		
Pre-treatment	345	316	91.59
Treatment	833	767	92.07

fertilized by spermatozoa one to seven days old.

The data presented in Figure 1 shows that sperm one to four days of age are about equally capable of fertilizing eggs produced by the three groups of females, while the fertility of the eggs fertilized by sperm five to seven days of age tend to decline rapidly, especially in the case of group-D and group-E females. The fertility of the group-F eggs remained at a high level until the sixth day and then decreased slightly.

Following the last insemination, 12 weeks subsequent to the initiation of treatment in trial II, the eggs produced by the three groups of females were collected over a period of three weeks and incubated. The percentage fertility of these eggs, determined for three periods of seven days each, are summarized in Table 2. Both the group-D and the group-F females gave better fertility in the first week following the last insemination than did the females in group E. However, in the second week, the group-F females gave better fertility than did the other two groups of females. During the third week there was very little difference in the fertility of the three groups of females. The control and the thyroprotein-fed females produced no fertile eggs after the sixteenth day, while the thiouracil-fed females produced fertile eggs up until the twentieth day after the last insemination.

Hatchability. The percentage hatchability of the fertile eggs produced by the females in trial I and trial II are shown in Tables 3a and 3b, respectively.

In trial I, thyroprotein at the level fed had no affect upon the hatchability of the eggs produced by the group-B females. During the

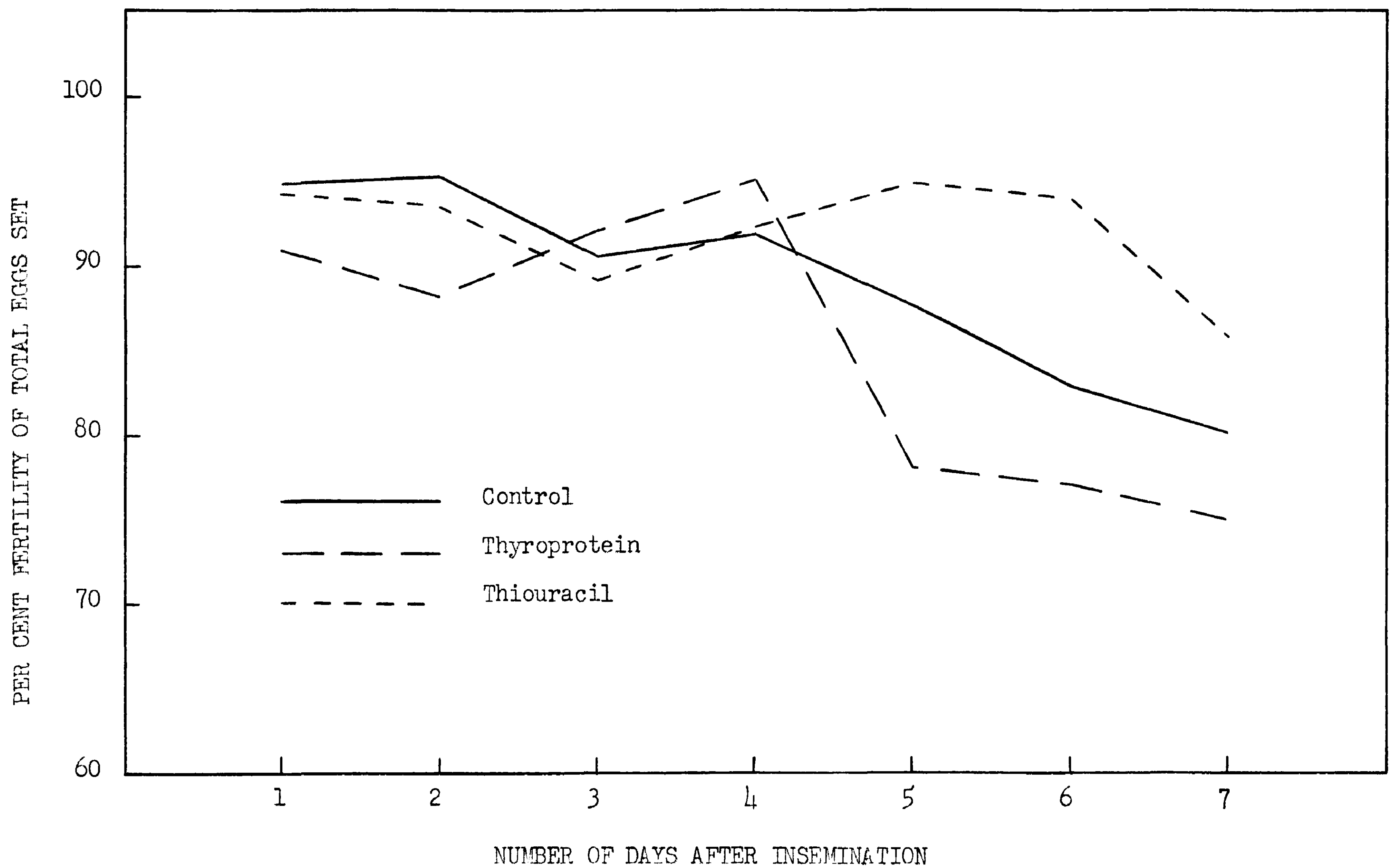

Figure 1. - Fertility of eggs laid 1 to 7 days after insemination

Table 2. - Effect of treatment on duration of fertility in trial II

Treatment	Period (days)	Number Set	Number Fertile	Per Cent Fertile
Control (group D)	1-7	105	100	95.23
	8-14	90	42	46.67
	15-21	108	8	7.40
Thyroprotein (group E)	1-7	87	73	83.91
	8-14	84	34	40.47
	15-21	97	11	11.34
Thiouracil (group F)	1-7	82	78	95.12
	8-14	79	56	70.89
	15-21	79	7	8.86

Table 3a. - Hatchability of eggs produced by the females in trial I

Period of Treatment	Number Fertile	Number Hatch	Per Cent Hatch
	CONTROL (group A)		
Pre-treatment	135	112	82.96
Treatment	1187	947	79.78
Post-treatment	141	126	89.36
	THYROPROTEIN (group B)		
Pre-treatment	127	104	81.89
Treatment	910	729	80.12
Post-treatment	90	68	75.55
	THIOURACIL (group C)		
Pre-treatment	144	115	79.86
Treatment (0.3%)	299	162	54.18 **
Treatment (0.1%)	1144	786	68.71
Post-treatment	182	159	87.36

** The difference between these values and thiouracil pre-treatment or post-treatment are highly significant, as determined by the method of Snedecor, 1946.

Table 3b. - Hatchability of eggs produced by the females in trial II

Period of Treatment	Number Fertile	Number Hatch	Per Cent Hatch
	CONTROL (group D)		
Pre-treatment	282	245	86.88
Treatment	860	707	82.21
	THYROPROTEIN (group E)		
Pre-treatment	322	280	86.95
Treatment	706	597	84.56
	THIOURACIL (group F)		
Pre-treatment	316	240	75.95
Treatment	767	587	76.53

pre-treatment period, the hatchability of the group-C females was 79.86 per cent. However, as soon as 0.3 per cent thiouracil was added to the ration of these females, it dropped to approximately 55 per cent and, during the next six weeks of the treatment period was 54.18 per cent. Hatchability improved after the level of thiouracil was reduced to 0.1 per cent and soon approached that of the control females, being 68.71 per cent for the remainder of the treatment period. During the post-treatment period, the hatchability returned to normal and was 87.36 per cent for this four-week period. The difference between the hatchability of the eggs produced by the thiouracil-fed females during the treatment period (54.18 per cent or 68.71 per cent) and the pre-treatment period (79.86 per cent) or the post-treatment period (87.36 per cent) are highly significant.

It is apparent from the results presented in Table 3b that neither thyroprotein nor thiouracil, at the levels fed, had any significant affect upon the hatchability of the fertile eggs produced by these two groups of females in trial II. The percentage hatchability of the eggs produced by each of these groups of females during the 12-week treatment period was comparable with the percentage hatchability of the eggs produced by the same groups of females during the 4-week pre-treatment period. However, there was a tendency for the hatchability of the thiouracil-fed females to decrease slightly towards the end of the treatment period.

It has been suggested that eggs fertilized by stale sperm are more apt to terminate their development prior to hatching than are eggs fertilized by fresh sperm. Therefore, in trial II, hatchability percentages were determined for seven periods of one day each. The results summarized in Figure 2 indicate that eggs fertilized by sperm seven days old tend to hatch equally as well as eggs fertilized by sperm one day old. The hatchability of the group-F eggs was consistently lower for each of the seven one-day periods than was the hatchability of the other two groups.

The fertile eggs saved during the two weeks following the last insemination were incubated and the percentage hatchability determined for two periods of seven days each. These results show that the eggs fertilized during the second week after the last insemination did not tend to hatch as well as eggs fertilized during the first week.

Embryonic Mortality. In trial I, during the 24-week treatment period, the date of embryonic mortality of all fertile eggs set was determined at the termination of each hatch. Figure 3 presents the distribution of embryonic mortality of the fertile eggs produced by the control females and the females fed thyroprotein or thiouracil. During the entire incubation period, the mortality of the fertile eggs produced by the thiouracil-fed females greatly exceeded that of the eggs produced by the control females, especially on the second and third days, on the eighteenth day of incubation, and for those embryos classified as dead-in-shell (D.I.S.). D.I.S. refers to a chick that was alive and fully developed at the conclusion of the hatch, but was apparently too weak to emerge from the partially pipped shell.

In Table 4, the distribution of embryonic mortality, shown in Figure 3, is broken down into percentages for three periods of seven days each and for the embryos classified dead-in-shell. These results show that the per cent embryonic mortality in each of the four periods was the greatest for the fertile eggs produced by the thiouracil-fed

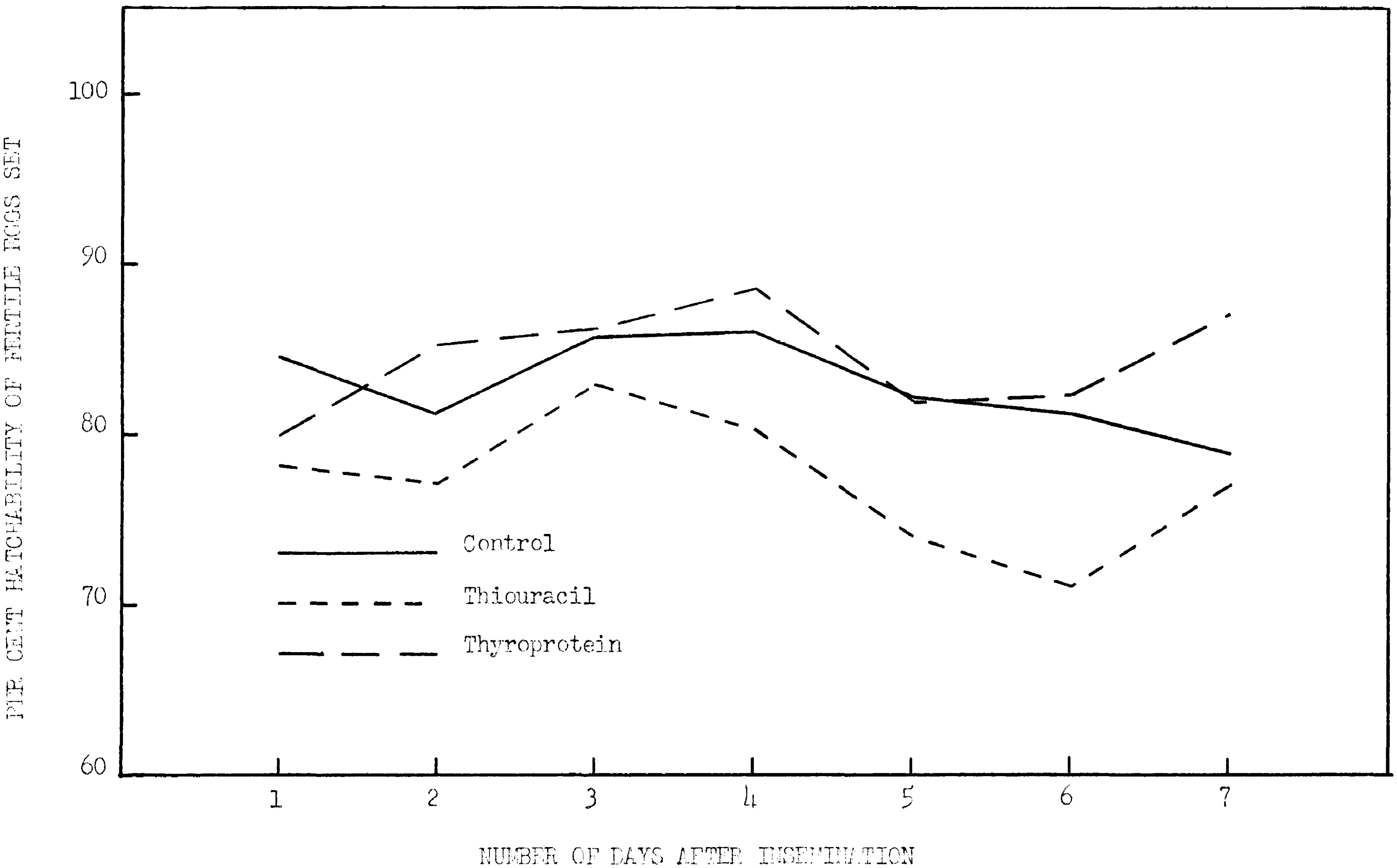

Figure 2. - Hatchability of eggs laid 1 to 7 days after insemination

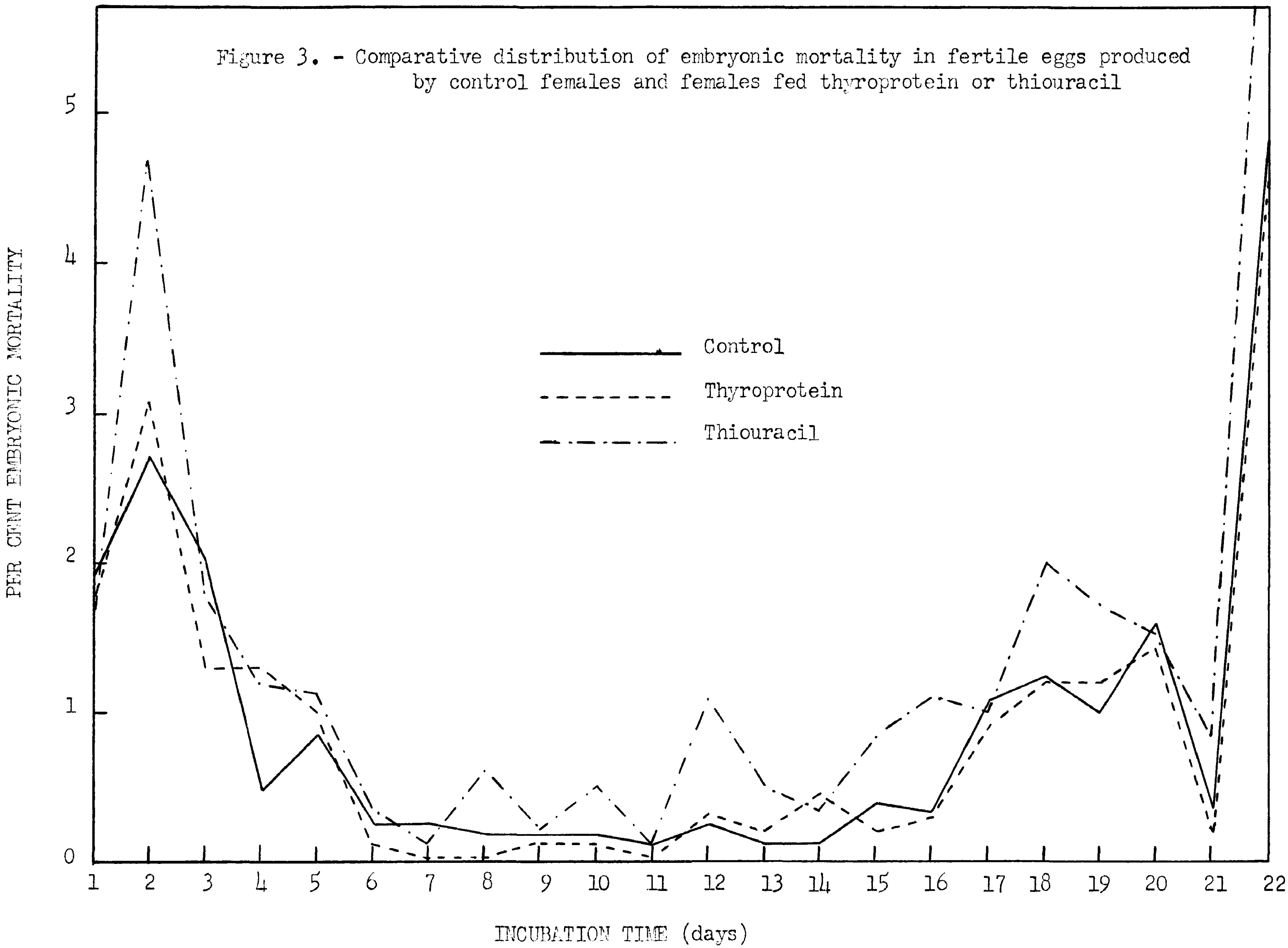

Figure 3. - Comparative distribution of embryonic mortality in fertile eggs produced by control females and females fed thyroprotein or thiouracil

Table 4. - Per cent embryonic mortality of fertile eggs produced by the females in trial I

Treatment	1-7 days	8-14	15-21	D.I.S.*
Control	8.51	1.01	6.06	4.62
Thyroprotein	8.57	1.21	5.49	4.61
Thiouracil (0.3%)	16.29	1.92	12.77	12.14
Thiouracil (0.1%)	10.17	3.90	7.81	5.20

* Dead-in-shell

females, especially during the 4-week period that these females were receiving 0.3 per cent thiouracil.

Egg Production. The egg production of the six groups of females, calculated in percentage, is shown in Figures 4a and 4b. The egg production of these females was analysed by means of the analysis of variance method of Snedecor (1946).

In trial I (groups A, B, and C), percentage egg production was determined, at bi-weekly intervals, over a 38-week period beginning September 13, 1947. The statistical analysis, for each of the experimental periods, revealed that there was no significant differences in egg production among the three groups of females. However, for a period of approximately ten weeks after the initiation of treatment, the egg production of group C was considerably lower than that of either of the other two groups. Due to this decreased production, four weeks subsequent to the beginning of treatment, the level of thiouracil in the ration of group C was reduced from 0.3 per cent to 0.1 per cent. Six weeks after this reduction in the level of thiouracil, the egg production of this group had returned to normal. However, for a period of four weeks following the change in the level of thiouracil, the average production of group-C females was 6.56 ± 0.69 eggs and of group-A females was 12.53 ± 1.29 eggs. The difference (5.97 ± 1.46) between these groups is statistically significant.

In trial II (groups D, E, and F), percentage egg production was determined, at weekly intervals, over a 16-week period beginning September 1, 1948. During the experimental period, thiouracil in the ration of group E and group F, respectively, lowered egg production. However, the difference between the egg production of these groups and

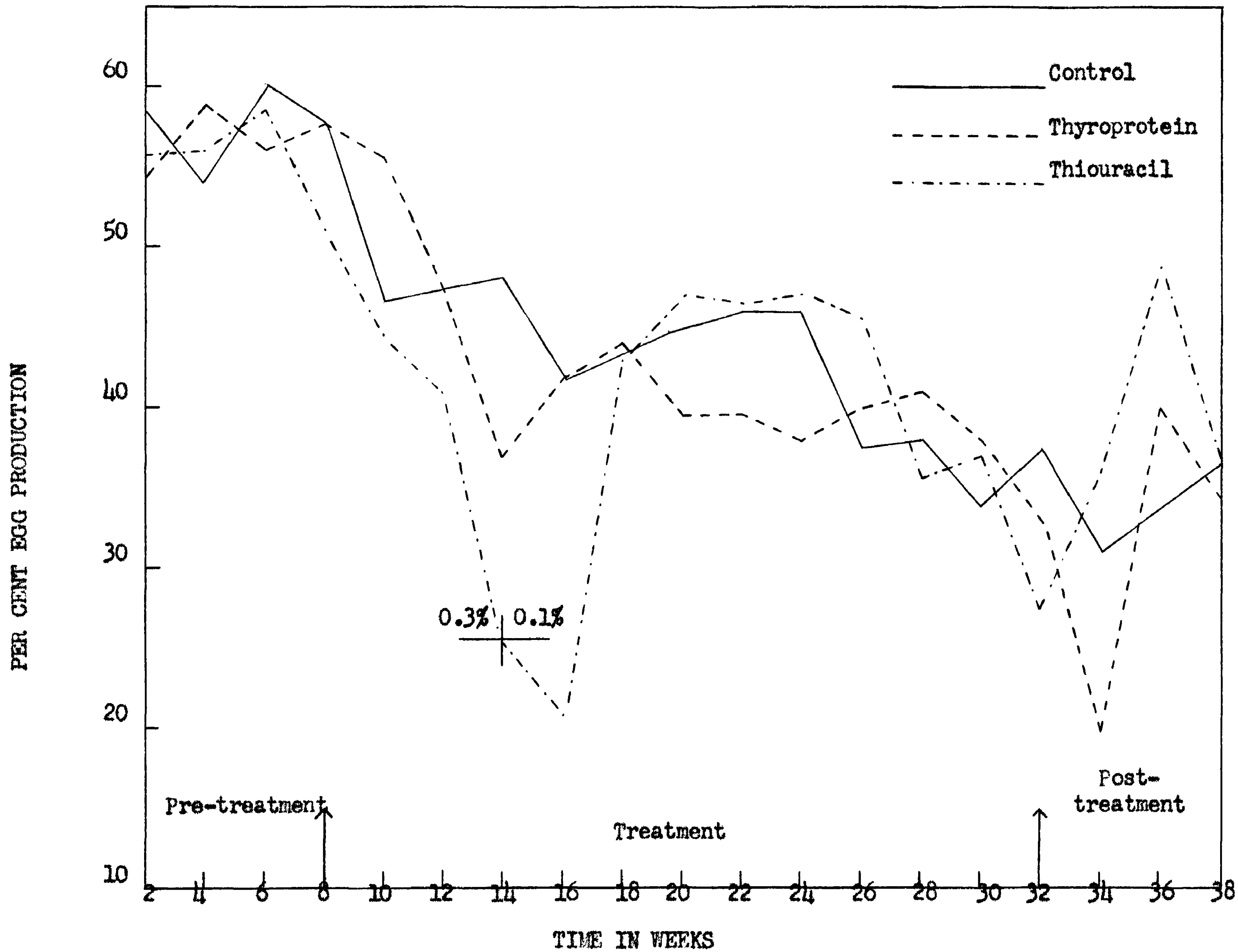

Figure 4a. - Rate of egg production of females in trial I

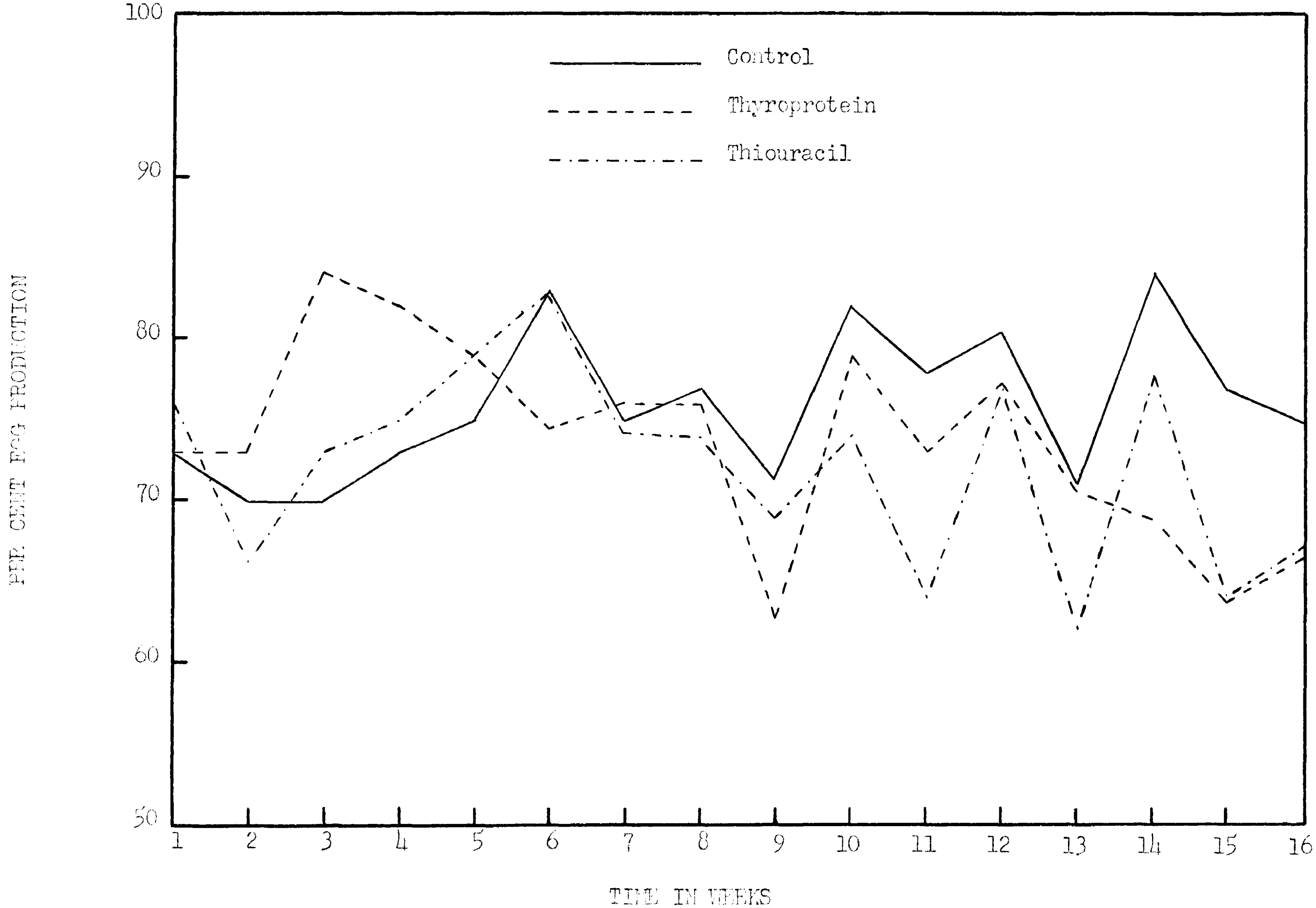

Figure 4b. - Rate of egg production of females in trial II. Treatment was initiated after a 4-week pre-treatment period.

that of the control group (D) were not statistically significant.

Egg weight records were kept on all eggs laid by the females in trial I. Analysis of the data obtained on the weight of the eggs produced in March indicates that the eggs laid by the females receiving thyroprotein or thiouracil did not differ significantly in size from those from the control females.

Comb Development. Since comb development has been shown to be an indicator of gonadal activity in the female fowl, records were kept in trial II on comb size as an additional measure of the female's ovarian activity. The average increase in comb area of the controls between September and November was $3.46 \pm 2.39 cm^2$, $10.16 \pm 1.76 cm^2$ in the group receiving thyroprotein, and $3.19 \pm 1.95 cm^2$ in the group receiving thiouracil. The increment in comb development of the thyroprotein-fed females of $10.16 cm^2$ is highly significant statistically.

Body Weight and Feed Consumption. During trial II, beginning with the initiation of treatment, monthly records were kept on body weight. During the treatment period, the females receiving thiouracil gained more than the controls, while the females receiving thyroprotein weighed slightly less at the end of this period than they did at the beginning. The gain in weight of 260.00 ± 80.08 grams for the thiouracil-fed females is highly significant statistically. Similar observations were also made during trial I.

In trial II, during the experimental period, records were kept on feed consumption to determine if thyroprotein or thiouracil, at the levels fed, had any effect upon the amount of feed consumed. The average individual daily feed consumption was as follows: Control - 0.282 pound; thyroprotein - 0.299 pound; thiouracil - 0.355 pound.

From these results, it appears that the feeding of 0.1 per cent thiouracil considerably increased feed consumption. The thiouracil-fed females consumed approximately 18 per cent more feed than the control females during this 12-week period.

Resting Metabolism. By securing data on the rate of metabolism, it was hoped to determine how effective thyroprotein and thiouracil, at the levels fed, were in altering the thyroidal activity of the females so treated. The average oxygen consumption of the control females and the females receiving thyroprotein or thiouracil during trial II is shown in Figure 5. These data indicate that the addition of 0.022 per cent thyroprotein to the ration of group E brought about a small but significant increase in the rate of resting metabolism. After twelve weeks of treatment, the average rate of resting metabolism of the thyroprotein-fed females was about 12 per cent more than that of the controls. Feeding 0.1 per cent thiouracil in the ration of group-F females caused a marked decrease in the rate of metabolism. The average rate of resting metabolism of the thiouracil-fed females was approximately 24 per cent less than that of the controls. The difference in oxygen consumption of 204.22 $\pm$ 30.53 ccs. per kilogram of body weight per hour between the control females and the thiouracil-fed females is highly significant statistically. These results provide sufficient evidence to indicate that mild hyperthyroidism and hypothyroidism were experimentally induced in the females receiving thyroprotein and thiouracil, respectively, in their ration.

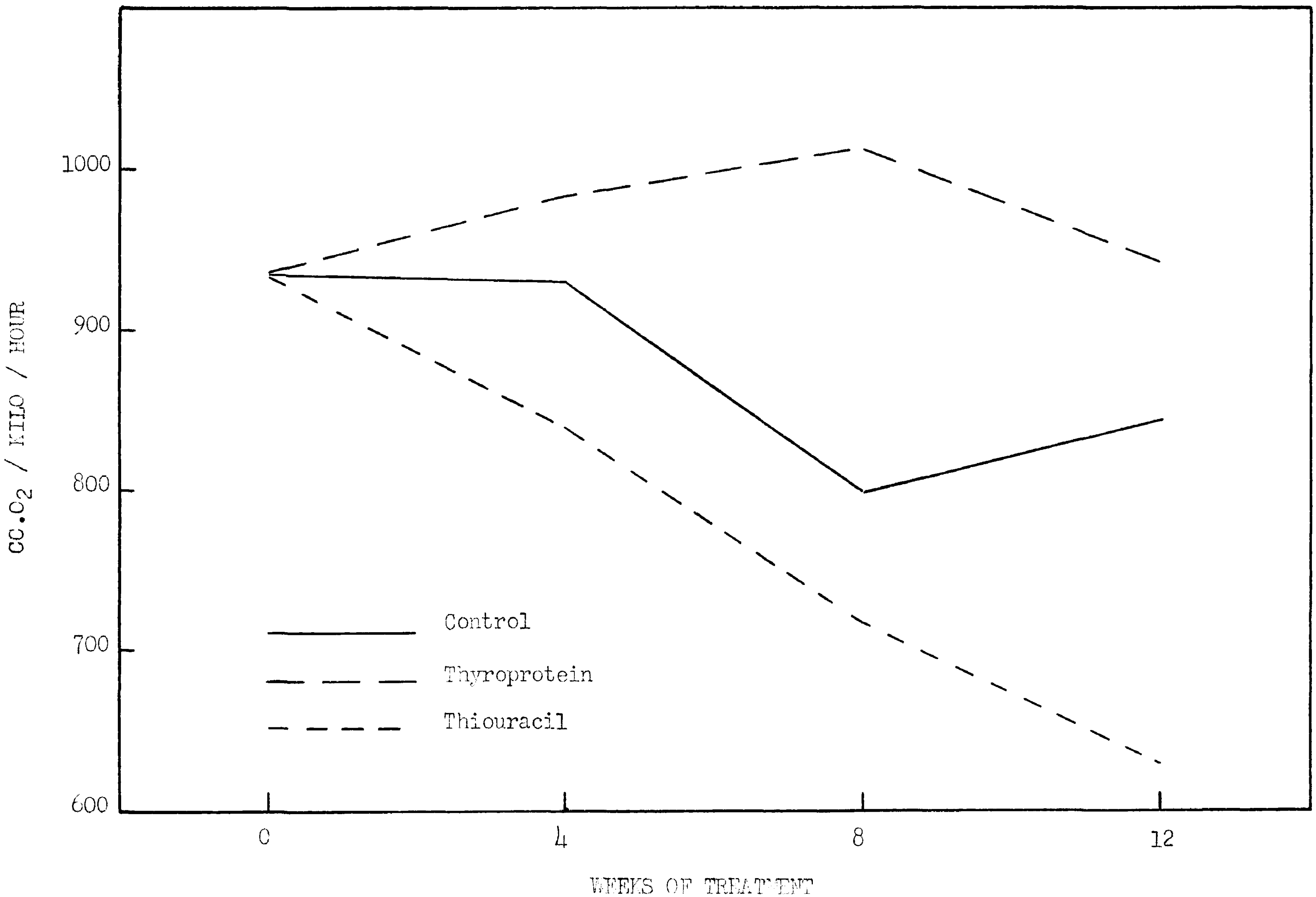

Figure 5. - Resting metabolism (trial II)

PART II

Observations on Chicks Produced by Females with Altered Metabolism

Chick Thyroid Size. In trial I, day-old chicks hatched from thyroprotein-fed, thiouracil-fed, thyroxine-injected and normal females were sacrificed and their thyroid glands weighed on a precision balance. These observations were first made five weeks after the initiation of treatment. Thyroid gland weights were also obtained on a limited number of chicks hatched from thyroxine-injected and thyroprotein-injected eggs.

The chick thyroid gland weights are shown in Table 5. Chicks with enlarged thyroids were produced by the thyroprotein-fed and the thiouracil-fed females, while a decrease in thyroid size resulted when thyroxine was injected into control eggs. These differences in thyroid gland weights are highly significant. The thyroid gland weights of the chicks hatched from eggs produced by the thyroxine-injected females were comparable in size with the glands of normal chicks. In this trial, the data obtained on the thyroid gland weights of the chicks hatched from the thyroprotein-injected eggs were too limited to determine its affects on thyroid size. However, subsequent studies revealed that thyroprotein injected to control eggs at levels equivalent to 9, 12, 20, and 40 micrograms of d,l-thyroxine had no affect upon chick thyroid size. Highly significant differences were also shown to exist in thyroid gland size between the chicks produced by the same females receiving thiouracil at a level of 0.3 and 0.1 per cent, respectively.

Table 5. - Mean thyroid weight of day-old chicks from females fed thyroprotein, thiouracil, or females and eggs injected with thyroxine

Treatment	No. of Hatches	No. of Chicks	Mean Thyroid Weight (mgs.)
Control	1[1]	41	5.66 ± 0.50
Thyroprotein	1	41	9.52 ± 0.44**
Thiouracil (0.3%)	1	28	15.65 ± 1.15**+
Control	9[2]	97	5.10 ± 0.11
Thyroprotein	9	99	9.08 ± 0.34**
Thiouracil (0.1%)	9	98	7.87 ± 0.26**
40 γ thyroxine injected, daily, to females	3	35	5.24 ± 0.28
12 γ thyroxine injected to control eggs	2	25	4.08 ± 0.18**

[1] The hatch in this series was five weeks after the initiation of treatment.

[2] The hatches in this series were one week apart, the first being seven weeks after the initiation of treatment.

** The difference between these values and the control are highly significant.

+ The difference between this value and thiouracil (0.1%) is highly significant.

In trial II, the thyroid glands of the chicks hatched from eggs injected with 6, 12, 20, and 40 micrograms, respectively, of d,l-thyroxine were consistently smaller than the thyroids of normal chicks. Since chicks with goiterous thyroid glands are produced by thyroprotein-fed females, eggs laid by these females were injected with thyroxine, at levels of 2, 4, 6, 8, and 10 micrograms. The results of these thyroxine-injection studies revealed that about 6.5 micrograms of exogenous thyroxine is necessary to effect thyroids of normal size in chicks produced by thyroprotein-fed females.

During an 11-week period following the initiation of treatment in trial II, groups of ten day-old chicks were sacrificed at weekly intervals and their thyroid glands weighed to the nearest 0.1 milligram on a Roller-Smith balance. The results of this study, as presented in Figure 6, indicate that the thyroid glands of the chicks produced by the thiouracil-fed females had not attained their maximum size after eleven weeks of treatment, while the thyroids of the chicks produced by the thyroprotein-fed females had apparently reached their greatest size of about 11.0 milligrams after 6-8 weeks of treatment.

The lines shown in Figure 7 represent the change occurring in the thyroid weight from day-old to three weeks of age of chicks produced by the females in trial II. The thyroid weights of the chicks produced by the thyroprotein-fed females decreased markedly during the first week, while those of the thiouracil-fed females increased somewhat. By the end of the second week the thyroid glands of the chicks from treated dams were comparable in size with those of the controls. The thyroids of the three groups of chicks increased slightly in size during the third week of the post-hatching period.

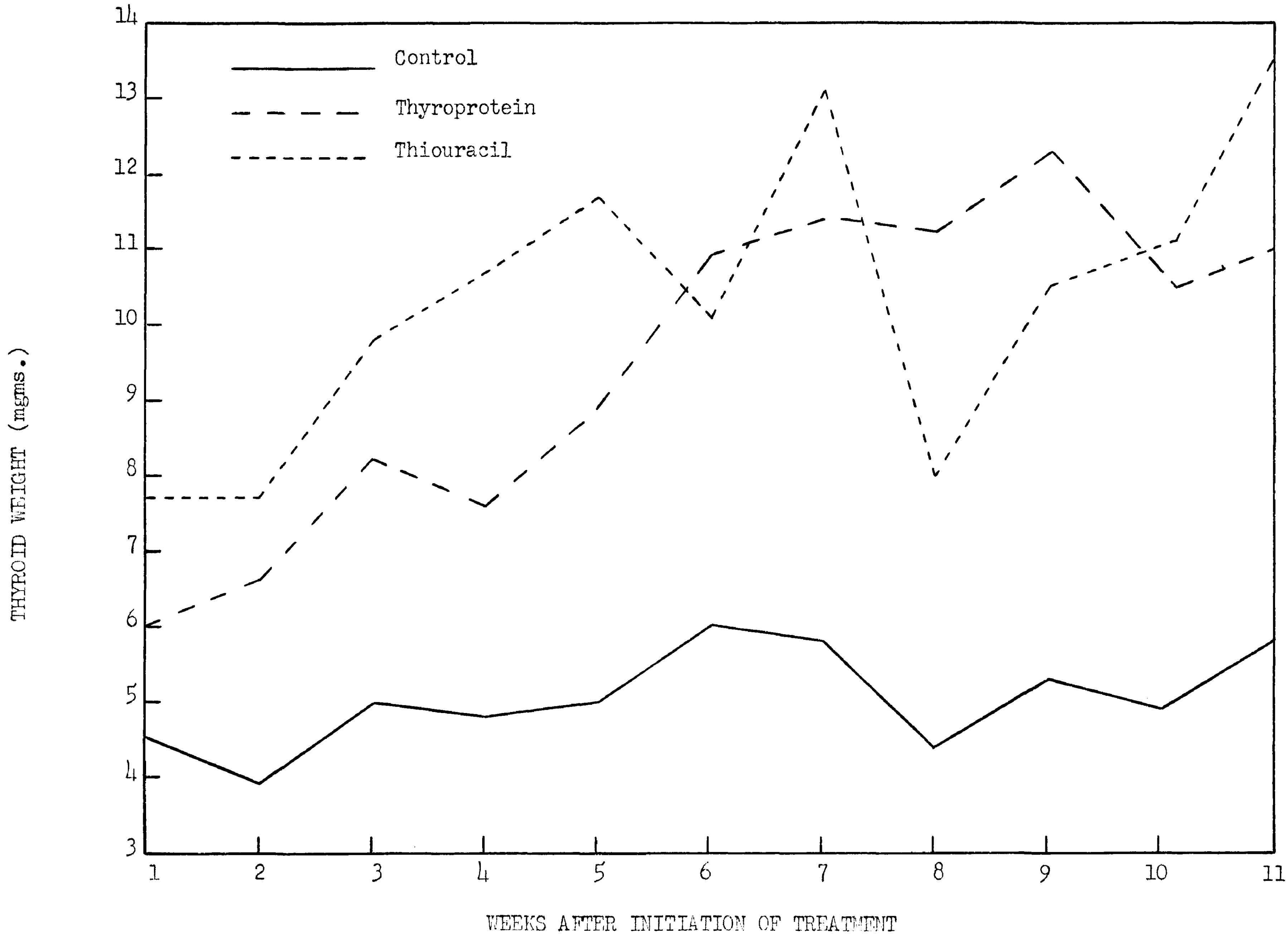

Figure 6. - Thyroid weight of day-old chicks from females in trial II

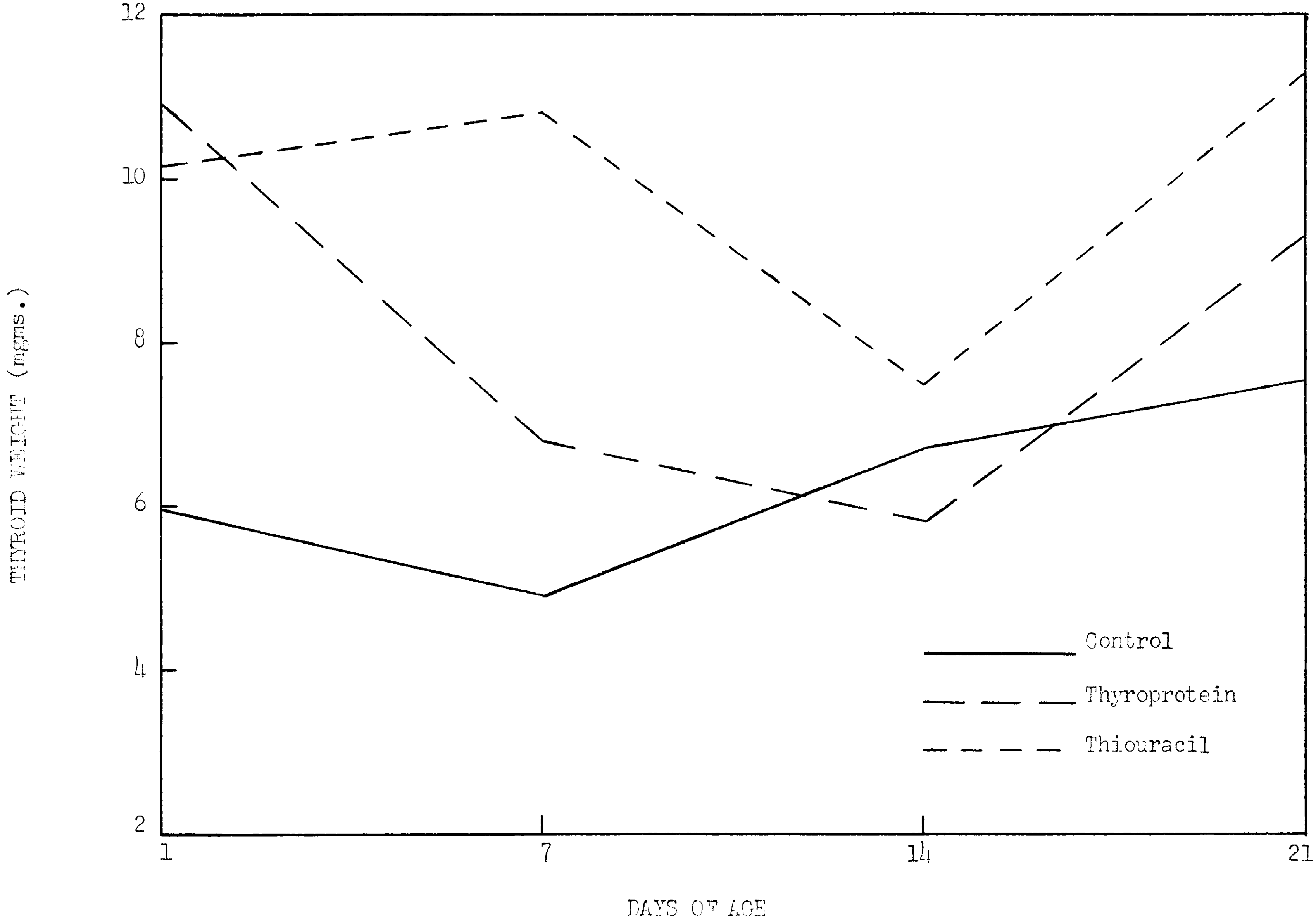

Figure 7. - Change in chick thyroid weight (day-old to 21 days of age)

Incubation Period. The effect of feeding thyroprotein and thiouracil (0.3 and 0.1 per cent) on the incubation period of eggs from females so treated is shown in Figure 8. The irregularity of the lines in this figure is not the result of treatment, but primarily due to fluctuations of the incubation temperature and humidity. It will be noted that the week-to-week variation in the length of the incubation period of the eggs from the treated females corresponds to that of the controls. The average incubation period of the control eggs was 20 days plus 18.7 hours. The incubation period of the eggs from the thyroprotein-fed females was 12.3 hours longer than those from the control females, while the incubation period of the eggs produced by the females receiving thiouracil at a level of 0.3 and 0.1 per cent was 32.4 and 11.9 hours, respectively, longer than the eggs from control females. The difference of 3.7 hours in incubation period between the eggs produced by the thyroxine-injected females and the control females is non-significant. No material alteration in the length of the incubation period was observed in the case of the thyroxine-injected eggs.

Chick Survival Time and Metabolism. The chick survival time results, as determined by the closed-vessel technique, are summarized in Table 6. This table also gives the thyroid gland weights of the chicks upon which the survival time observations were made. The data show an increase in survival time of the chicks produced by the females fed thyroprotein or thiouracil (0.1 per cent) and of the chicks produced by females injected with thyroxine. These increases in survival time in each case are highly significant. The survival-time observations of the chicks hatched from the thyroprotein-injected and the thyroxine-

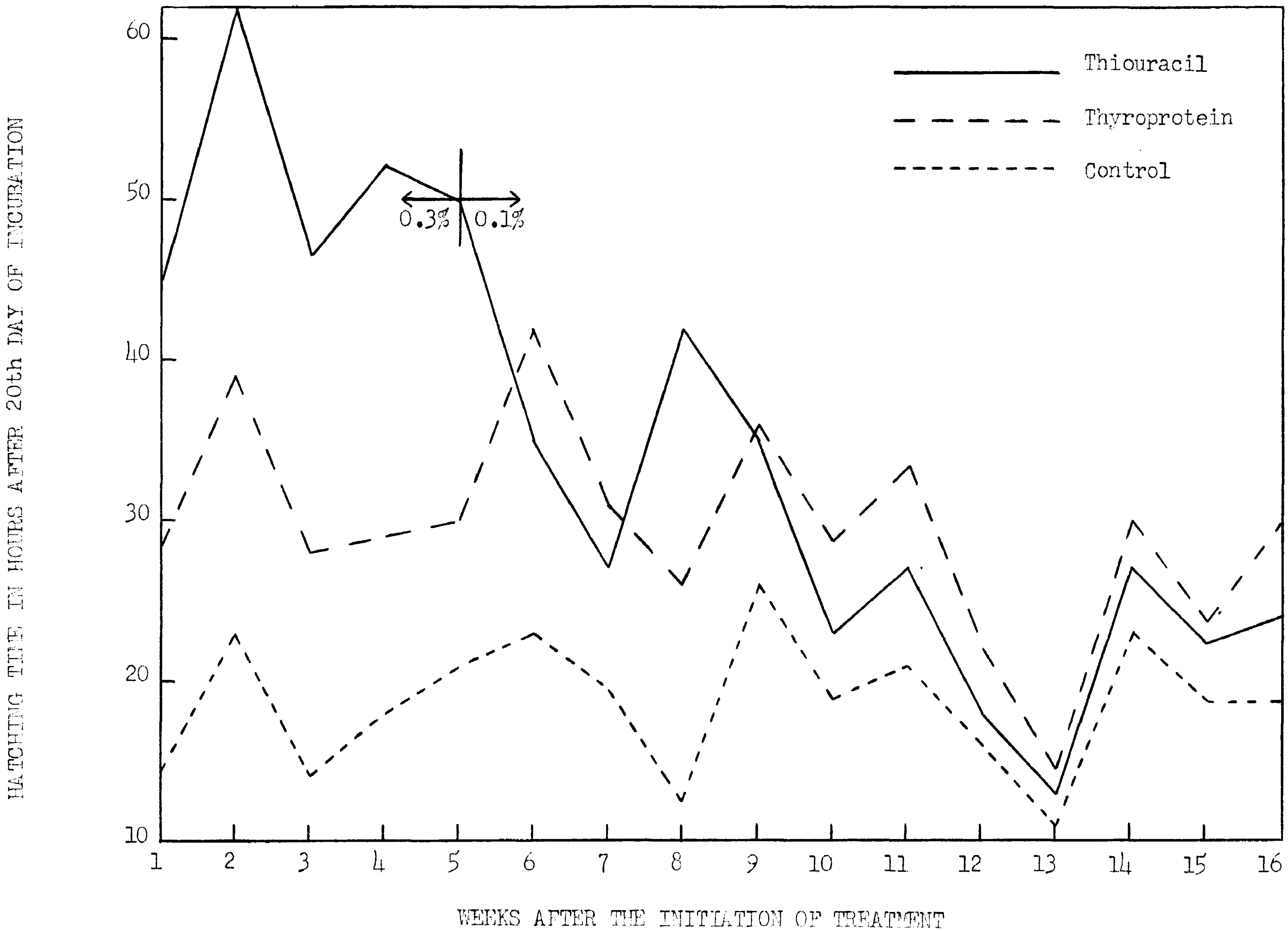

Figure 8. - Incubation period of chicks hatched from control females and females fed thyroprotein (10 gms./100 lbs.) or thiouracil (0.3 and 0.1 per cent)

Table 6. - Mean survival time and thyroid weight of day-old chicks from females fed thyroprotein, thiouracil, or injected with thyroxine

Treatment	No. of Chicks	Survival Time (mins.)	Mean Thyroid Weight (mgs.)
Control	42	68.4 ± 1.5	4.77 ± 0.20
Thyroprotein	34	79.5 ± 1.9**	8.17 ± 0.47**
Thiouracil (0.1%)	36	98.5 ± 1.9**	6.98 ± 0.36**
40γ thyroxine injected, daily, to females	30	82.4 ± 3.0**	5.32 ± 0.21

** The difference between these values and the control are highly significant.

injected eggs were too limited to make definite conclusions. However, the results indicate a slight increase in the survival time of the chicks hatched from the eggs injected with thyroxine.

In trial II, the resting metabolism of eight groups of eighteen day-old chicks each were obtained. The average oxygen consumption of the control chicks was 1981.00 $\pm$ 73.92 ccs. per kilogram of body weight per hour. The chicks produced by the thyroprotein-fed and the thiouracil-fed females consumed 16.2 and 16.5 per cent, respectively, less oxygen than the control chicks. In each case, the decrease in oxygen consumption of the experimental chicks is statistically significant.

DISCUSSION

The results obtained on the rate of resting metabolism of the females in trial II indicate that mild hyperthyroidism and hypothyroidism were experimentally induced in the females receiving thyroprotein and thiouracil, respectively, in their ration. The average rate of resting metabolism of the thyroprotein-fed females, after 12 weeks of treatment, was about 12 per cent more than that of the control females. This increase in metabolism is comparable with the changes in metabolism obtained in other animals by Reineke and Turner (1945), Blaxter (1948), and Thorbek (1948). However, the reduction in the rate of resting metabolism in the thiouracil-fed female chickens was not as rapid as that found in the rat by Reineke et al. (1945), Meyer et al. (1945), and Gordon et al. (1946). The decrease of approximately 30 per cent in the resting metabolism of the thiouracil-fed female chickens during a period of 12 weeks is somewhat less than that reported by Shaffner and Andrews (1948) for male chickens during a similar period of time. Since females normally have a lower metabolic rate, these results suggest that there may be a sex difference in the rate of absorption and utilization of thiouracil or that the difference may be due entirely to the higher level of thiouracil received by the males.

From the results obtained in these experiments, it is clear that thyroprotein and thiouracil, at the levels fed, had no adverse affect upon the fertility of the eggs produced by females so treated. These results are in accord with the findings of Wheeler and Hoffman (1948b) and Andrews and Schnetzler (1945). The data secured on duration of fertility shows that the sperm from normal-fed males were capable of surviving in the oviducts of the experimental females as long or longer than the sperm in the oviducts of the control females. Apparently the changes in oviduct environment of the experimental females were too small to have any delirious affect upon sperm viability.

In both trials, thyroprotein, at the level fed, did not adversely affect hatchability, providing that the eggs produced by the thyroprotein-fed females were kept in the incubator for an additional day or two. The mean incubation time of the eggs produced by these females proved to be 12.3 hours longer than that of the control females.

In trial I, thiouracil, fed at levels of 0.3 and 0.1 per cent, caused a marked reduction in the hatchability of the fertile eggs produced by group-C females. This decreased hatchability was due to increased embryonic mortality throughout the entire incubation period. Embryonic mortality was particularly increased at the two critical peaks of incubation and for the chicks classified as dead-in-shell. Since thiouracil has been shown to be transmitted through the egg of the domestic fowl to the developing embryo, it seems likely that this increased embryonic mortality may result from an interference in embryonic metabolism during the critical periods of incubation. In order to obtain maximum hatchability, it was necessary to keep the eggs from the thiouracil-fed females in the incubator for an extra two days because the incubation period of the eggs produced by the females receiving thiouracil at a level of 0.3 and 0.1 per cent was 32.4 and 11.9 hours, respectively, longer than the control eggs.

Thiouracil did not significantly reduce hatchability during trial II. However, there was some evidence of a slight decrease toward the end of the 12-week treatment period, indicating that the amount of thiouracil passing from the dams to their eggs was approaching the level required to effect lowered hatchability.

During both trials thyroprotein had no apparent beneficial effect upon egg production. These results are similar to the findings of Hutt and Gowe (1948), who questioned the stimulatory effect of thyroprotein upon egg production. Temperton and Dudley (1947) and Gutteridge and Novikoff (1947) were also unable to increase egg production by feeding thyroprotein. This indicates that mild hyperthyroidism, as characterized by a slight but significant increase in resting metabolism by feeding 0.022 per cent thyroprotein, is not an important factor in controlling this physiological characteristic in the female domestic fowl.

Feeding thiouracil at a level of 0.1 per cent to the females in both trials apparently had no adverse affect upon egg production. Although egg production of the females in trial II was lower than that of the controls, the difference was not significant. Feeding 0.3 per cent thiouracil to the females in trial I caused a marked depression in egg production. However, the egg production of these females returned to normal shortly after the level of thiouracil was reduced to 0.1 per cent. These results indicate that the higher level of thiouracil was detrimental to egg production. This lowered egg production may have been the result of decreased metabolism and reduced thyroid function or may have been due to the storage of thiouracil in the ovary, limiting ovarian activity.

Comb growth in the female fowl is coincident with the increased activity of the ovary, being influenced by the androgen secretion of the ovary's medulla. Therefore, the marked increase in the comb development of the females receiving thyroprotein in their ration indicates that the androgen production of these females was greatly stimulated. This increased secretion of androgen may have been brought about in two ways, (1) by the exogenous thyroxine from the thyroprotein acting directly upon the ovary or (2) indirectly by acting through the anterior pituitary gland to increase the secretion of the luteinizing hormone which in turn stimulates the ovary to produce more androgen.

The slight loss in body weight observed in the thyroprotein-fed females during the 12 weeks of treatment is characteristic of a state of hyperthyroidism. These birds did not lose enough weight to affect egg production but on handling they appeared in poor condition as compared to the control females. The females receiving thiouracil made marked gains in body weight, having considerably more subcutaneous and abdominal fat than the controls. This excessive storage of body fat is associated with hypothyroidism in all animals.

Feed consumption was apparently increased by feeding thiouracil. However, if allowance is made for the difference in body weight part of this increased consumption may be accounted for. The thiouracil-fed females weighed about 12 per cent more than the control females at the end of the 12-week treatment period. Taking this factor into consideration, it is doubtful if thiouracil-feeding increased feed consumption significantly in these females. In this respect, Berg and Bearse (1948) found that the addition of 0.1 per cent thiouracil in the ration of White Leghorn females resulted in a marked daily decline in feed consumption for about one week, followed by a considerable increase in the amount of feed consumed. In males, Shaffner and Andrews (1948) concluded that feeding 0.2 and 0.5 per cent thiouracil did not affect feed consumption.

In discussing the results on chick thyroid size, it is assumed that the size of the thyroid gland of the embryo is controlled by the thyrotropic secretion of its anterior pituitary gland and that the secretion of the thyrotropic hormone in turn is governed by the amount of thyroxine in the blood stream. The thyroid glands of the chicks were enlarged by the feeding of thiouracil to the females. This is in accord with the results reported by Andrews and Schnetzler (1945). The feeding of 0.3 per cent thiouracil to the females resulted in a greater enlargement of the chick thyroid than did the feeding of 0.1 per cent. This would indicate that at the higher level more thiouracil and/or less thyroxine was transmitted from the female to her egg. These conditions would result in an increased stimulation of the chick thyroid gland by way of its anterior pituitary gland.

The production of chicks with goiterous thyroid glands by females fed thyroprotein may have been the result of a decreased secretion of thyroxine by the females, there consequently being less thyroxine transmitted to the egg than normally. This implies that the thyroprotein either is not transmitted to the egg in the same levels as natural thyroxine or that thyroprotein has a different reaction in the chick than in the adult. Another possible explanation might be that a small amount of thyroprotein is transmitted to the egg and this acts to cause hypertrophy. A basis for this explanation is the fact that Moreng and Shaffner (1948) have reported that thyroprotein added in very small quantities to a ration containing thiouracil caused an increase in thyroid size. Wheeler and Hoffman (1948) have also reported that goiterous chicks were produced by females receiving thyroprotein at a level of 0.02 per cent of the ration. The results of these workers, based on a total of 66 observations, are in agreement with the results reported herein.

Chicks with normal sized thyroids were produced by the thyroxine-injected females, indicating that the thyroxine injections were too small to have much effect on the normal endocrine balance and consequently the normal titer of naturally occurring thyroxine was transmitted to the developing chick. However, the thyroid glands of the chicks hatched from the eggs injected with thyroxine were smaller than normal, indicating that these small thyroid glands were the result of the action of the injected thyroxine upon the anterior pituitary gland of the developing chick embryo. Since thyroxine was administered to the females at levels much higher than the normal thyroid hormone secretion rate estimated by Turner (1948a), these results suggest that the exogenous thyroxine was not transmitted from the female to her eggs. A basis for this contention is the fact that Monroe and Turner (1946) concluded that the mammary gland epithelium of the rat was impermeable to thyroxine. However, if this was the case, one would expect the thyroxine-injected females in this experiment to produce chicks with enlarged thyroid glands; the exogenous thyroxine should completely supplement the natural thyroxine and no hormone would be transmitted to the eggs. Therefore, possibly only the normal titer of synthetic thyroxine passed into the eggs from the females, producing thyroids of normal size in the chicks hatched.

Thyroprotein injected into control eggs at levels as high as 40 gamma of d,l-thyroxine had no affect on chick thyroid size, indicating that the thyroxine available in thyroprotein can not be assimulated and utilized by the developing chick embryo or that the shell membrane is impermeable to the thyroprotein solution. Booker and Sturkie (1949) injected thyroprotein into the albumen of incubating eggs at the level of 33 gamma (equivalent to 1 gamma of d,l-thyroxine) and obtained a slight decrease in thyroid size. However, in view of the limited number of observations in this experiment, the difference between the thyroid size of the chicks from the control eggs and the injected eggs could have been easily due to sex. Males have significantly smaller thyroids than females (Aberle and Landauer, 1935).

The injection of thyroxine into eggs produced by thyroprotein-fed females consistently reduced thyroid size, indicating that there was actually a deficiency of thyroxine in these eggs. Approximately 6.5 micrograms of exogenous thyroxine was necessary to effect thyroid glands of normal size in chicks from eggs produced by females receiving 0.022 per cent thyroprotein in their ration.

According to Wheeler and Hoffman (1948c), the thyroid enlargement of chicks from thyroprotein-fed hens is maximum about 14 days after the initiation of treatment. These results are not in accord with the findings reported herein because in this experiment thyroid size did not attain its greatest enlargement of approximately 11.0 milligrams until 6 to 8 weeks after the females were put on the experimental ration. This indicates that the maximum reduction in deposition of maternal thyroxine was not reached until several weeks after the initiation of treatment. However, chicks with goiterous thyroids occurred concurrently with the addition of thyroprotein to the ration, the thyroids being significantly larger within one week from the beginning of treatment.

The thyroid glands of the chicks produced by the thiouracil-fed females were still increasing in size after eleven weeks of treatment. This progressive increase in thyroid size would indicate that more thiouracil and/or less thyroxine was transmitted from the female to her eggs as the treatment advanced. Since the resting metabolism of these females progressively decreased during the experimental period, it is apparent that a relationship exists between the degree of hypothyroidism of the female and the rate of thyroid enlargement of her chicks.

The increase in thyroid weight, during the first post-hatching week, of the chicks produced by the females receiving thiouracil suggests that thiouracil was stored in the chick's yolk sac. The thyroid weight of the chicks produced by the thyroprotein-fed females decreased during the first week, indicating that there is little or no carry over in the yolk sac of the factor or factors causing thyroid enlargement in these chicks.

It seems plausible to assume that a close relationship exists between the function of the chick embryo thyroid gland and the rate of embryonic development, since evidence is submitted indicating that the increase in length of the incubation period of the chicks produced by the thyroprotein-fed and the thiouracil-fed females was proportional to the increase in their thyroid gland size. The independent studies of Wheeler and Hoffman (1948c) have shown that the length of the incubation period is almost proportional to the amount of thyroprotein fed to the hens. Mean incubation time increased 6-11, 13-21, and 23-37 hours, respectively, when thyroprotein was fed at levels of 0.02, 0.04, and 0.08 per cent in the ration.

Riddle (1930) suggested that normal embryo development and length of incubation period in the pigeon are contingent upon an adequate supply of maternal thyroxine entering the egg from the dam, an inadequate supply of the hormone resulting in a prolongation of time required for the completion of embryonic growth. This hypothesis was later substantiated by Hollander and Riddle (1943 and 1946), who observed that young pigeons hatched from goiterous females were themselves goiterous and exhibited a tendency for hatching to be delayed an extra day or more. Therefore, it seems logical to assume that an oversupply of thyroxine entering the egg from the dam might reduce the length of the incubation period and decrease thyroid size. However, no observable decrease in incubation period time accompanied the decrease in thyroid size of the chicks hatched from the thyroxine-injected eggs, indicating that the rate of embryonic development proceeds normally in the presence of additional thyroxine or that the supplemental effect of the exogenous thyroxine was incomplete. The incubation period and thyroid size of the chicks hatched from eggs produced by the thyroxine-injected females were not affected, indicating that the exogenous thyroxine in the female's blood stream is not transmitted to the egg in the same manner as the naturally occurring thyroxine.

Since survival time has been shown to be inversely proportional to metabolic rate, the increased mean survival time of the chicks hatched from the thyroprotein-fed and the thiouracil-fed (0.1 per cent) females suggests that the metabolic rate of these chicks was lower than that of the controls. Chicks produced by two comparable groups of females receiving the same experimental rations required about 16 per cent less oxygen each than the control chicks. Both survival time and resting metabolism results are in accord with the increase in thyroid size of these chicks, thus supplying further evidence that the chicks produced by both the hyperthyroid and the hypothyroid females are themselves hypothyroid.

SUMMARY

Three groups of twenty New Hampshire females each were maintained, respectively, on a control ration or a ration containing thyroprotein or thiouracil for 24 weeks in one experiment and for 12 weeks in a second experiment. In the first trial, treatment was initiated on the following basis: Group A - control, group B - 0.022 per cent thyroprotein, and group C - 0.3 per cent thiouracil. Four weeks later the level of thiouracil was reduced to 0.1 per cent. In the second trial group D served as control and groups E and F received 0.022 per cent thyroprotein and 0.1 per cent thiouracil, respectively.

Percentage fertility, hatchability, and embryonic mortality of the eggs produced by these six groups of females was determined on an individual basis. Egg production records were kept on all females of both trials, while egg weight records were obtained on the eggs produced by the females in trial I. In the second trial, data were secured on comb development, body weight, feed consumption, and rate of resting metabolism.

Observations were made on the incubation period of the eggs and the thyroid gland weights of the chicks produced by normal females and females fed thyroprotein or thiouracil and females injected with thyroxine (20, 40, and 100 gamma, daily). Eggs from untreated females were injected with thyroxine or thyroprotein and similar observations made on the chicks hatched. Eggs from thyroprotein-fed females were also injected with thyroxine and their thyroid glands weighed. Samples of day-old chicks and chicks 7, 14, and 21 days of age were sacrificed

and their thyroid glands weighed in order to ascertain the change existing in thyroid size during the first three weeks of the chick's life. The rate of thyroid enlargement was determined by sacrificing samples of ten day-old chicks weekly and weighing their thyroid glands.

The thyroidal activity of representative samples of all chicks in the first trial was determined by the closed-vessel technique. During the second trial, the rate of resting metabolism of groups of eighteen day-old chicks was determined by means of the indirect calorimetry method. This measurement was made by recording the cubic centimeters of oxygen consumed per kilogram of body weight per hour.

CONCLUSIONS

1. The addition of either thyroprotein or thiouracil to the control ration had no affect upon subsequent fertility.

2. The hatchability of the fertile eggs produced by the thyroprotein-fed females was as good as that of the control females. Both levels of thiouracil reduced hatchability significantly in the first trial. In the second trial, however, hatchability was not adversely affected until towards the end of the treatment period.

3. The embryonic mortality of the fertile eggs produced by the thiouracil-fed females greatly exceeded that of the eggs produced by the control females, especially at the peaks of mortality.

4. Egg production was not stimulated by the addition of 0.022 per cent thyroprotein to the ration. Apparently, the inclusion of 0.1 per cent thiouracil to the ration of the females in these experiments was not harmful to egg production. However, egg production declined markedly when 0.3 per cent thiouracil was included in the ration.

5. The marked increase in comb development of the thyroprotein-fed females indicates that the androgen production of these females was greatly stimulated.

6. The thiouracil-fed females gained considerably more in weight than the controls, while a slight loss in body weight resulted in the females fed thyroprotein.

7. Thiouracil did not affect feed consumption when allowance was made for differences in body weight. The feed intake of the thyroprotein-fed females was comparable with that of the control females.

8. The addition of 0.022 per cent thyroprotein to the ration of group E produced a small but significant increase in the rate of resting metabolism, while feeding 0.1 per cent thiouracil to group F caused a marked decrease in metabolism. These results on the rate of resting metabolism show that mild hyperthyroidism and hypothyroidism were experimentally induced in the females receiving thyroprotein and thiouracil, respectively, in their ration.

9. The thyroid glands of the chicks hatched from the thyroprotein-fed and the thiouracil-fed females were 78.0 and 54.3 per cent, respectively, heavier than those of the chicks from the control females. The thyroid glands of the chicks hatched from the thyroxine-injected females and the thyroprotein-injected eggs were comparable in size to the thyroid glands of normal chicks. Thyroxine injected into control eggs and eggs produced by thyroprotein-fed females consistently reduced chick thyroid size.

10. The incubation period of the eggs from the thyroprotein-fed females was 12.3 hours longer than that of the control eggs. The incubation period of the eggs from females receiving thiouracil at a level of 0.3 and 0.1 per cent was 32.4 and 11.9 hours, respectively, longer than that of the control eggs.

11. Resting metabolism and closed-vessel survival time observations indicate that the thyroidal activity of the chicks hatched from the thyroprotein-fed and the thiouracil-fed females was lower than in normal chicks, suggesting a lowered metabolic rate and a functional hypothyroid condition. Therefore, chicks produced by both hyper- and hypothyroid females were themselves hypothyroid.

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