THE INFLUENCE OF VARYING LEVELS OF THYROID ACTIVITY ON SEMEN PRODUCTION IN THE DOMESTIC FOWL

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THE INFLUENCE OF VARYING LEVELS OF THYROID ACTIVITY ON SEMEN PRODUCTION IN THE DOMESTIC FOWL

By

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TABLE OF CONTENTS

INTRODUCTION

Investigations relative to the functions of the thyroid gland, its relationships with the other elements of the endocrine system, and its influence on the animal economy have gained impetus in the last few years. This is due in a large degree to the discovery of the goitrogenic action of a series of chemical compounds on the one hand and the development of a new source of thyroidal substance on the other.

The goitrogenic nature of sulguanidine was first described in a preliminary report by MacKenzie, MacKenzie and McCollum in 1941. In a later report by the MacKenzies (1943) the action of sulfanilamide and thiouracil on the hormone secreting mechanism of the thyroid gland was elucidated.

At almost the same time, Astwood et al. (1943) published the results of their investigation on the action of certain sulfanilamides and thiouracil on the thyroid of rats.

If to this discovery is added the development of a thyroid active substance by Reineke and Turner (1942), which made possible the use of the thyroid hormone at a lower cost, the great number of assays made and the works published on these topics in the last few years is easily explained.

Certainly, the history of the thyroid gland dates back to antiquity, but it was only with the discovery of Gull (1874) relative to the symptoms observed in some women

with spontaneous atrophy of the thyroid, that this matter fully came into the annals of medicine. Later, in 1882, Reverdin corroborated the observations of Gull.

In 1895, Baumann discovered the presence of iodine in the thyroid secretion, and this fact marked an epoch in the iodine therapy of the disturbances due to abnormalities of the thyroid gland.

If it is true that the discovery of the last author cited constitutes a great event, it is also true that the discovery of the goitrogenic action of the sulfa drugs and thiourea derivatives, and the methods developed for the preparation of thyroactive iodinated casein have constituted the lever which has moved the enthusiasm of investigators in the fields of endocrinology, therapeutics, and zootechnique.

The development of these new products now makes it possible to alter the level of thyroid function of experimental animals at will from the hypothyroid level to extreme hyperthyroidism. By means of this technique a number of the physiologic processes of domestic animals that are of economic importance have been studied. Only a limited amount of attention has been given to the possible role of the thyroid gland in reproduction, however.

The research to be reported was devised to determine the relation of the thyroid secretion to semen production in the domestic fowl.

REVIEW OF LITERATURE

Since both thiouracil and thyroprotein were used in the research problem as a means of altering the thyroid function it is of interest to review some of the fundamental investigations that have been done to establish the physiologic action of these compounds.

The role of the thyroid in the reproductive processes of both the male and female of the various species is still not well understood, though it apparently plays a part in both sexes. Very little information has been reported on the relation of the thyroid to semen production in the chicken. Therefore, the subject will be reviewed quite broadly, taking into account the work published on both sexes of the various species on which research has been done, in order to provide the background of information that led to the inception of the present investigation.

Goitrogenic Drugs.

Thyroid-inhibitory properties: In a preliminary report, MacKenzie, MacKenzie and McCollum (1941) announced that rats, to whose diet was added one per cent sulfaguanidine, invariably showed hypertrophy and hyperemia of the thyroid gland. At the same time they observed histological changes in the gland.

Two years after this, the MacKenzies (1943) administered thioureas and sulfanilamides to rats, mice and dogs and found hyperemia and enlargement of the gland. Histologically, there was a reduction in colloid and an increase in

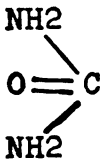
the height of the thyroid epithelium. They also pointed out that the B.M.R. in sexually mature male rats decreased. These effects on the B.M.R. are prevented by thyroxine at a dosage level of one microgram per 10 grams of body weight. Finally, the modification of the thyroid produced by thioureas and sulfanilamides did not occur in hypophysectomized animals.

At almost the same time, Astwood et al. (1943) arrived at the same conclusion as the MacKenzies did. Nevertheless, we come to a new statement in this report. In fact, they informed us that the phenomena that occur in the thyroid due to goitrogenic substances were not influenced by a large supplement of iodine, but were abolished by the administration of thyroid powder or hypophysectomy.

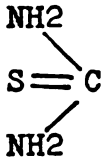
Astwood (1943-a) reported the first treatment performed with thiourea in a human subject. Thus the drug became useful in human therapy. A subject who was undergoing hyperthyroidism was administered one to two grams thiourea daily. After a latent period of one to two weeks, the administration resulted in a relief of the symptoms and the B.M.R. became normal. When therapy was discontinued the hyperthyroid symptoms returned.

Chemistry of the thiourea derivatives:

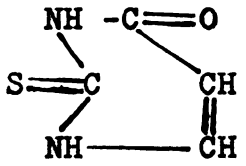
Thiourea derivatives have their origin in urea: CO (NH2)2.



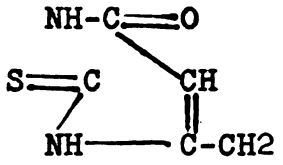
By replacing the O in the urea by sulfur, thiourea or sulfocarbamide is obtained: CS (NH2)2:



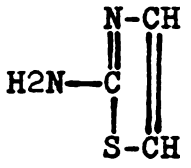
Then, from sulfocarbamide, comes thiouracil:



In 1944, methylthiouracil was obtained in Switzerland:



Aminothiazole is the fourth one of the best known goitrogenic drugs, whose formula is:



Thirty-two thiourea derivatives were tested in 1943 by Astwood. Until that time the most important drugs of this family were: thiourea: CS (NH2)2; 2-thiouracil: CS(NH2)2-CO-CH-CH; and 2-thiobarbituric acid: CS(NH2)2.COCH-CO.

All of them have in common the group NH-CS-NH. The essential components are S and the group NH.

In 1945(a) Astwood, Bissell and Hughes reported that thiobarbital or 5,5-diethyl-2 thiobarbituric acid, regarding their antithyroid effects, were second only to thiouracil, among 103 of these compounds. Later on, the same authors (1945-b) continued their trials in order to determine the relative antithyroid activity of 220 compounds. They used rats as experimental animals, thiouracil as a standard and the thyroid enlargement and the decrease of thyroid iodine concentration as a method to test the goitrogenic activity.

The results were the following:

115 substances possessed detectable activity.

25 proved to have as much or more active power than thiouracil.

The relative activity of some of them, were as follows:

6-n propyl	11	6-n buthyl	3
6-benzyl	10	5-isopropyl	2.5
6-tetrabutyl	9	5-n propyl	2
6-isopropyl	9	5.6-diethyl	2
6-ethyl	8	5,5-diethyl-2-thiobarbituric acid	1.7
5-methyl	6		
6-sec.butyl	6	2-mercaptomidazole	1.5
6-isobutyl	5	2-mercapthiazoline	1.3
5-ethyl	3.5	5-amino-2-mercapto	1.3
6-ethyl	3.5	4-thiadozole	1.2

Mechanism of action: Several investigators have given theories in order to explain the mechanism of action of the thiourea derivatives. The majority of them are based on the results of experiments carried out on different species of animals and on the observation of the effects that goitrogenic drugs produce on man.

The opinions of the authors differ much from one to another; that is why we rather prefer to make a brief survey of the literature in connection with this matter.

In 1943(b) Astwood suggested as a hypothesis that the aniline derivatives may inhibit the enzyme system responsible for the conversion of diiodotyrosine into thyroxine.

Astwood, Sullivan, Bissell and Tyslowitz in the same year (1943) reported that the primary action of the thiourea compounds is to avoid the formation of new amounts of thyroid hormone by means of a mechanism not yet well explained.

In 1944, Higgins studied the action of thiourea and its derivatives, thiouracil, and sulfonamide compounds, notably sulfaguanidine and promizole, and concluded that when administered to experimental animals these are goitrogens. They produced hyperplasia of the thyroid gland and decreased the B.M.R. These phenomena were reversible when the administration of the drugs was discontinued.

The action of the drugs appeared to be exerted on the formation of the thyroid hormone. It seems that the thyroid gland becomes unable to take up the iodine from the blood stream and at the same time, loses its normal iodine content.

Thyroxine or desiccated thyroid administered concurrently with the goitrogens, inhibits their action on the thyroid, but large amounts of iodine are ineffective to prevent the gland changes. Thus the goitrogens do not act upon the thyroxine previously formed, but they inhibit the new formation.

Another attempt to explain the way that thiourea acts was carried out by Bauman, Navinette and Marine (1944). They tried to avoid the enlargement of the thyroid gland of rabbits caused by thiourea by the administration of iodine, but the iodine was promptly excreted in the urine.

They concluded that "thiourea produces a functional thyrostasis of the thyroid cells."

In 1944, Hughes injected thiouracil in rats at a 0.1 to 0.2 per cent dosage level, from the time of birth of the animals. He observed a marked growth retardation, arrested development, mild anemia and changes similar to those seen in cretinism. These symptoms were not observed when thyroxine was administered concurrently with thiourea.

Hughes and Astwood (1944) reported that thiouracil in 1:2000 concentration inhibited the metamorphosis of R.Clamitans tadpoles. Nevertheless, the action of thyroxine in inducing metamorphosis was not inhibited by thiouracil.

Franklin, Lerner and Chaikoff (1944) performed experiments on rats to see the effects of thiouracil and thiourea on the capacity of the thyroid gland to take up injected radioactive inorganic iodine. As a conclusion, the authors pointed out that those drugs depressed the

capacity of the thyroid gland to convert iodine into thyroxine and diiodotyrosine. The thyroid was restored to its normal performance two weeks after the administration of thiouracil ceased.

Astwood and Bissell (1944) reported that in young rats, thiouracil feeding produced a complete disappearance of the thyroid iodine in five days and a three-fold increase in the size of the gland in two weeks. Hypophysectomy or injection of thyroxine prevented these effects. If the administration of thiouracil was discontinued for eight days the thyroid gland returned to normal.

In continuation of his investigations concerning the action of thiouracil, Astwood (1944) pointed out that the influence of this compound on the B.M.R. depended mainly on the amount of thyroid hormone which has been already formed. He said that it is known that thiouracil has no effect over this hormone but only over the ability of the gland to synthesise its particular product. That is why a prolonged treatment is necessary to get a decrease in the B.M.R.

He treated six cases (human subjects) over a forty-two day period with 0.4 to 0.6 grams of thiouracil and there were no effects on metabolic rate, pulse rate, or body weight, and no detectable changes in the thyroid gland. In two cases a little decrease in the B.M.R. was observed after fifty-two to sixty days.

Campbell, Landgrebe and Morgan (1944) also attempted to explain the mechanism of action of thiourea. In fact,

they said that when the drug in powdered form is added to a solution of iodine in hexane, the violet color is immediately discharged. On the basis of this it was suggested that when thiourea is in contact with the thyroid iodine it would form "formamidine disulphide hydriodide," thus tieing up the iodine and preventing the synthesis of diiodotyrosine and so of thyroxine.

Larson and his associates (1945) conducted experiments in chickens and concluded that thiouracil produced hypertrophy and hyperplasia of the thyroid gland in the same manner as injections of thyrotropic hormone does. However, thiouracil acted after five days of administration while thyrotrophin exerted its action rapidly.

The thiouracil-treated chick's thyroid, when made goitrous, showed a great decrease in its capacity to collect radioactive iodine, but when the drug was withdrawn the gland rapidly recovered its power of collecting iodine. The amount of iodine actually collected was larger than in the controls. Perhaps this property of thiouracil, to inhibit the thyroid in the collection of iodine, is its essential and primary effect.

Further investigations conducted by Larson, et al. (1945) led them to the conclusion that thiouracil inhibited the capacity of the thyroid gland of the chick to collect injected radioactive iodine rapidly after the injection. The maximum inhibition is reached in an hour.

In 1945, Paschkis, et al. observed that thiouracil induced hyperplasia of the thyroid gland in 24 hours and that

this cell multiplication reached its high level at 10 to 15 days, only to decrease after this period.

Recently, in 1946, Malkiel concluded that thiouracil and sulfaguanidine did not have any antagonistic effect against the thyroid hormone previously formed. These drugs do not act by destroying or inactivating the circulating thyroxine.

Vanderlaan and Bissell (1945) tested the effectiveness of a group of goitrogenic compounds on chickens. The birds were fed for fourteen days in concentrations of 0.001 to 0.3 per cent of the goitrogenic drugs in the food. When they administered the largest doses during a period of 14 to 25 days, simultaneously with potassium iodide, it was observed that the drug did not prevent the thyroid accumulation of iodine.

Very recently, in 1947, Albert and his collaborators showed that the action of goitrogenic compounds is in close association with the affinity of these substances for iodine.

The same authors in a later report (1947), said that when pituitary extract was exposed to various goitrogens the thyrotropic activity of the hormonal material was increased.

Dosage level and physiologic effects of goitrogens: Various authors have conducted assays in order to determine the effective dosage of thiourea and its derivatives. Due to the different animals used as experimental subjects, and also to the several fields influenced by the drugs, and the large amount

of drugs herein studied, it is hard to make a definitive statement in connection with dosage. However, at the present time we have the results of the many experiments carried out to make this point clear. We will try to review briefly the reports on this subject. The physiologic effects observed are all ascribed to the thyroid-inhibitory action of the drugs.

In 1944, Mixner, Reineke and Turner concluded that 0.1 per cent of thiouracil or thiourea in the diet of White Plymouth Rock chickens, one to two days old, during a period of fourteen days, produced maximal enlargement of the thyroid gland. At the same time, they reported that increased dosages of thyroxine decreased the enlargement and that the female required greater amounts of thyroxine to obtain the same results as in male.

Leblond and Hoff (1944) reported on a comparison made on rats between the effects of thiourea, thiouracil, sulfathiazole, sulfadiazine, and thyrodectomy. They gave thiourea as one per cent of the drinking water and obtained the same symptoms as with thyroidectomy; in other words: bradycardia, histologic changes in the hypophysis and atrophy of kidneys, adrenals and heart. Sulfathiazole given as one per cent of the food or thiouracil as a solution in the drinking water produced the same syndrome but to a lesser degree. Sulfadiazine in the drinking water had no significant influence on the thyroid gland.

Juhn (1944) fed ten Brown Leghorn capons with 0.4 gm. daily of thiouracil. The effect of the compound on the

feathers was reported, textually: "to cause the replacement of black feather segments by red pigment and to effect a reduction in barbulation. These changes are similar to those obtained following thyrodectomy in this breed of fowl."

Reineke, Mixner and Turner (1945) reported on albino rats that received one-tenth per cent of thiouracil in their drinking water for two weeks. After this period the authors determined the effect of thiouracil alone, and thiouracil plus graded doses of thyroxine, on the B.M.R. and the thyroid gland weight. In the rats on thiouracil alone, they observed a reduction of 23.7 per cent in the B.M.R. and the gland weight was more than doubled.

To return the B.M.R. to normal, daily injection of 4.75 micrograms of d-l-thyroxine was required, and the thyroid gland weight became normal again with 4.8 micrograms of d-l-thyroxine.

Leathem (1945) performed some experiments on male rats that were fed 1.0 per cent thiourea during a 20 to 22 day period. He observed a rise in the blood plasma protein concentration entirely due to an increase of plasma globulin. The plasma albumin concentration did not change. A loss in body weight was also produced; however, the food intake by the treated rats was only slightly less than that of the normal controls. The expected increase of thyroid gland weight occurred.

Meyer and Virginia Ranson (1945) observed that after the thyroid gland of rats was removed, the B.M.R. fell rapidly. However, sometimes, due to the presence of accessory

thyroid glands, the metabolic rate did not change. On rats fed 0.05 per cent of thiouracil in the diet or drinking water, the B.M.R. decreased more slowly. This is probably due to the amount of hormone stored in the gland, upon which the drug does not have any influence.

Barker (1945) tested the effects of thyrotrophin on the metabolism of thiouracil-treated rats. The drug was administered in 200 to 250 mgm. per kg. of body weight, daily. The results could be outlined as follows:

a) At the twenty-fourth day, the B.M.R. was depressed an average of 12 per cent. When the observation was made on the ninety-fourth day now more changes were discovered.

b) A certain amount of thyrotrophin was injected in normal rats and rats treated 14 to 21 days with thiouracil. In normal controls there was an increase in the B.M.R. of 24.8 per cent, while in the treated rats, there was only 15.7 per cent increase. The treated animals were administered thiouracil for 10 to 40 more days. The rise in the B.M.R. in response to thyrotrophin was then decreased to 6.3 per cent.

c) Desiccated thyroid was administered to thiouracil-treated rats, and oxygen consumption recovered its normal level.

This last conclusion gives a further support to the concept that the thiouracil action is exerted mainly in blocking the production of thyroid hormone.

Andrews and Schnetzler (1945) fed 0.2 per cent thiouracil to adult female fowl. They concluded that at

that dose, the drug does not influence egg production, egg size, fertility or hatchability.

In the Department of Dairy Husbandry, University of Missouri, an experiment on chickens was conducted by Turner (1946) to compare the goitrogenic effect of thiobarbital and thiouracil. Doses of 0.1-, 0.05-, 0.025-, 0.0125-, and 0.00625 per cent were fed to one-day-old chicks for a three-weeks period. After the feeding period the birds were weighed, killed, the thyroid glands weighed and the sex determined.

Turner concluded that thiobarbital at the 0.1 per cent level restricted body weight and that at lower levels it increased the average thyroid weight regularly. For thiouracil, it was found that the 0.05 per cent level produced the maximum increase in actual thyroid weight. As another conclusion, it was suggested that thiouracil at the 0.05 per cent level is equally as effective as thiobarbital at 0.025 per cent, regarding their goitrogenic effect.

Mixner et al. (1946) reported that when they fed thiouracil 0.2 per cent in the ration to New Hampshire cockerels for a period of 16 to 35 days, more gain in body weight was produced than in normal controls and that the treated birds showed greater efficiency in the utilization of food.

Glazener and Jull (1946) conducted an experiment on the effect of thiouracil on naturally occuring molt in hens. A group of fourteen New Hampshire pullets was fed with 0.45 gm. of thiouracil daily during 26 weeks. Egg records were

kept daily, body weight and feathering were checked every two weeks during the first 14 weeks and every 4 weeks during the rest of the period, and, at the same time, compared with a control group of fourteen hens.

It was concluded that there was no difference in egg records, despite the fact that at the end of the 26-week experimental period the treated group laid a little more than the control. No significant difference was found in body weight. In the thiouracil-fed group, retarded molting was observed.

In 1944, Goldsmith, Ross, Gordon, Charipper, and M. Gordon reported an experiment carried out on fish. When these vertebrates were immersed in thiourea solution, the growth was stopped and the development of secondary sex characters inhibited. It seemed that the drug produced these abnormalities by means of an inhibition of thyroid hormone secretion in the same manner as in mammals.

Gordon, Goldsmith and Charipper (1945) observed the effects of thiouracil and para-aminobenzoic acid on rats. They fed 0.20 per cent of thiouracil and 3.0 per cent of PABA in the food during a period of 19 to 45 days. Both produced marked hyperplasia, basophilia and appearance of thyroidectomy cells in the anterior lobe of the pituitary, and more resistance of the animals against the effects of low barometric pressure. Despite the fact that the results were quite similar for both compounds, thiouracil effects were more pronounced than those of PABA. The drugs exerted an equal depression of the B.M.R.

Nevertheless, there were some differences: thiouracil produced an inhibition in body weight gain and after 38 days a slight anemia and granulocytopenia. These phenomena were not observed in rats treated with PABA.

In 1945, DiPalma and Dreyer conducted a trial in order to determine the effect of thiouracil on the autonomic nervous system in cats and rats. The administration of the compound for 7 to 21 days failed to influence the pupillary reaction to sympathetic stimulation, the salivary secretion and intestinal movements from vagus stimulation. Blood pressure and pulse after the injection of 100 to 200 mg. per kilogram, did not change in either cats or rats. From this experiment the worker concluded that thiouracil does not affect the autonomic mechanisms in the intact animal.

Eagle and Aranow (1945) reported that after treatment with 0.1 to 0.2 per cent of thiouracil for 12 weeks, hyperplasia of the thyroid gland of Rhesus Monkeys did not occur, but when the dose was increased to 0.8 gm. daily for 73 days the biopsy showed extreme hyperplasia. In 34 days after thiouracil treatment was withdrawn, the thyroid recovered its normality and in 49 days showed itself histologically normal in shape, colloid content and height of the epithelium.

Lerner and Chaikoff (1945) made an attempt to observe the influence of sulfanilamides and its derivatives and thiourea and its derivatives on respiration of thyroid tissue. They concluded that the tested drugs had no marked effect on the oxygen consumption of surviving sheep thyroid.

Baumann and Marine (1945) observed that the adrenal cortex of rats fed with thiouracil underwent a reduction in its size after a 3 to 4 month period of treatment; this involution resulted sometimes in glands that were half the normal size.

Higgins and Ingle (1946) reported that the toxic effect of the goitrogen, promizole, on the thyroid colloid was the same on hypophysectomized rats as on intact ones. However, the enlargment of the thyroid gland and the hyperplasia of the thyroid cells produced on normal animals by the promizole was not observed in hypophysectomized animals.

In 1946, Danowsky, Man and Winkler found some differences between dogs and human subjects in the behavior of the thyroid gland and in the concentration of blood precipitable iodine. In dogs this iodine is much lower and sometimes is too low to be detectable. In proportion to the body weight, the dog possesses much more ability to inactivate or to destroy desiccated thyroid or injected thyroxine. This capacity appears not entirely dependable on the thyroid but primarily on the other tissues. By attempting to prove that the metabolic processes of the dog are not completely dependent on the thyroid hormone, they blocked out the thyroxine production by thyrodectomy or by administration of thiourea or thiouracil. The reported not to have observed symptoms of myxedema, such as have been observed in human subjects.

Tipton and Nixon (1946) reported that the effect of thiouracil in any peripheral tissue is a consequence of the primary action of the drug over the thyroid gland.

Drill and Truant (1947) demonstrated that vitamin A, supplied to rats fed on a diet that was otherwise devoid of vitamin A, prevented xerophtalmia in both control and thyroidectomized groups. However, when carotene was given instead of vitamin A, xerophtalmia appeared in the thyroidectomized group and not in the controls. This proves that the thyroid gland plays a very important role in the conversion of carotene into vitamin A.

Influence of the Thyroid on Reproduction.

Laboratory animals: Williams, Albert, Grosvernor, Bissell and Peters (1944) conducted experiments on rats and reported that "No inhibition of the effect of chorionic gonadotrophin by thiouracil was demonstrated."

In 1945, Ershoff also worked on female rats. To these animals' diet was added 0.05 to 0.1 per cent of desiccated thyroid, from the time of birth to maturity, in an attempt to see the effect of hyperthyroidism on the ovaries. The ovaries remained infantile both in weight and histological appearance.

In another experiment carried out by Aranow, Engle and Sperry (1946), four adult female Rhesus monkeys were used. The animals were administered with increasing amounts of thiouracil up to 0.8 gm. daily for 14 months. Menstrual irregularities with frequent periods of amenorrhea were observed during the treatment period.

Seegar Jones, Debfs and Foote (1946) studied the action of thiouracil on reproduction in male and female adult rats. They concluded that hypothyroidism induced by this drug has no effect on the reproductive system in male adult rats, regarding their ability to sire litters. In the females, sterility due to hypothyroidism induced by thiouracil did not occur, but when the treatment was prolonged, an interference of the gestation was produced, and in 100 per cent of the cases the resorption of the foetus was the result.

When the treatment was administered during a period of less than 100 days, some of the females delivered normal litters in growth and development and reproduced themselves normally.

Cattle: Erb, Wilbur and Hilton (1940), in a study of the breeding efficiency of the Purdue University dairy herd for a period of 20 years, observed the highest average efficiency for the year during the month of May, with a 74.3 per cent conception rate.

Seasonal differences in the rate of conception were observed in dairy herds in both northern and southern Louisiana by Seath and Staples in 1941. In both herds the summer months suggests the influence of factors related to climatic changes which in turn are believed to result in differences in thyroid activity.

Petersen, Spielman, Pomeroy and Boy (1941) reported that a male Jersey was thyroidectomized at 4 months of age. After 60 days myxedemic symptoms developed rapidly. The skin became thick and puffy, the hair became dry, brittle and sparse. One of the most striking effects was that of reduced activity.

While the gonads appeared to develop normally, there was complete absence of libido at sexual maturity. His reaction was tested at frequent intervals with female in estrus and in no instance could he be induced to mate.

Periodic ejaculations were obtained by rectal manipulation of the ampulla. Semen samples obtained by

manipulation were used in artificial insemination of cows and pregnancy resulted.

Oral administration of 25 gm. of desiccated thyroid over a period of three days restored sexual activity and sexual behavior.

From this study it appears that thyroidectomy of the male bovine causes a complete inhibition of libido but had no effect upon spermatogenesis or normality of the sperm. Desiccated thyroid restored sexual activity and sexual behavior.

Erb, Andrews and Hilton (1942) made a study of the characteristics of the semen from bulls in the Purdue University dairy herd. The following seasonal variations were noted. The average semen volume was least in July, August and September. The average initial motility was least in July, August and September. The average concentration of sperm and total sperm per ejaculation was maximum during April, May and June.

The average period of sperm survival was least in August and lower in July, September and November, than during any other months. The average number of abnormal sperm was 25 per cent greater during July, August and September than during the next highest month (in temperature) of the year. The quality of the semen produced by the bulls in this study was significantly superior during the spring and significantly inferior during the summer.

Brody and Frankenbach (1942) presented a comparison of the relative growth and development, metabolism and

cardio-respiratory activities, general appearance and behavior of a thyroidectomized Jersey heifer with that of a normal Jersey heifer.

At the age of 40 months, the thyroidectomized animal was about half normal weight and metabolism per unit surface area about 40 per cent below normal. Estrus cycles were not observed.

Following the feeding of thyroprotein, the metabolism was increased, skeletal growth as indicated by an increase in the height at withers was observed, and the hair coat improved in appearance. She began to appear more feminine. There was considerable external development of her udder. Her horns began to grow and several estrus cycles were observed.

Reineke and Turner (1943) discussed the possibilities in the use of synthetic thyroprotein to influence some of the body processes of farm animals. They pointed out that after the growth of domestic animals is completed, the secretion of the thyroid gland gradually slows down; the energy metabolism of such animals decreases; they gradually fatten; breeding animals (both male and female) may become sluggish and their reproductive organs less effective. In males, the semen may lose its fertility, and in females the ovaries may become dormant and estrus cycles become irregular. By replacement therapy with thyroid material, the slightly hypo-thyroid animal can be returned to a normal condition of metabolism, and accompanying this change there is usually a marked improvement in reproductive ability. In males the

normal sexual drive returns and the quality of the semen improves. In females, the dormant ovaries return to an active condition and normal estrus cycles are resumed.

In 1943, Turner suggested the use of thyroprotein as a possible remedy for sterility in cattle due to deficiency in thyroid gland secretion. As methods of determining the energy metabolism of animals which indicates deficiencies of thyroid secretion are difficult to perform, it was suggested that it would be easier to try feeding thyroid for a period of several months to infertile cows or sluggish or infertile bulls.

Phillips, Knapp, Heemstra and Eaton (1943) made observations on motility, volume, number of sperm per c.c., total number of sperm, and the proportion of abnormal heads, necks, middle pices, tails, and total abnormal spermatozoa in the semen from three beef Shorthorn and three milking Shorthorn bulls collected at intervals of two weeks throughout a year.

Significant or highly significant differences were observed between seasons in six of the measures. The variation due to season was not significant in motility, volume, total abnormal sperm, and survival in storage.

Examination of results of 1,135 matings during the period from 1935 to 1942 revealed that the highest percentage of fertile matings occurred in April (59.6%) and the lowest in August (40.8%). It appears that the decrease noted in certain measures of semen quality during the summer months is reflected in decreased breeding efficiency in the herd.

Spielman, Petersen, Fitch and Pomeroy (1945) reported experiments on three completely and one partially thyroidectomized cows. One cow thyroidectomized 37 days after breeding aborted a dead fetus on the 225th day of the gestation period. A second cow receiving the operation 45 days after conception demonstrated no apparent effect on the duration of pregnancy. Without exception all the completely thyroidectomized cows failed to manifest the normal physical signs of estrus. Absence of the normal physical expressions of estrus was confirmed by the complete lack of interest shown these cows by normal males. Oral administration of fresh thyroid to one cow elicited marked increases in milk and fat yield and restored normal estrual behavior. Thyrotherapy of another cow failed to evoke a response in either milk secretion or sexual behavior.

Although estrus was absent, rectal examination of the ovaries indicated that ovulation occurred and that it was accurately determined by manual examination was shown by the fact that, of the four anestrus conceptions, two required one insemination each and the other two required two inseminations each.

Artificial insemination on the 161st, 242nd, and 453rd days after complete thyroidectomy, with semen from a completely thyroidectomized male resulted in the birth of three normal offspring.

Reineke (1946) reported that records have been compiled regarding the effect of feeding thyroprotein to fourteen bulls which had unsatisfactory breeding performance

records. Of this number, improvement in vigor and libido was observed in ten. The time required for an observable effect to occur ranged from 7 to 40 days, and averaged 16 days.

Definitive evidence of improvement in the conception record was obtained in only four cases. All of the bulls which responded in this series had become rather sluggish and lethargic, and showed lessened sex interest. In this type of bull, thyroprotein feeding rather consistently caused an improvement in sexual drive and vigor. The limited conception records are suggestive of an improvement in spermatogenesis.

The thyroprotein was fed at the rate of about 0.5 to 1.0 gm. per 100 lbs. body weight. At these levels no losses in condition or other deleterious symptoms were observed.

Sheep and Goats: Asdell (1926), in an extensive study of the season of conception of milk goats in Great Britain, observed a gradual increase in the rate of conception from August to October. This was followed by a gradual decline, reaching a minimum in May. The number of conceptions during the four months, April, May, June and July was very small. Evidence was presented suggesting that a cool summer produced early estrus while a hot summer had the opposite effect.

Turner (1936) analyzed the registry data on the birth rate of the milking goat in the United States. The

season of birth of 37,047 kids of the four breeds of milk goats was tabulated to determine the season of breeding. It was observed that about 5 per cent of the total goat population is born in January, and that the number increases rapidly until the first half of March when over 16 per cent kid (30 per cent for the month of March). From that time the number of births declines each month until July, when about 3 per cent kidded. From January to June over 93 per cent of all kids were born.

These data are interpreted as indicating that the milk goats of the United States are seasonal breeders. Considering the period of pregnancy as about 150 days, the anestrus period of the goat covers at least the months of April, May, June and July. In favorable seasons some does will come in heat in August.

McKenzie and Berliner (1937), measured the seasonal variation in sperm production in eight Shropshire and eight Hampshire rams. Libido was observed to be relatively independent of spermatogenesis for sterile rams might exhibit normal sex desire and fecund rams very little. The Shropshire rams showed three periods: a distinct breeding season with high sperm production from October to January; a period of somewhat lower sex activity during the spring; and a season of greatly impaired breeding capacity in summer. The Hampshire rams showed a much less fluctuating spermatogenic process with a breeding season from August to January and very slightly impaired sex activity in summer.

An increase in the number of abnormal sperm was produced artificially during late winter by keeping rams in rooms at high temperatures. The Shropshires were affected much more than the Hampshires and in much the same way as they were during the summer.

Berliner and Warbritton (1937), observed twenty-four rams during two years. The animals showed a rather distinct seasonal variation in sperm production, summer being the low season. All Shropshires produced semen of poor quality during the hot months, but only the poorer Hampshires declined noticeably during the summer. It was suggested that the poor summer sperm production was due to the decline in thyroid hormone secretion due to the high temperatures prevailing during the summer.

The effect of high temperatures was studied by placing animals in a hot room in the late winter. Sperm production failed almost completely in all the Shropshires on test and was scarcely affected in the Hampshires.

Hampshire rams totally thyroidectomized in June fell off in sperm production as did those of the intact Shropshires.

Injections of thyroxine were tried in both intact and thyroidectomized rams. A ram thyroidectomized as a lamb, after 75 mg. of thyroxine in 10 weeks, gave an ejaculation consisting of spermatids and pin-head sperm. The dose was increased to 4 mg. every other day and in August normal sperm appeared in the ejaculation. He continued to produce sperm after the thyroxine treatment was discontinued in September.

An intact Shropshire ram treated with thyroxine produced more normal sperm than usual, though in low concentration, during the summer. Two intact and one thyroidectomized Hampshire ram with high abnormality counts returned to normal after two doses (2 mg.) of thyroxine in August.

In several experiments good results were obtained by combined treatment with the gonadotrophic hormone in pregnant mares' serum and thyroxine.

They concluded that in regions in which high temperatures prevail before and during the onset of the breeding season rams with a high range of thyroid activity are better able to reproduce than animals with thyroids of low activity. Thyroxine therapy in a relatively normal animal with a less active thyroid can prevent the summer decline in normal sperm production or can restore sperm production early in the fall. Thyroxine in late summer or early fall, just before the breeding season should start, is probably preferable.

Green (1940) pointed out that the quality of the semen decreased slightly but steadily from January to May. During June and July the ejaculation became quite inferior, presenting an increased number of head abnormalities, tailless heads, and minute sperm. From July to October a very rapid readjustment toward higher quality was under way. The density likewise started to decline in May, reaching its lowest level in August. The rate of recovery lagged about one month behind the change in normal cells, the maximum density not being attained until December.

The results from the above work seem to indicate that more attention should be given to the study of the ability of rams to enter the early portion of the breeding season in a high degree of fertility.

Phillips, Schott, Eaton, and Simmons (1943) used twelve rams and two bucks in a study of seasonal changes in the amount and quality of semen produced. Semen samples were obtained at intervals of two weeks throughout a year in artificial vaginas and observations were made on motility, volume, number of sperm per c.c., total sperm produced, and the proportion of abnormal heads, necks, middle pieces, tails, and total abnormal sperm. Differences between seasons were found to be significant in all the items studied except volume. The rams within a breed differed significantly in all the characteristics studied. The findings substantiate the results of earlier investigations which indicate that warm summer temperatures may be an important factor in reducing fertility in sheep. The extent to which it influences fertility in goats could not be determined from available data. Further work is needed on methods of counteracting the effects of hot weather on fertility.

Turner, Mixner, and Reineke (1943) published a report of an experiment in which a ram which had good sex drive but deficient sperm was fed thyroprotein. The sperm improved in quality and afterwards settled several ewes.

A Toggenburg buck showed a definite lack of interest in the does as they came in heat. With the feeding of 1 gm. daily of thyroprotein, he again displayed sexual interest and afterwards settled five out of six does.

Bogart and Mayer (1946, abc) also worked on the relation of temperature and the thyroid to mammalian reproductive physiology.

During periods of high summer temperature, there was a decrease in total spermatozoa per ejaculation, a lowered volume and a greater percentage of morphologically and physiologically abnormal cells in the semen of rams. Injection of thyroxine or thyroprotein feeding during this period of high temperature caused the production of more spermatozoa and a lower percentage of anormal cells. In the fall (normal breeding season) reproductive activity was at its peak. Creation of hypothyroid state by feeding thiouracil practically eliminated spermatogenesis and caused the production of greater percentage of anormal cells. These results are similar to those induced by high summer temperatures. Thyroxine restores spermatogenesis to its normal level in rams whose fertility was lowered by thiouracil administration.

It was concluded that the thyroid gland is of major importance in the reproductive physiology of the ram. Further, it was concluded that the changes in environmental temperature produce variations in reproductive activity in the ram indirectly through the thyroid gland.

Man: Mills (1939), in a study of the influence of the season upon the conception rate in man, presents data obtained in various cities in Canada, along the United States northern coast and southern cities with high summer temperatures.

As the average summer temperature increases, the conception rate is markedly reduced. In the north and in the coastal cities with less variation in seasonal temperature, the conception rate is far more constant. Since high temperatures depress the thyroxine secretion rate, these data may be interpreted as indicating the depressing effect of lack of thyroxine upon reproduction.

Martin (1930) made a review of the literature upon the mechanism by which man adjusts himself to the external environment. In a trip from England to Australia by way of the Suez Canal, Martin observed that with rise in temperatures in the Red Sea and in Indian Ocean, his energy metabolism declined markedly, whereas with cooler temperatures approaching Australia his metabolism again increased toward the normal.

Birds.

1) Egg production: Crew (1925) reported rather phenomenal results with the feeding of thyroid in the rejuvenation of five cocks and seven hens, five to eight years of age. To each of these birds desiccated thyroid was administered daily for six months (May to October). Soon after treatment was begun all the birds passed through a molt, and when new plumage appeared it was characteristic of younger fowls. The head furnishings became red and turgid. The very low egg production of 6.67 eggs per hen in 6 months previous was increased during the thyroid treatment to 34 eggs for the same period and during the 6 months period following the treatment, production was 24 eggs per hen.

Turner, Irwin and Reineke (1945) reported on forty-eight 2-year-old White Leghorn hens that were divided into 4 groups of twelve hens each of equal potential productive ability as judged by physical characteristics. One lot fed the basal ration alone served as control, whereas thyroprotein (containing 2.7% thyroxine) was added in increasing amounts to the other three lots as follows: Lot II, 5 gm.; Lot III, 10 gm.; and Lot IV, 20 gm. per 100 lbs. of complete battery feed.

Lots II and III reached a much higher level of egg production and the summer seasonal decline was less marked. The average per cent egg production for the year was as follows: Lot I control 22.6 per cent; Lot II, 5 gm. 38.1 per cent; Lot III, 10 gm. 40.6 per cent and Lot IV, 20 gm. 30.7 per cent. It was concluded that the optimum level of thyroprotein in the feed ranged between 5 and 10 gm. per 10 lbs. of feed. These amounts of thyroprotein fed were without effect on the average weight of the eggs produced.

No evidence was obtained that the feeding of thyroprotein caused the passage of this hormone into the eggs.

Turner, Kempster, Hall and Reineke (1945) fed a group of twenty-four year-old White Leghorns for a full year upon a ration containing 10 gm. of thyroprotein per 100 lbs. of feed. The thyroprotein contained 2.7 per cent thyroxine by chemical analysis. At this level of feeding there was present more than twice the amount of thyroxine necessary to prevent the enlargement of the thyroids of six-month old chickens fed 0.1 thiouracil.

During the fall and winter, the egg production of the experimental group followed the production of the control group. However, after May 7, the control group gradually declined in egg production whereas those fed thyroprotein continued to lay at the winter level until August when egg production fell off precipitously. Thus the control group fell off 43.8% in production during the second half of the year in comparison with only 6.4% for the birds that were fed thyroprotein.

In comparison with their first and second year production, the hens surviving three years in the control produced 81.3 per cent and 89.5 per cent of their previous years' production, whereas those fed thyroprotein the third year produced 72.5 per cent and 102.3 per cent of the previous years' production.

Two groups of 12 Massachusetts Red Pullets were fed 10 and 20 gm. of thyroprotein per 100 lbs. feed in comparison with a control group. At the 10 gm. level, egg production was again maintained at a higher level during the second half of the year. During this period the control decreased 25.6 per cent in egg production in comparison with the fall and winter level of production, whereas those fed 10 gm. of thyroprotein declined only 6.1 per cent. The 20 gm. level of thyroprotein-feeding was slightly above the optimum so egg production was below that of the control group.

These results are interpreted as indicating that the seasonal cycle of egg production is due in part to a reduced secretion of thyroxine during the summer months.

When the thyroid hormone is maintained at a uniform level in the feed and presumably in the body by the feeding of thyroprotein, egg production was maintained on a more uniform level during this period.

2) Testes and sperm production:

(a) Thyroid feeding: Jaap (1933) said that during late winter and early spring months, the testes of Mallard drakes normally increase in size and spermatogenic activity. During this period, an artificial increase much greater than that of the normal drakes is obtained by feeding daily doses of 0.25 to 1.0 gm. of desiccated thyroid. The testis size ranged from 2 to 10 times that of the non-thyroid-fed controls. Microscopically, the testes of the thyroid fed drakes exhibited a marked increase in spermatogenesis, which was proportional to the size of the testis. The largest testes have a large number of fully formed sperm in the lumen of each tubule, while the smaller testes showed only division figures.

Castrated drakes molted profusely when as small a daily dose as 0.25 gm. of desiccated thyroid was administered, while non-castrated drakes lost very few feathers under the same treatment.

Aron and Benoit (1934) observed that when immature male ducks were fed with thyroid tissue or injected with thyroxine they became sexually stimulated.

Titus and Burrows (1940) fed 100 mg. of desiccated thyroid to White Leghorn cockerels about six months old,

three times per week, starting in May, for a period of six weeks. The seasonal decline in semen volume as measured three times per week was increased by this treatment. When treatment was stopped, there was a temporary increase in semen volume. It would appear from these results that this dosage of thyroid was excessive.

(b) Effect of thyroidectomy: Benoit and Aron (1934) reported that thyroidectomy delayed the normal testicular growth in chickens and ducks. Testes of White Leghorns rapidly decreased in size. Losses were as great as 80 per cent of the testes size in 11 days and 90 per cent in 20 days. Similar results were obtained with drakes.

Benoit (1936) showed that thyroidectomy reduced considerably the testicular growth in immature ducks exposed for 15 hours per day during three weeks, to the stimulating action of electric light.

Benoit (1937) observed that thyroidectomy of the duck decreased spermatogenetic activity of the testes which is stimulated by artificial light. The inhibition was very pronounced.

Benoit (1937) reported that the penis of ducks after thyroidectomy attained a development inferior to that attained by normal ducks with testicles of equal size. When the thyroid regenerates to some extent normal growth of the penis follows. Within the limits of this experiment a functioning thyroid appears to be necessary for the normal development of secondary sex characteristic.

Benoit (1937) found that thyroidectomy exerted an inhibiting effect on the growth of the testes of the ducks stimulated by electric light. This effect is very marked during 3-4 weeks but diminishes later on. It is not yet possible to say whether it disappears completely after a sufficient length of time. It also exerts an inhibitory effect on the development of the penis. This inhibition is more marked and more prolonged than that of the testes.

Greenwood and Chu (1939) observed that the effect of thyroidectomy on the male Brown Leghorn was very marked. There was a regression in testis size, together with the cessation of spermatogenesis. Expressing the testes weight as percentage of body weight, they gave 1.030 per cent for the normal adult and 0.026 per cent for the thyroidectomized birds of similar age, while in the juvenile group the comparative figures for normal and experimental birds were 0.55 per cent and 0.047 per cent, respectively.

Histological examination of the testis of thyroidectomized birds showed that the loss in weight was followed by a marked reduction in the diameter of the tubules and inhibition in the progress of sperm production.

A young thyroidectomized cockerel was fed 20 mg. of dried thyroid substance daily for a period of 80 days immediately following the operation. During this time the comb of the bird increased in size by 72 mm., while untreated birds showed an average increase in comb size of only 8 mm. Following the cessation of thyroid treatment the comb underwent regression, decreasing in size by 26 mm. during the succeeding 80 days.

Blivaiss and Domm (1942) found a total of 30 birds to be completely thyroidectomized from a large series of Brown Leghorn cockerels operated on at 4 to 20 days. Body weight of adult roosters were 40-45% below those of normals. Combs averaged 62-68% less than those of controls. At 8 months of age, histological observations indicated a delay in spermatogenesis. The testes had smaller tubules and no stages beyond spermatocytes.

Glazener and Morley (1946) fed 0.2 and 0.1 per cent thiouracil and 1.0 per cent desiccated thyroid to six-week-old chickens during 10 weeks. They reported that the weight of testes in both levels of thiouracil and desiccated thyroid was appreciably less than in the controls.

Recently in 1947, Mixner and Upp (86) reported that in "double cross" chickens (Rhode Island Red and S.C.W. Leghorn) there was an increased level of thyroid gland secretion in these hybrid subjects above the inbred controls. They suggested that this is possibly a factor contributing to the development of hybrid vigor.

Shaffner and Andrews (1947) fed thiouracil as 0.5 per cent of the ration to male fowl. They observed that this drug in such a dose produced after treatment for 16 weeks a decrease to 60 per cent of the B.M.R. of that of the control. The fertility showed by the treated roosters was 0.0 to 10 per cent while that of the controls was 35 to 45 per cent. An impairment of the semen volume, motility, sperm concentration, methylene blue reduction time and survival of sperm at 5. C. was also observed.

EXPERIMENTAL PROCEDURE

A. Selection of subjects: The Rhode Island Red male fowls used in this experiment were bred and raised in the Poultry Department of Michigan State College. They were hatched in the summer of 1946. On the 16th of March, 1947, the birds were placed in individual cages.

The room temperature was maintained at 60° to 70° F. by means of a thermostatically controlled radiator, during March and April. After that, we thought that the laboratory temperature would not produce ill effects on the roosters. During the March and April, artificial light was supplied from 8 a.m. to 7 p.m. - natural sunlight was sufficient thereafter.

The cockerels were fed a mash recommended by the Poultry Department of Michigan State College and mixed by the King Milling Company of Lowell, Michigan. It contains the following ingredients:

690 lbs.	Corn Meal
400 lbs.	Ground Oats
300 lbs.	Bran
200 lbs.	Middlings
60 lbs.	17% Dehydrated alfalfa meal
60 lbs.	Meat scraps
40 lbs.	Dry milk
50 lbs.	Fish meal
50 lbs.	Soybean meal
100 lbs.	Oyster shell flour
30 lbs.	Steamed bone meal
12 lbs.	Salt
8 lbs.	Fish liver oil (400 A. 2000 D.)
2000 lbs.	Total

The birds had the mash "ad libitum." They also had running tap water all the time.

B. Selection of dosage: Since there were no references about the relation of thyroid and semen production on birds we selected the doses of thyroprotein according to the amount required to stimulate feather growth and egg production, as reported by Turner, Irwin and Reineke (1943). Thus the different groups received the drugs at the levels indicated below:

Group I. Normal Control
Group II. Thiouracil 0.1%
Group III. Thiouracil 0.1% and thyroprotein 0.01%
Group IV. Thiouracil 0.1% and thyroprotein 0.02%
Group V. Thiouracil 0.1% and thyroprotein 0.04%
Group VI. Thyroprotein 0.02%

Since the mixing of the drugs with the feed is such an important factor in the results, taking into account the small amounts used we gave special attention to the mixing process, to obtain even distribution. We mixed the respective amount of drugs with 20 lbs. of the mash. The thiouracil was kindly supplied by Lederle Laboratories, Inc., Pearl River, New York. The thyroprotein used was "Protamone," a highly active iodinated protein supplied by Cerophyl Laboratories, Inc., 2438 Broadway, Kansas City, Missouri.

C. Semen samples: To obtain the semen samples the Burrows and Quinn (1937) method was used. When we began the trials on April 1, we failed; consequently, we had to give the roosters a long period of training in order to obtain good samples. We handled the subjects once every day. In this way, one after another gave better and better samples until all of them were working quite well in approximately twenty days. We noticed that the first roosters to ejaculate

during the training period were the best semen producers during the experimental period. We also found that some of them did not react regularly and others, two or three, did not react at all. We also observed that some of them gave better samples when a preliminary collection was made 48 hours earlier. Parker, McKenzie and Kempster (1942) pointed out that a constant interval between ejaculations is desirable in this kind of experiment with cocks. Thus we decided to take two samples a week, according to the schedule below.

DAYS	GROUPS Vol. Mot. Conc. Total N.Sperm	GROUPS Vol. and Motil.
Monday	II	V
Tuesday	III and IV	I and VI
Wednesday	V	
Thursday	I and VI	
Friday		
Saturday		II
Sunday		III and IV

Vol. = volume; Motil. = motility; Conc. = concentration; Total N. of Sperm. = Total number of sperm.

D. General Plan: From the first collection, we tested only the volume and the motility. From the second one, we did, beside the volume and the motility, the sperm concentration per cubic milimeter and the total number of sperm per ejaculation.

a) Volume - The volume was measured by means of a 1 c.c. tuberculin syringe. It gave us a reasonably accurate measurement.

b) Motility - Soon after the volume was obtained the motility was examined. Most of the authors advised the use of the hanging drop technique, but we did not find any difference between the above and the use of the common slide. Therefore, we decided to use the latter. We put a small drop of the sample on the slide and it was covered by a cover glass to be examined under the microscope. For scoring the motility, the table employed by Parker, McKenzie and Kempster (1942) was used. This is shown below.

Motility Rating	DESCRIPTION
5.	Semen containing sperms showing a vigorous and progressive motility.
4.	Semen containing a few sperms with vigorous motility. There may be a few inactive sperms.
3.	Semen containing many sperms showing moderate motility. There may be a number of inactive sperms.
2.	Semen containing many sperms showing slight motility. There may be a number of inactive sperms.
1.	Semen containing few sperms showing slight motility.
0.	No motility observed.

C. Sperm Concentration: To determine the sperm concentration we employed the same hematocytometer used in counting red blood cells. The technique is the same, but as we found the sperms still alive when we made the counts, we added a few drops of alcohol to the Tyrode's solution used to dilute the semen.

In the mixing pipette we took undiluted semen up to the 0.5 mark and Tyrode's solution up to the 101 mark. Then we shook the pipette for three and a half minutes in a mechanical shaker. We observed that this time was sufficient to get a very good dispersion of the sperms. When we used more time in shaking we observed that a large number of sperms lost their tails or presented different kinds of abnormalities.

Two counts of each sample were taken and the average was used as the value for the sperm concentration per cubic milimeter.

D. Total number of sperm: As we knew the volume in cubic centimeters and the sperm concentration in cubic milimeters, we used the following formula to determine the total number of sperm per ejaculation:

$$T.N. = C \times V \times 1{,}000.$$

EXPERIMENTAL RESULTS

After the 22nd of April, when the roosters had started to produce good semen samples regularly, we began to record the results. On May 7, we added an equal dose of thiouracil to groups II, III, IV, and V (0.1%). Group I was the normal control and to group VI was added thyroprotein as 0.02 per cent of the ration. Unfortunately, groups I and VI were too irregular to be considered in the results. Two of the normal control roosters died during the experimental period. In group VI we have the best semen producer, but the other two roosters were such poor producers that we decided to eliminate this group as we did with the normal control.

A. Volume: The results in the volume test show the average range of between 0.2 and 0.8 c.c. A good number of the samples are between 0.8 and 1.0 c.c., and only few of them surpass the one cubic centimeter mark. The largest sample we recorded was 1.56 c.c. We also noticed that a few of the collections were below 0.2 c.c.

Group I - As we said above, this normal control group was very irregular, because two of the birds died. We replaced the first one, but when the second went the same way as the first it was necessary to omit this group. However, during the time that we worked on this group, we obtained samples with volumes ranging between 0.20 and 0.51 c.c. (Table 1).

SEMEN VOLUME cc.

Date	Birds			
	3736	3709	3732	Average
April 25	0.06	0.51	0.38	0.32
29	0.53	0.68	----	----
May 1	0.57	0.66	0.30	0.51
6	0.65	0.54	0.32	0.50
8	0.21	0.62	----	----
13	0.31	0.25	----	----
15	0.38	0.55	0.50	0.48
20	0.41	0.48	0.35	0.41
22	0.33	0.67	0.23	0.41
27	0.29	----	0.18	----
29	----	0.48	----	----
June 3	0.24	0.55	0.20	0.33
5	0.33	0.52	0.37	0.41
10	0.19	0.19	0.21	0.20
12	0.31	0.65	----	----
17	0.45	0.49	0.10	0.34
19	0.41	0.44	----	----
24	0.62	0.53	----	----
26	0.21	0.44	----	----

On May 6, bird 3732 died, and on June 18, bird 3740 died.

Group II - This group was fed 0.1 per cent thiouracil from May 7th until the end of the experimental period on June 26th. The average (Table II) was like that of the normal control group, between 0.20 and 0.50 cc.; in other words, a variation in volume was not produced with this dose of the drug.

TABLE 2. The influence of thiouracil given as 0.1 per cent of the ration on the volume of semen produced by roosters.

Date	Birds				Remarks
	3778	3537	9789	Average	
April 22	0.51	0.27	0.26	0.35	No treatment
26	0.03	0.21	0.02	0.09	
28	0.26	0.21	0.30	0.26	
May 3	0.05	0.43	0.30	0.26	
5	0.26	0.38	0.36	0.33	
10	0.06	0.20	0.42	0.23	Thiouracil 0.1 per cent
12	0.45	0.38	0.67	0.50	
17	0.29	0.21	0.50	0.33	
19	0.39	0.41	0.36	0.39	
24	0.41	0.42	0.52	0.45	
26	0.32	0.21	0.24	0.26	
31	0.15	0.39	0.18	0.23	
June 2	0.41	0.43	0.22	0.35	
7	0.33	0.35	0.20	0.29	
9	0.39	0.40	0.34	0.38	
14	0.38	0.46	0.41	0.42	
16	0.36	0.48	0.25	0.33	
21	0.29	0.42	0.33	0.35	
23	0.29	0.31	0.33	0.31	

SEMEN VOLUME cc.

Group III - This group received 0.1 per cent thiouracil from May 7 until June 5, and then during the next three weeks, 0.01 per cent thyroprotein. At the end of the thiouracil period (Table III) we observed a very slight decrease in the semen volume. After we started feeding thyroprotein they recovered normal volume. The average figures in this group ranged from 0.27 to 0.57 cc. before the thiouracil period; 0.32 to 0.49 during the thiouracil period; and 0.43 to 0.59 cc. during the thyroprotein period. We can say that the influence of 0.01 per cent thyroprotein on the semen volume is negligible.

TABLE 3. The effect on semen volume of thiouracil followed by 0.01 per cent thyroprotein.

SEMEN VOLUME cc.					
Date	Birds				Remarks
	3708	9792	3706	Average	
April 22	0.52	0.18	0.18	0.29	No treatment
29	0.33	0.31	0.10	0.25	
May 4	0.32	0.31	0.27	0.30	
6	0.32	0.51	0.45	0.43	
11	0.50	0.32	0.65	0.49	Thiouracil 0.1
13	0.52	0.36	0.83	0.57	per cent
18	0.31	0.57	0.49	0.46	
20	0.38	0.48	0.47	0.44	
25	0.54	0.39	0.55	0.49	
27	0.32	----	0.28	----	
June 1	0.42	0.41	0.31	0.38	
3	0.22	0.34	----	----	
8	0.42	0.39	0.34	0.38	Thyroprotein 0.01
10	0.27	0.39	0.31	0.32	per cent
15	0.47	0.58	0.61	0.59	
17	0.45	0.36	0.47	0.43	
21	0.32	0.53	0.79	0.55	
24	0.71	0.42	0.38	0.50	

Group IV - This group was fed 0.1 per cent of thiouracil from May 7 until June 5 and then 0.02 per cent thyroprotein.

The average figures were the following:

Before the treatment: 0.52 to 0.96 cc.
Thiouracil 0.1 per cent: 0.37 to 0.78 cc.
Thyroprotein 0.02 per cent: 0.48 to 0.67 cc.

We should point out that the largest values in the thiouracil period were obtained at the beginning of treatment with a considerable decrease occurring toward the end of this period.

TABLE 4. The effect on semen volume of thiouracil followed by 0.02 per cent of thyroprotein.

SEMEN VOLUME cc.

Date	Birds				Remarks
	3769	9798	3742	Average	
April 23	0.64	0.66	0.29	0.53	No treatment
29	0.40	0.98	0.59	0.66	
May 4	0.42	0.65	0.48	0.52	
6	0.52	0.85	0.58	0.65	
10	0.58	0.83	0.62	0.68	Thiouracil 0.1
13	0.74	1.56	0.59	0.96	per cent
18	0.66	0.96	0.72	0.78	
20	0.43	0.65	0.52	0.53	
25	0.43	0.96	0.51	0.63	
27	----	0.49	0.30	----	
June 1	0.42	0.44	0.18	0.35	
3	----	0.30	0.20	----	
8	0.38	0.56	0.57	0.50	Thyroprotein 0.02
10	0.55	0.69	0.40	0.55	per cent
15	0.54	0.99	0.42	0.65	
17	0.67	1.02	----	----	
22	0.62	0.59	0.60	0.60	
24	0.20	0.91	0.33	0.48	

We notice here that in the thiouracil period we attained a slight decrease in volume, and a slight increase in the thyroprotein period time.

Group V. - This group was fed with the largest amount of thyroprotein that we used in this experiment; 0.04 per cent in the diet during three weeks after the thiouracil treatment. The average volume figures were as follows:

Before the treatment: 0.29 to 0.69 cc.
Thiouracil 0.1 per cent: 0.39 to 0.73 cc.
Thyroprotein 0.04 per cent: 0.63 to 0.88 cc.

TABLE 5. The effect on semen volume of thiouracil followed by 0.04 per cent of thyroprotein.

SEMEN VOLUME in cc.					
Date	Birds				Remarks
	3726	3735	3745	Average	
April 24	0.21	0.39	0.63	0.41	No treatment
28	0.27	0.12	0.47	0.29	
30	0.52	0.29	0.53	0.45	
May 5	0.34	0.22	0.67	0.41	
7	0.45	0.45	0.74	0.55	
12	0.57	0.57	0.91	0.69	Thiouracil 0.1 per cent
14	0.85	0.85	0.71	0.73	
19	0.81	0.81	0.63	0.63	
21	0.49	0.49	0.44	0.47	
26	0.31	0.31	0.51	0.39	
28	0.51	0.51	0.58	0.46	
June 2	0.48	0.48	0.63	0.53	
4	0.35	0.35	0.56	0.41	
9	0.68	0.68	0.94	0.76	Thyroprotein 0.04 per cent
11	0.55	0.55	0.79	0.64	
16	0.66	0.66	0.79	0.68	
18	0.72	0.72	1.04	0.88	
23	0.68	0.68	1.16	0.84	
25	0.51	0.51	0.82	0.63	

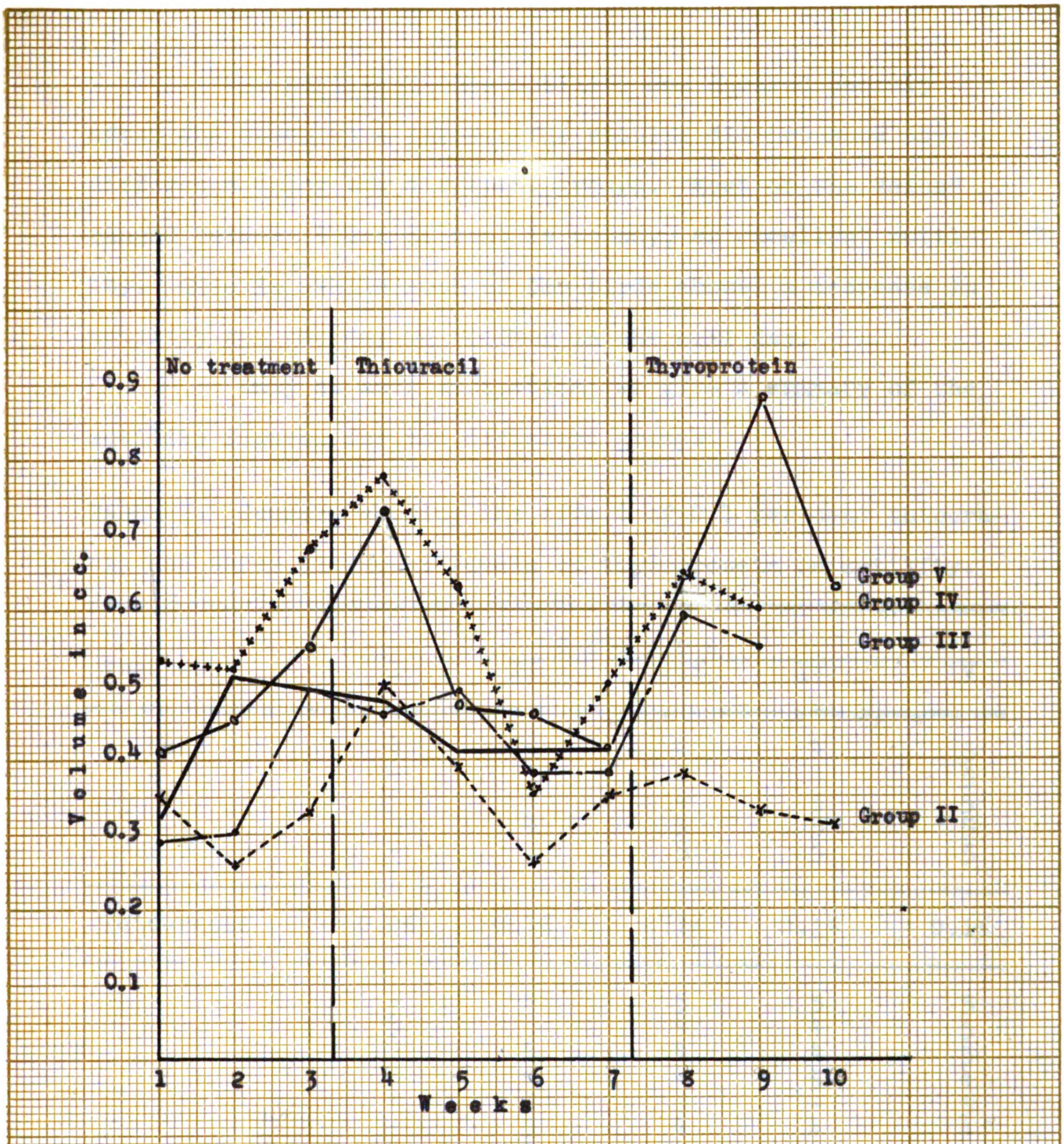
As in group IV, the largest volumes in the thiouracil period were obtained at the beginning, that is to say, we cannot consider the increase as an effect of the drug, but as a normal daily variation as it was very often observed. The

high values obtained in the thyroprotein period, with 0.04 per cent dose level, must be considered as a definitive influence of the drug on the semen volume.

A general table of the average figures of the volume of the different groups and a graph will better illustrate the results.

TABLE 6. Summary showing the effect of 0.1 per cent thiouracil and three different levels of thyroprotein on semen volume.

Treatment	Groups				
	I	II	III	IV	V
None	0.32	0.35	0.29	0.53	0.41
	----	0.09	0.25	0.66	0.29
	0.51	0.26	0.30	0.52	0.45
	0.50	0.26	0.43	0.65	0.41
	----	0.33	0.49	0.68	0.55
Thiouracil	0.0%	0.1%	0.1%	0.1%	0.1%
	----	0.23	0.57	0.96	0.69
	0.48	0.50	0.46	0.78	0.73
	0.41	0.33	0.44	0.53	0.63
	0.41	0.39	0.49	0.63	0.47
	----	0.45	----	----	0.39
	----	0.26	0.38	0.35	0.46
	0.33	0.23	----	----	0.53
	0.41	0.35	0.38	0.50	0.41
Thiouracil	0.0%	0.1%	0.0%	0.0%	0.0%
Thyroprotein	0.0%	0.0%	0.01%	0.02%	0.04%
	0.20	0.29	0.32	0.55	0.76
	----	0.38	0.59	0.65	0.64
	0.34	0.42	0.43	----	0.68
	----	0.33	0.55	0.60	0.88
	----	0.35	0.50	0.48	0.84
	----	0.31	----	----	0.63



Graph 1. The effect of 0.1 per cent thiouracil and three different levels of thyroprotein on semen volume.

B. Motility: The motility was checked shortly after the semen were taken. The results did not show any difference among the different groups. Most of the values ranged between 4 and 5; that is to say, an excellent motility. Once in awhile some of the birds showed a decrease in motility for one day to recover in the next day.

Only as an illustration, we give a summary of the motility figures for the different groups.

TABLE 7. Summary showing the effect of 0.1 per cent thiouracil and three different levels of thyroprotein on motility.

Treatment	Groups I	II	III	IV	V
None	4	5	4	4	5
	-	1	4	4	5
	5	4	4	5	5
	5	4	5	5	5
	-	5	5	5	5
Thiouracil	0.0%	0.1%	0.1%	0.1%	0.1%
	-	4	5	5	5
	5	5	4	4	5
	5	5	4	4	5
	5	4	4	5	4
	-	3	3	-	5
	-	5	4	4	4
	5	3	-	-	5
	5	4	5	5	5
Thiouracil	0.0%	0.1%	0.0%	0.0%	0.0%
Thyroprotein	0.0%	0.0%	0.01%	0.02%	0.04%
	5	5	4	4	5
	-	5	5	4	5
	4	5	4	-	5
	-	5	5	4	5
	-	3	4	3	4
	-	5			5

C. Concentration: As we said above, the red blood cell count technique was used to determine the sperm concentration. We carried out two counts of each sample; we logically assumed that in this way the errors should be considerably less. We recorded the average value in each case.

GROUP I - This was the normal control group, but as it was so irregular we cannot consider it to make a good comparison with the rest of the groups. The data for this group are shown in Table 8.

TABLE 8. Sperm concentration of the normal control birds.

Date	Sperm Concentration in Millions				Remarks
	3736	3709	3732 3740	Average	
April 25	3.980	-----	3.010	3.495	
May 1	4.560	3.310	3.220	3.696	
8	-----	4.255	-----	-----	
15	3.110	3.035	1.945	2.696	
22	3.325	2.850	1.465	2.546	
29	-----	2.065	-----	-----	
June 5	1.745	1.670	1.425	1.613	
12	1.995	2.335	-----	2.165	
19	2.570	1.975	-----	2.272	
26	2.955	1.940	-----	2.447	

GROUP II - This group was fed all through the experimental period on 0.1 per cent thiouracil. It maintained an average concentration of 2.5 millions, with a low concentration of 2.016, and the highest was 3.466. No effect of thiouracil was observed at this dosage level.

TABLE 9. The effect on sperm concentration of 0.1 per cent thiouracil.

Date	Sperm Concentration in Millions				Remarks
	3778	3537	9789	Average	
April 22	2.275	2.020	3.225	2.506	No treatment
28	3.010	1.180	1.860	2.016	
May 5	3.510	3.570	3.320	3.466	
12	3.220	2.750	2.050	2.673	Thiouracil 0.1
19	2.785	2.960	2.285	2.676	per cent
26	3.625	1.855	1.570	2.350	
June 2	2.350	1.970	2.310	2.210	
9	3.010	2.890	3.040	2.980	
16	2.425	1.950	1.945	2.106	
23	2.890	3.160	1.850	2.633	

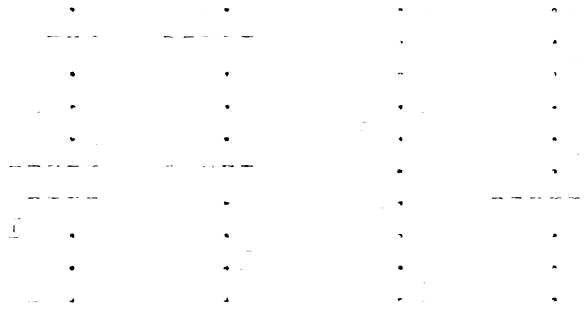
Regarding these figures, it is very easy to observe that there was no influence of the thiouracil on the sperm concentration at the percentage indicated above.

GROUP III - This group received thiouracil 0.1 per cent from May 7 until June 5, and thereafter thyroprotein 0.01 per cent until June 25, the end of the assay. Neither a decrease with the former drug nor an increase with the latter was observed. The average concentration was about 2.6 millions with the lowest figure of 2.2 millions and 3.75 as the highest.

TABLE 10. The effect on sperm concentration of thiouracil followed by 0.01 per cent of thyroprotein.

Date	Sperm Concentration in Millions				Remarks
	3706	3708	9792	Average	
April 22	1.290	3.320	3.175	2.595	No treatment
29	0.400	3.500	-----	-----	
May 6	1.580	3.065	3.055	2.566	
13	2.660	3.625	2.365	2.833	Thiouracil 0.1
20	3.040	4.710	2.430	3.393	per cent
27	1.660	3.060	-----	-----	
June 3	-----	2.735	2.835	-----	
10	0.380	2.510	1.525	2.611	Thyroprotein 0.01
17	1.205	2.320	1.760	2.626	per cent
24	1.875	1.905	2.850	2.210	

The unrecorded figures are because the cockerels in those days did not give a sample, or else the sample was not suitable to determine the concentration due to contamination with fecal material.



GROUP IV - This group was fed 0.1 per cent thiouracil from May 7 to June 5 and thereafter 0.02 per cent thyroprotein until June 25, that is to say, the end of the experiment. The lowest figure in concentration was 1.38 millions and the highest average figure 3.093 millions. Almost all through the experimental period, this group kept an average concentration around 2.0 millions.

TABLE 11. The effect on sperm concentration of thiouracil followed by 0.02 per cent of thyroprotein.

Date	Sperm Concentration in Millions				Remarks
	3769	9798	3742	Average	
April 23	3.230	2.165	2.540	2.645	No treatment
29	2.555	3.325	3.400	3.093	
May 6	3.765	2.995	1.950	2.903	
13	2.545	1.780	2.455	2.260	Thiouracil 0.1
20	1.935	2.485	2.665	2.361	per cent
27	-----	2.280	1.530	1.905	
June 3	-----	2.265	1.770	2.017	
10	1.915	2.120	1.495	1.843	Thyroprotein 0.02
17	1.040	1.720	-----	1.380	per cent
24	0.055	2.180	2.955	1.730	

We should say from these figures that 0.02 per cent thyroprotein did not make a significant influence in concentration; the decrease that is observed may be by chance and not a result of the drug action.

GROUP V - Fed 0.1 per cent thiouracil from May 7 to June 5, and then thyroprotein, 0.04 per cent, until the end of the experimental period, that is to say during the three following weeks. The average figures of this group show very regular values during the time before the treatment and during thiouracil administration. Shortly after the thyroprotein feeding started, a significant increase in sperm concentration was noticed, rising to the highest figures at the end of the assay. 2.045 millions and 3.100 millions are the lowest and the highest values obtained in this group.

TABLE 12. The effect on sperm concentration of thiouracil followed by 0.04 per cent of thyroprotein.

Date	Sperm Concentration in Millions				Remarks
	3726	3735	3745	Average	
April 24	2.875	2.690	3.390	2.985	No treatment
30	3.235	1.830	3.625	2.896	
May 7	2.770	1.345	2.020	2.045	
14	2.410	2.240	2.465	2.371	Thiouracil 0.1
21	1.730	2.390	3.365	2.495	per cent
28	2.910	2.190	1.570	2.223	
June 4	2.730	2.225	2.290	2.415	
11	3.625	1.580	3.755	2.986	Thyroprotein 0.04
18	2.725	2.580	3.600	2.968	per cent
25	3.020	2.740	3.540	3.100	

It is interesting to observe that the highest figures of this group correspond to the period of thyroprotein administration.

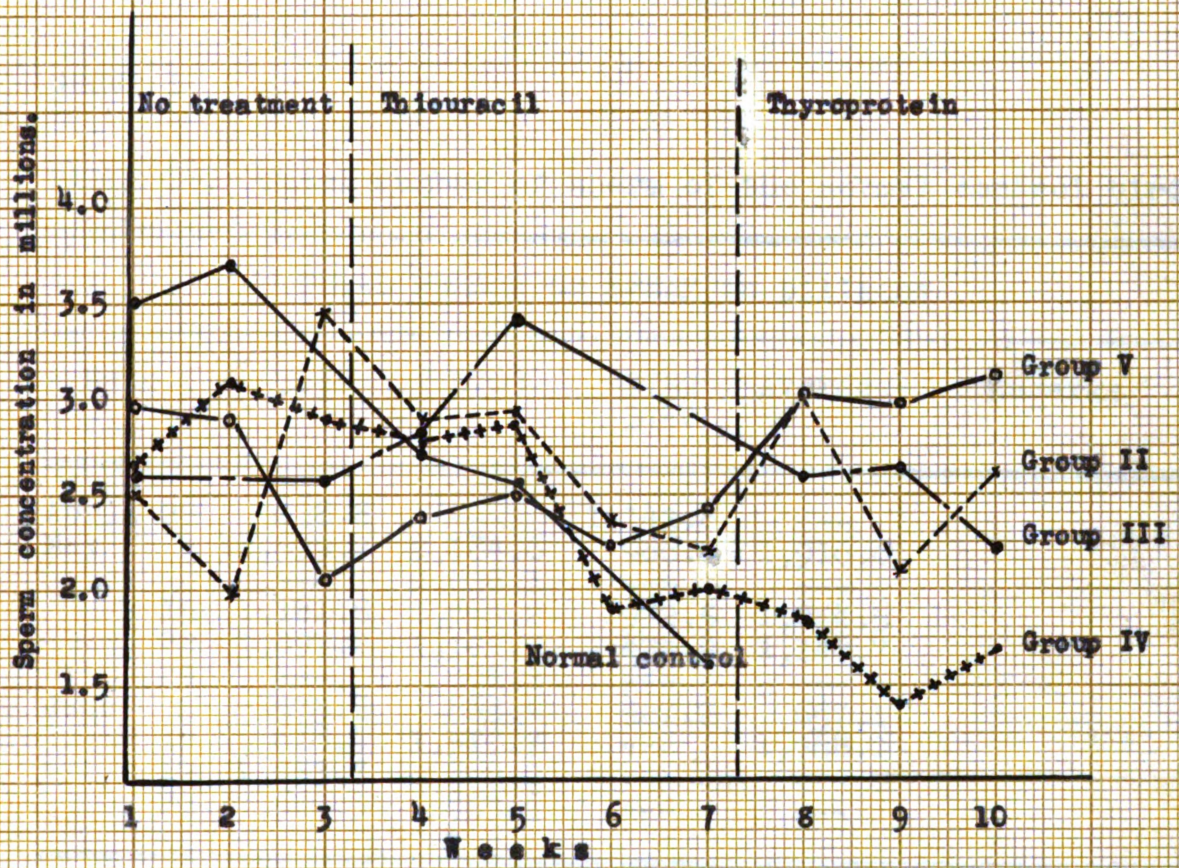
In the groups described earlier the values show that no influence on the sperm concentration is obtained with 0.1 per cent thiouracil or 0.01 and 0.02 per cent thyroprotein. But when we come to this dose (0.04%) of thyroactive substance a very considerable increase in concentration is realized. Unfortunately, the small number of subjects in the groups (3 in each one) does not allow us to come to statistically important conclusions. Nevertheless, these results suggest the possibility that 0.04 per cent thyroprotein exerts an improvement on birds' sperm concentration.

A general table of the average values and a graph will illustrate better the above statement.

TABLE 13. Summary showing the effect of 0.1 per cent thiouracil and three different levels of thyroprotein on sperm concentration.

Treatment	Groups				
	I	II	III	IV	V
None	3.495	2.506	2.595	2.645	2.985
	3.696	2.016	-----	3.093	2.896
	-----	3.466	2.566	2.903	2.045
Thiouracil	0.0%	0.1%	0.1%	0.1%	0.1%
	2.696	2.673	2.833	2.260	2.371
	2.546	2.676	3.393	2.361	2.495
	-----	2.350	-----	1.905	2.223
	1.613	2.210	-----	2.017	2.415
Thiouracil	0.0%	0.1%	0.0%	0.0%	0.0%
Thyroprotein	0.0%	0.0%	0.01%	0.02%	0.04%
		2.980	2.611	1.843	2.986
		2.106	2.626	1.380	2.968
		2.633	2.210	1.730	3.100

On the graph included on the next page, it is possible to corroborate the matter of this table.



Graph 2. Showing the effect of 0.1 per cent thiouracil and three different levels of thyroprotein on sperm concentration.

D. Total number of sperm: The total number of sperm has illustrated better than any other point, the effect of the drugs used in this experiment. In fact, as in this matter are involved the volume and the sperm concentration, it is easier to observe the variation produced. We have given earlier a simple formula to get the total number of sperm.

GROUP I

TABLE 14. Total number of sperm of the normal control birds.

Date		TOTAL NUMBER OF SPERM in billions				
		3736	3709	3732 3740	Average	Remarks
April	25	238.8	---	1,143.8	---	
May	1	2,599.2	2,184.6	966.0	1,916.6	
	8	---	2,638.1	---	---	
	15	1,181.8	1,669.2	972.5	1,274.5	
	22	1,097.2	1,909.5	434.2	1,146.7	
	29	---	991.2	---	---	
June	5	575.8	868.4	527.2	657.1	
	12	618.4	1,517.7	---	1,068.0	
	19	1,053.7	869.0	---	---	
	26	620.5	853.6	---	---	

In the third column there are two roosters because of the death of the first one on May 6. He was replaced by the rooster 3740, who died on June 18.

GROUP II - This group was fed thiouracil during the entire experimental time. The dose used was 0.1 per cent. The variations observed in total number of sperm were insignificant. Nevertheless, at the end of the assay there was a decrease in the average figures. It appears that the dose used was not sufficient to produce a wide difference to be considered as a result of thiouracil influence on the semen production. The last values are almost identical with the values obtained before the treatment.

TABLE 15. The effect on total number of sperm of 0.1 per cent thiouracil.

Date		TOTAL NUMBER OF SPERM in billions				
		3778	3537	9789	Average	Remarks
April	22	1,160.2	545.4	838.5	848.0	No treatment
	28	782.6	247.8	558.0	529.5	
May	5	912.6	1,356.6	1,251.0	1,173.4	
	12	1,449.0	1,045.0	1,373.5	1,286.4	Thiouracil 0.1
	19	1,086.1	1,213.6	822.6	1,040.8	per cent
	26	1,160.0	389.5	376.8	642.1	
June	2	963.5	847.1	508.2	772.9	
	9	1,173.9	1,156.0	1,033.6	1,121.2	
	16	630.5	936.0	486.2	684.2	
	23	838.1	979.6	610.5	809.4	

GROUP III - After two weeks of recording the data without treatment, this group was fed thiouracil 0.1 per cent during four weeks, that is to say, from May 7 to June 5, and then was fed thyroprotein 0.01 per cent until June 24. We already saw that the dose of the goitrogenic drugs was not sufficient to exert an influence on semen production. We corroborated this statement in this group, since it was fed the same dose and significant changes in the total number of sperm were not produced. Regarding the dose of thyroprotein (0.01%), according to the results, we realized that such a dose did not improve the semen production as we had expected.

TABLE 16. The effect on total number of sperm of 0.1 per cent thiouracil followed by 0.01 per cent of thyroprotein.

	TOTAL NUMBER OF SPERM in billions				
Date	3706	3708	9792	Average	Remarks
April 22	670.8	597.6	571.0	613.1	No treatment
29	132.0	1,085.0	---	---	
May 6	505.6	1,563.2	1,374.8	1,147.8	
13	1,383.2	1,305.0	1,962.9	1,550.3	Thiouracil 0.1
20	1,155.2	2,260.8	1,142.1	1,519.4	per cent
27	531.2	---	---	---	
June 3	---	929.9	---	---	
10	102.6	978.9	472.8	518.1	Thyroprotein 0.01
17	542.2	835.2	827.2	734.9	per cent
24	1,331.2	800.1	1,083.0	1,071.4	

GROUP IV - Like Group III, this one was on thiouracil, 0.1 per cent, after two weeks of recording normal data; in other words, from May 7 to June 5, and then until June 24 this group was fed thyroprotein 0.02 per cent.

The values obtained in this group show us that such a dose of thyroprotein is insufficient to exert an improvement in semen production. Nevertheless, just after we started the feeding of the thyroid active substance we noticed an increase in the total number of sperm, but this was only to a level similar to that before the treatment.

TABLE 17. The effect on total number of sperm of thiouracil followed by 0.02 per cent of thyroprotein.

	TOTAL NUMBER OF SPERM in billions				
Date	3769	9798	3742	Average	Remarks
April 23	2,067.2	1,428.9	736.6	1,410.9	No treatment
29	1,022.0	3,258.5	2,006.0	2,095.5	
May 6	1,957.8	2,545.7	1,131.0	1,878.2	
13	1,883.3	3,829.8	1,448.4	2,387.2	Thiouracil 0.1
20	832.0	1,615.2	1,385.8	1,277.7	per cent
27	---	1,117.2	459.0	---	
June 3	---	679.5	354.0	---	
10	1,053.2	1,462.8	598.0	1,038.0	Thyroprotein 0.02
17	696.8	1,754.4	---	---	per cent
24	11.0	1,983.8	975.1	989.9	

GROUP V - This group was fed thiouracil from May 7 to June 5 at a 0.1 per cent dose level and after that thyroprotein, 0.04 per cent, until June 25. The dose of thiouracil used in this group, as in the others, was unable to produce any effect on semen production or on the total number of sperm, since the values obtained from the beginning of the experiment until thyroprotein started were quite similar. But, shortly after the treatment with this drug began, a very significant increase in total number of sperm was noticed. The three figures attained during the thyroprotein treatment are the highest averages in all the groups. Thus, we can say that 0.04 per cent of thyroprotein does exert a marked influence on the total number of sperm.

TABLE 18. The effect on total number of sperm of thiouracil followed by 0.04 per cent of thyroprotein.

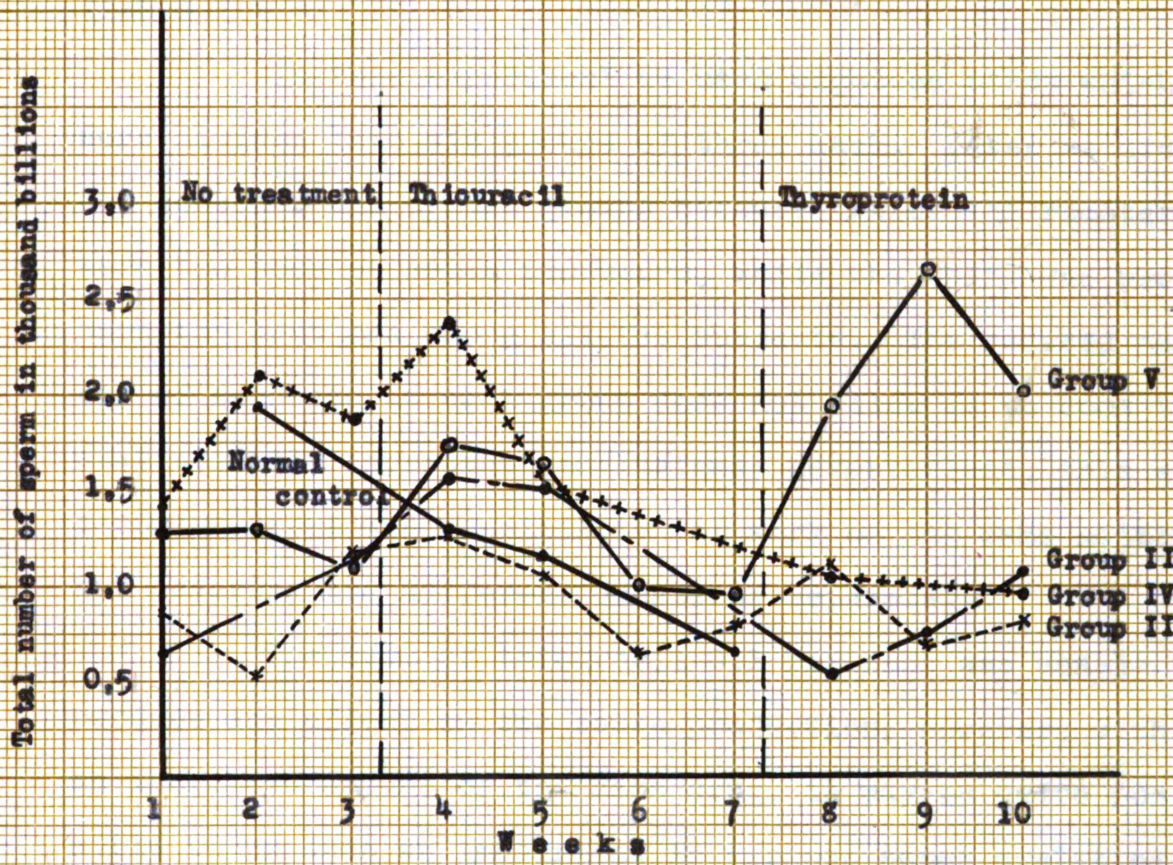
	TOTAL NUMBER OF SPERM in billions				
Date	3726	3735	3745	Average	Remarks
April 24	603.7	1,049.1	2,135.7	1,262.8	No treatment
30	1,682.2	219.6	1,921.2	1,274.3	
May 7	1,246.5	605.2	1,494.8	1,115.5	
14	2,048.5	1,411.2	1,750.1	1,736.6	Thiouracil 0.1
21	847.7	2,558.4	1,480.6	1,628.9	per cent
28	1,484.1	657.0	910.6	1,017.2	
June 4	955.5	712.0	1,282.4	983.9	
11	1,933.7	932.2	2,966.4	1,944.1	Thyroprotein 0.04
18	1,962.0	2,244.6	3,744.0	2,650.2	per cent
25	1,540.2	1,507.0	2,902.8	1,983.0	

A general table of the average figures of all the groups will give us a better illustration about the influence of thyroprotein at different doses.

TABLE 19. Summary showing the effect of 0.1 per cent thiouracil and three different levels of thyroprotein on total number of sperm.

Treatment	Groups				
	I	II	III	IV	V
None	---	848.0	613.1	1,410.9	1,262.8
	1,916.6	529.5	---	2,095.5	1,274.3
	---	1,173.4	1,147.8	1,878.2	1,115.5
Thiouracil	0.0%	0.1%	0.1%	0.1%	0.1%
	1,274.5	1,286.4	1,550.3	2,387.2	1,736.6
	1,146.7	1,040.8	1,519.4	1,277.7	1,628.9
	---	642.1	---	---	1,017.2
	657.1	772.9	---	---	983.9
Thiouracil	0.0%	0.1%	0.0%	0.0%	0.0%
Thyroprotein	0.0%	0.0%	0.01%	0.02%	0.04%
		1,121.2	518.1	1,038.0	1,944.1
		684.2	734.9	---	2,650.2
		809.4	1,071.4	989.0	1,983.0

On the next page, a graph in connection with this table is included which shows clearly the results of the treatment on the total number of sperm.



Graph 3. The effect of 0,1 per cent thiouracil and three different levels of thyroprotein on total number of sperm.

SUMMARY AND DISCUSSION

The influence of the thyroid gland on the semen production of mammals has been shown by many investigators, but no reports of the same matter on birds have been published.

In this experiment we used eighteen birds which were divided into six groups of three each. Later we did not consider one of the groups because two of the roosters were too poor as semen producers, while the third was perhaps the best one among all the subjects. The reduced number of subjects in each group does not allow us to come to statistically important conclusions; nevertheless, this experiment opens the possibility for work on the same matter, but covering the different questions separately.

The dose of thiouracil 0.1 per cent was unable to produce any effect on the semen production; however, with an increased amount of the same drug, it may be quite possible to produce an impairment in various semen qualities, according to the first report on this matter published by Shaffner and Andrews (1947).

The increased doses of thyroprotein (0.01, 0.02 and 0.04 per cent) used, have shown that the largest dose was the only one able to produce an improvement in the semen production. The effect of still larger dosages still remains to be determined.

Sperm motility was not affected by the administration of thyroprotein. Since motility depends mainly on the

accessory organs, this suggests that thyroid has little or no effect on the accessory organs of birds. On the other hand, sperm motility was checked very soon after the samples were taken and this is perhaps another reason why differences among the various groups were not seen.

In semen volume, there was a very slight increase or none at all in Group V, which was fed with the largest amount of thyroprotein. In the rest of the groups no changes in the different phases of the experiment were noticed. The semen volume, like the sperm motility, depends on the accessory sexual glands, and so this is another point which suggests that, in birds, the thyroid has little or no influence on the accessory glands.

On sperm concentration and consequently on the total number of sperm per ejaculation, a marked increase with 0.04 per cent of thyroprotein was observed immediately after the treatment started. Since sperm concentration depends on the process of spermatogenesis, it may be assumed that thyroprotein at certain dose levels stimulates the spermatogenetic activity.

CONCLUSIONS

1. Thiouracil fed to the domestic fowl as 0.1 per cent of the ration for a period of four weeks does not produce significant changes in the semen volume, motility or sperm concentration.

2. Thyroprotein fed as 0.01 and 0.02 per cent of the ration does not influence semen production of spermatogenesis in the domestic fowl.

3. Thyroprotein fed as 0.04 per cent of the ration causes a definite stimulation of spermatogenesis. Both the semen volume and the sperm concentration are increased quite markedly. Consequently the total number of sperm per ejaculation is increased by approximately 65 per cent.

REFERENCES

Albert, Alexander; Rulon W. Rawson; Priscilla Merril; Beatrice Lennon; and Charlotte B. Riddell, 1947. "The effect of goitrogenic and other reducing agents on inactivated thyrotropic hormone extract." Endocrinology, 40:299.

Andrews, F. N. and E. E. Schnetzler, 1945. "The effect of feeding thiouracil to hens upon the thyroid gland of chicks." Endocrinology, 37:382.

Aranow, Henry Jr.; Earl T. Engle and Warren M. Sperry, 1946. "Some effects of the administration of thiouracil to monkeys." Endocrinology, 38:331.

Aron, M.; and J. Benoit, 1934. Comptes rendus Soc. Biol. 117:218.

Asdell, S. A., 1926. "Variation in onset of the breeding year in the goat." J. Agr. Sci., 16:632.

Astwood, E. B., 1943-a. "Treatment of hyperthyroidism with thiourea and thiouracil." J. A. M. A., 22:78.

Astwood, E. B., 1943-b. "Chemical nature of compound which inhibit the function of the thyroid gland." J. Pharmacol. and Exper. Therap., 78:79.

Astwood, E. B., 1944. "Thiouracil treatment in hypothyroidism." J. Clin. Endocrinol., 4:229.

Astwood, E. B. and Adelle Bissell, 1944. "Effect of thiouracil on the iodine content of the thyroid gland." Endocrinology, 34:282.

Astwood, E.B.; Adelle Bissell and A. M. Hughes, 1945-a. "The antithyroid activity of thiobarbital (5,5-diethyl - 2 thiobarbituric acid)." Endocrinology, 36:72.

Astwood, E. B.; Adelle Bissell and A. M. Hughes, 1945-b. "Further studies on the chemical nature of compounds which inhibit the function of the thyroid gland." Endocrinology, 37:456.

Astwood, E. B.; J. Sullivan; A. Bissell and R. Tyslowitz, 1943. "Action of certain sulfonamides and of thiourea upon the function of the thyroid gland of the rat." Endocrinology, 32:210.

Barker, S. B., 1945. "Effects of thyrotrophin on metabolism of thiouracil-treated rats." Endocrinology, 37:230.

Baumann, E., 1896. "Ueber das normale vorkommen von jod im thierkorper." Z.Physiol.Chem., 21:319.

Baumann, J. Emil and David Marine, 1945. "Involution of the adrenal cortex in rats fed with thiouracil." Endocrinology, 36:400.

Baumann, J. Emil; Navinette Metzer and David Marine, 1944. "Mode of action of thiourea on the thyroid gland of rabbits." Endocrinology, 34:44.

Benoit, J. and Aron, M., 1934. "Comptes rendus." Soc.Biol., 116:221.

Benoit, J., 1936. "Role de la thyroide dans la gonado-stimulation par la lumiere artificielle chez le canard domestique." Soc. Biol., 123:243.

Benoit, J., 1937-a. "Thyroide et croissance testiculaire chez le canard domestique." Comptes rendus Soc. Biol., 125:459.

Benoit, J., 1937-b. "Thyroide et croissance du penis chez le canard domestique." Comptes rendus Soc. Biol., 125:461.

Benoit, J., 1937-c. "Relation between thyroid and growth of testes and penis when stimulated by electric light." Proc. Soc. Exp. Biol. and Med., 36:782.

Berliner, V. and Warbritton, V., 1937. "The pituitary and thyroid in relation to sperm production in rams." Proc. Am. Soc. Animal Prod. 30 ann. meeting, p. 137.

Best, C. H. and N. B. Taylor, 1943. "The Physiological Basis of Medical Practice." Williams and Wilkins Co., p. 18.

Blivaiss, B. and Domm, L. V., 1942. "Relation of thyroid gland to plumage pattern and gonad function in the Brown Leghorn male. Anat. Rec., 84:529.

Bogart, R. and Mayer, D. T., 1946-a. "The relation of temperature and the thyroid to mammalian reproductive physiology." Am. Soc. of Zoologists, A.A.A.S. Meeting, St. Louis, Missouri.

Bogart, R. and Mayer, D. T., 1946-b. "Environmental temperature and thyroid gland involvement in lowered fertility of rams." Mo. Agr. Exp. Sta. Res. Bul. 402, April.

Brody, S. and Frankenbach, R. F., 1942. "Age changes in size, energy, metabolism, and cardio-respiratory activities of thyroidectomized cattle." Mo. Agr. Exp. Sta. Res. Bul., 349.

Burrows, W. H. and J. P. Quinn, 1937. "The collection of spermatozoa from the domestic fowl and turkey." Poul. Sci., 16:19.

Campbell, David; Landgrebe, F. W. and Morgan, T. N., 1944. "Pharmacology and thiourea." Lancet, 1:630.

Crew, F. A. E., 1925. "Rejuvenation of the aged fowl through thyroid medication." Proc. Roy. Soc. Edin., 45:252.

Danowski, T. S.; E. B. Man and A. W. Winkler, 1946. "Tolerance of normal and thyroidectomized of thiourea and thiouracil treated - dogs, to oral desiccated thyroid and intravenous thyroxine." Endocrinology, 38:230.

Di Palma, R. Joseph and Nicholas B. Dreyer, 1945. "Failure of thiourea to alter the anatomic responses of intact animals." Endocrinology, 36:236.

Drill, Victor A. and Aldo P. Truant, 1947. "Effect of thyroidectomy on the conversion of carotene to vitamine A." Endocrinology, 40:259.

Engle, Earl T. and Henry Aranow, 1945. "Hyperplasia of the thyroid gland of Rhesus monkeys after thiouracil treatment." Endocrinology, 38:325.

Erb, R. E.; Wilbur, J. W. and Hilton, J. H., 1940. "Some factors affecting breeding efficiency in dairy cattle." J. Dairy Sci., 23:549.

Erb, R. E.; Andrews, F. N. and Hilton, J. H., 1942. "Seasonal variation in semen quality of the dairy bull." J. Dairy Sci., 25:815.

Ershoff, H. Benjamin, 1945. "Effects of thyroid feeding on ovarian development in the rat." Endocrinology, 37:215.

Franklin, A. L.; S. R. Lerner and I. L. Chaikoff, 1944. "The effect of thiouracil on the formation of thyroxine and diiodotirosine by the thyroid gland of the rat with radioactive iodine as indicator." Endocrinology, 34:265.

Glazener, Edward W. and Morley A. Jull, 1946-a. "Effects of thiouracil, desiccated thyroid, and stilbestrol derivatives on various glands, body weight, and dressing appearance in the chicken." Poul. Sci., 25:236.

Glazener, W. Edward and Morley A. Jull, 1946-b. "Effect of thiouracil on naturally occurring molt in the hen." Poul. Sci., 25:533.

Goldsmith, E. D.; Ross F. Nigrelli; Albert S. Gordon; Harry A. Charipper and Myron Gordon, 1944. Endocrinology, 35:132.

Gordon, Albert S.; E. D. Goldsmith and Harry A. Charipper, 1945. "Effects of para aminobenzoic acid and thiouracil on thyroid function and resistance to low pressures." Endocrinology, 37:223.

Green, W. W., 1940. "Seasonal trends of sperm cell types in sheep." Proc. Am. Soc. An. Prod., p. 207.

Greenwood, A. W. and Chu, J. P., 1939. "On the relation between thyroid and sex gland functioning in the Brown Leghorn fowl." Quart. J. Exp. Physiol., 29:111.

Gull, W. W., 1874. "On a cretinoid state supervening in adult life in women." Tr. Clin. Soc. London, 7:180.

Higgins, George M., Ph.D., 1944. "A consideration of the physiologic action of thio-uracil and other goitrogens." Minnesota Med., 27:997.

Higgins, George M. and Dwight J. Ingle, 1946. "The relation of the hypophysis to certain changes induced in the rat by the goitrogen Promizole." Endocrinology, 38:110.

Hughes, A. M., 1944. "Cretinism in rats induced by thiouracil." Endocrinology, 34:69.

Hughes, A. M. and E. B. Astwood, 1944. "Inhibition of metamorphosis in tadpoles by thiouracil." Endocrinology, 34:138.

Jaap, R. G., 1933. "Testis enlargement and thyroid administration in ducks." Poul. Sci., 12:(Sept.).

Juhn, Mary, 1944. Endocrinology, 35:278.

Larson, A. Roger; F. Raymond Keating, Jr.; Wendell Peacock and Rulon W. Rawson, 1945. "A comparison on the effect of thiouracil and injected thyrotropic hormone on the collection of radioactive iodine and the anatomic changes induced in the thyroid of the chick." Endocrinology, 36:149.

Larson, A. Roger; F. Raymond Keating, Jr.; Wendell Peacock and Rulon W. Rawson, 1945. "The effect of thiouracil on the collection of radioactive iodine by the thyroid of the chick." Endocrinology, 36:160.

Leathem, H. James, 1945. "Influence of thiourea on plasma proteins and organ weight in the rat." Endocrinology, 36:98.

Leblond, C. P., 1944. Endocrinology, 35:229.

Lerner, S. R. and I. L. Chaikoff, 1945. "The influence of goitrogenic compounds (sulfanilamides and its derivatives, thiourea and its derivatives) on respiration of thyroid tissue." Endocrinology, 37:362.

MacKenzie, Julia B.; C. G. MacKenzie and E. V. McCollum, 1941. Science, 94:518.

MacKenzie, C. G. and J. B. MacKenzie, 1943. "Effects of sulfanilamides and thioureas on the thyroid gland and basal metabolism." Endocrinology, 32:185.

Malkiel, Saul, 1946. "A note on the mode of thiouracil action." Endocrinology, 38:58.

Martin, C. J., 1939. "Thermal adjustments on man and animals to external condition." Lancet, 2:617.

McKenzie, F. F. and Berliner, V., 1937. "The reproductive capacity of rams." Mo. Agr. Exp. Sta. Res. Bul., 265.

Meyer, Arthur E. and G. Virginia Ransom, 1945. "The metabolism of rats after thyroidectomy or during thiouracil treatment, and the effect of thyroid feeding." Endocrinology, 36:259.

Mills, C. A., 1939. "Medical Climatology." Pub. C. C. Thomas, Springfield, Ill. p. 44.

Mixner, P. John and Charles W. Upp, 1947. "Increased rate of thyroxine secretion by hybrid chicks as a factor in heterosis." Poul. Sci., 26:389.

Mixner, J. P.; B. A. Tower and C. W. Upp, 1946. "The effect of feeding thiouracil on the body weight of New Hampshire cockerels." Poul. Sci., 25:536.

Mixner, J. P.; E. P. Reineke and C. W. Turner, 1944. "Effect of thiouracil and thiourea on thyroid gland of the chick." Endocrinology, 34:168.

Parker, Jesse E.; Fred McKenzie and H. L. Kempster, 1942. "Fertility in the male domestic fowl and turkey." Poul. Sci., 16:19.

Paschkis, K. E.; A. Cantarow; A. E. Rakoff and M. S. Rothenberg, 1945. "Mitosis stimulation in the thyroid gland induced by thiouracil." Endocrinology, 37:133.

Perez-Victoria, C., 1947. "El tratamiento de los estados de hiperfuncion tiroidea por el metiltiuracilo." Medicina Clinica, 8:242 (Barcelona, Espana).

Petersen, W. W.; Spielman, A.; Pomeroy, B. S. and Boyd W. L., 1941. "Effect of thyroidectomy upon sexual behavior of the male bovine." Proc. Soc. Exp. Biol. and Med., 46:16.

Phillips, R. W.; Knapp, B. Jr.; Heemstra, L. C. and Eaton, O. N., 1943. "Seasonal variation in the semen of bulls." Am. J. Vet. Res., 4:115.

Reineke, E. P. and C. W. Turner, 1942. "Formation in vitro of highly active thyroprotein, their biologic assay, and practical use." Mo. Agr. Exp. Sta. Res. Bul. 355(Nov.).

Reineke, E. P. and C. W. Turner, 1943. "Synthetic thyroprotein, a new drug available in veterinary practice." J. Am. Vet. Med. Assn., 102:105 (Feb.).

Reineke, E. P.; John P. Mixner and C. W. Turner, 1945. "Effect of graded doses of thyroxine on metabolism and thyroid weight of rats treated with thiouracil." Endocrinology, 36:64.

Reineke, E. P., 1946. "The effect of synthetic thyroprotein on sterility in bulls." Conference on fertility, Feb.2.

Reverdin, J. L., 1882. "Accidents consecutifs a l'ablation totale du goitre." Rev. Med. de la Suisse Romande, 2:539.

Schultze, A. B. and C. W. Turner, 1944. "The rate of thyroxine secretion by the thyroid glands of White Leghorn cockerels." Yale J. Biol. and Med., 17:269.

Seath, D. M. and C. H. Staples, 1941. "Some factors influencing the reproductive efficiency of Louisiana herds." J. Dairy Sci., 24:510.

Seegar, Jones G. E., M. D.; E. Debfs, M. D.; and E. C. Foote, M. A., 1946. "The effect of thiouracil hypothyroidism on reproduction in the rat." Endocrinology, 38:337.

Shaffner, C. S. and F. N. Andrews, 1947. "Influence of thiouracil on semen quality in the fowl." Poul. Sci., 26:555.

Spielman, A. A.; W. E. Petersen; J. B. Fitch and B. S. Pomeroy, 1945. "General appearance, growth and reproduction of thyroidectomized bovine." J. Dairy Sci., 28:329.

Tipton, Samuel R. and W. L. Nixon, 1946. "The effect of thiouracil on the succinoxidase and cytochrome oxidase of rat liver." Endocrinology, 39:300.

Turner, C. W., 1936. "Seasonal variation in the birth rate of the milking goat in the United States." J. Dairy Sci., 19:619.

Turner, C. W., 1943. "Sterility in sires and cows - synthetic thyroprotein is one answer." Guernsey Breeders' Jour., 63:712(May).

Turner, C. W.; M. R. Irwin and E. P. Reineke, 1945. "Effect of the thyroid hormone on egg production of White Leghorn hens." Poul. Sci., 24:171.

Turner, C. W.; H. L. Kempster; N. M. Hall and E. P. Reineke, 1946. "The effect of thyroprotein on egg production." Poul. Sci., 25:562.

Turner, C. W., 1946. "Comparison of the effect of feeding thiobarbital and thiouracil on the thyroid gland of the chick." Poul. Sci., 25:517.

Vanderlaan, W. P. and A. Bissell, 1945. "The influence of selected goitrogenic compounds on the thyroid gland of the chick." Endocrinology, 38:308.

Williams, Robert H.; Albert R. Weinglass; Grosvernor W. Bissell and Jean B. Peters, 1944. "Anatomical effects of thiouracil." Endocrinology, 34:317.