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M. F. MILLER, *Director*

The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals

A. B. SCHULTZE AND C. W. TURNER

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The Determination of the Rate of Thyroxine Secretion by Certain Domestic Animals

A. B. SCHULTZE AND C. W. TURNER

The ability to effect increased productive capacity in domestic animals by selective breeding has been demonstrated. What the changes in the physiologic mechanism of an animal are that result in increased productive ability have, until recent times, been unexplored. With the rapid development of endocrinology, it has been found that endocrine glands play an important role in the more direct mediation of productive ability. For example, an animal bred for fast growing capacity may attain this inherited expression because its pituitary has a high secretory activity with respect to the growth promoting hormone. Good reproductive function may be inherited but expressed in an individual because of high gonadotrophic production by the anterior pituitary. High milk production is inherited, but its expression is integrated with the inheritance of various physiologic mechanisms, one of which is undoubtedly high thyroid activity since thyroxine has been shown to increase milk production.

Thus when the balance of the activity of the endocrine glands is modified favorably by selective breeding the modification will result in improved productive ability in domestic animals.

In all domestic animals there is a more or less retention of seasonal variation in reproductive function and associated phenomenon that is probably a carry-over from the condition existing when the animals were in the wild state. The immediate expression of the seasonal variation is probably mediated by endocrine behavior. In the wild condition this seasonal fluctuation was probably necessary for the survival of the species. Under domestication, however, seasonal rhythm is sometimes detrimental to the efficient performance of the animal. For example, early lambs cannot be produced in some sections because of inability to get the ewes with lamb during the summer months. If the inability to breed in hot weather is related to subnormal thyroid activity in summer months, then by determining what thyroid activity level is necessary for reproductive function, the seasonal fluctuation could be eliminated by addition of proper levels of thyroidally active materials. Other variations of productive function such as milk and butterfat production, egg production, etc., with season, may be eliminated at least partially by proper endocrine stimulation.

Recent research on the relation of thyroid function to productive ability has been made. There are indications that increased thyroid function increases milk and butterfat production and egg production and stimulates growth in some species. On the other hand, decreased thyroid function apparently results in a tendency toward fattening.

With the recent availability of thyroidally active materials that are capable of increasing the thyroid hormone concentration in the blood on the one hand and of compounds capable of preventing the synthesis of thyroid hormone and thus reducing the concentration of the hormone in the blood on the other, it has become of increasing practical importance to determine the rate of thyroid hormone production in animals that vary in physiological activity.

This is true because the concentration of thyroid hormone in the blood has been shown to profoundly affect different functional processes. By determining the rate of thyroid hormone secretion in agricultural animals varying in milk production, growth rate, or fattening ability, the normal variation of thyroid secretion rate associated with the degree of productive ability can be determined. With these values determined, they will serve as a basis for the quantitative administration of thyroidally active materials to enhance those functions that are associated with high thyroid activity such as milk production and egg production, for example, and for the administration of anti-thyroid drugs such as thiourea or thiouracil to enhance those functions associated with relatively subnormal activity such as rapid fattening.

Added thyroxine increases milk production, but do high milk producing cows have high thyroid activity? In other words, does high milk production normally occur because of high thyroid activity? If not, possibly added thyroxine results in high milk production in an abnormal manner and would ultimately have, therefore, an unfavorable effect on the animal. On the other hand, if high thyroid activity is an essential factor for high milk production in the normal animal, then the addition of thyroidally active material in a quantity within the range of normal variation could not be considered to induce an abnormal condition and would be expected to have no unfavorable effect.

Determinations of the thyroid hormone secretion rate in rapidly fattening animals would serve as a basis for administering anti-thyroid compounds and the degree of administration would again be governed by normal variations associated with the fattening of animals possessing this function to a high degree.

It was with these considerations in mind that an initial study of the rate of thyroxine secretion in various animals was begun.

Normal Regulatory Mechanism of Thyroid Activity

Because of the important role which the thyroid hormone plays in animal physiology, many studies have been directed toward determining changes in thyroid activity associated with both internal and external environment. That the thyroid is capable of altering its activity to a great degree in response to changing environment is generally believed. Marine (1935b) noted that the thyroid is endowed with tremendous capacities for increasing or decreasing its functional activity as indicated by changes in weight, microscopic appearance, iodine content and blood supply. Also studies on the mitotic activity of the secreting cells of the thyroid, iodine content of the blood, histological appearance of the gland including the presence or lack of colloid in the gland, and changes of the basal metabolic rate (B.M.R.) have all indicated that the secretory activity of the thyroid is influenced by changing environment.

To interpret the meaning of such changes in the thyroid, an understanding of the normal regulatory mechanism is essential.

The primary regulatory mechanism controlling the activity of the thyroid has been revealed by experimental work within the past 20 years. Such work shows that the anterior pituitary secretes a thyrotrophic factor that regulates thyroid activity. Foster and Smith (1926) found that the B.M.R. was markedly lowered in rats by hypophysectomy and could be brought back to normal by pituitary implants or thyroid administration, thus showing a relationship between the anterior pituitary and the thyroid. Loeb and Bassett (1929 and 1930) and Aron (1930) showed that certain extracts of the anterior pituitary caused marked hypertrophy of the thyroid of guinea pigs. The hypertrophy was accomplished by a great number of mitoses followed by disappearance of the colloid and increased height and number of epithelial cells. Upon thyroid hypertrophy in the chick (Keating et al., 1945) there was a rapid loss of iodine store from the gland, indicating a high degree of thyroid activity. Hypertrophy of the thyroid appears to occur in autotransplants (Solomon and Sevringhaus, 1936), showing that nervous connections are not necessary to the action.

It is interesting to note that several authors including Dempsey (1944) have observed that pituitary activation of the thyroid by the thyrotrophic factor apparently results in the release of colloid from the gland with increased discharge into the blood stream, while suppression of the pituitary stimulus results in accumulation of colloid in the lumen of the follicles. Keating et al. (1945) present data on this point showing that thyrotrophic stimulation produces a prompt and early acceleration in the rate with which radioactive iodine previously stored in the thyroid is lost from it. The collection of radio-active iodine under the stimulus of the thyrotrophic factor did not increase in proportion to the increased thyroid weight induced. Thus the thyrotrophic factor seems to operate primarily as a stimulus for release of thyroid hormone into the blood stream upon a relatively sudden need for it by the body.

The anterior pituitary, as far as its release of thyrotrophic hormone is concerned, appears to have a basic rhythm under normal circumstances which is under humoral influences (Uotila, 1939a). Additional evidence by the same author indicates that the stimuli to the anterior pituitary thyrotrophic factor is mediated also by way of nerve connections passing through the pituitary stalk. The cervical sympathetics appear to be unnecessary for normal thyroid function (Uotila, 1939b). Reforzo-Membrives (1943) showed that thyroid hormone itself has a depressing effect on the release of thyrotrophic hormone. There exists a normal balance, therefore, between the thyroid by way of its hormone and the anterior pituitary by way of its thyrotrophic factor. This normal equilibrium is influenced by other humoral factors and by way of nervous connections through the pituitary stalk. Thus the thyroid is under the influence and stimulated by both internal and external environmental conditions. Direct nervous control of thyroid function has never been satisfactorily demonstrated; nevertheless, authorities on thyroid function and diseases have expressed the opinion that some such regulation of the thyroid exists. Salter (1940b) on this point stated that there is an ever increasing mass of clinical evidence in the general field of psychosomatic medicine that points to nervous regulation of endocrine activity. Means (1937) in his book "Thyroid and Its Diseases" says it is his belief that the nerves of the thyroid take some part in the regulation of secretion and storage, through the mediation of the vasculature; that there is no direct stimulation of the parenchymal cells by way of the nerve fibers.

CRITERIA INDICATIVE OF THYROID ACTIVITY

The stimulus to increased activity of the thyroid by way of the thyrotrophic factor is indicated by increased hyperplasia of the thyroid which results in increased weight of the gland, loss of colloid in the follicles and therefore a decreased iodine content of the gland, increased secretory cell height and increased secretory tissue, increased mitotic activity of the cells, increased organic iodine in the blood and an increase in B.M.R.

A lack of the thyrotrophic stimulus with a resulting decreased thyroid activity normally results in an atrophic thyroid, increased colloid in the gland acini and therefore increased iodine content of the gland, low cuboidal epithelial cells, decreased mitotic activity, decreased organic iodine in the blood and decreased B.M.R. These relationships are in agreement with the findings of Hertz and Roberts (1941) that cell height, relative thyroid size and B. M. R. vary essentially in parallel under the influence of thyrotrophic hormone stimulation.

IODINE CONTENT

Experimental work by various investigators lends support to the above stated relationships. Marine (1935b) observed that the iodine store of the thyroid varies inversely with the degree of active hyperplasia. In extreme degrees of thyroid hyperplasia the iodine store is exhausted. By hyperplasia he has reference to the histological picture associated with high epithelial cells, more infolding and less colloid. He observed further that when the thyroid contains much colloid, the epithelial cells are flattened and less active and the gland is usually rich in iodine. Salter (1940b) stated that when the thyroid is secreting hormone at full capacity, its total iodine reserve may be depleted to less than that of $\frac{1}{10}$ normal. Its thyroxine-like reserve may disappear, presumably because it is removed as fast as it is formed. These observations indicate that the more rapidly the thyroid hormone production is excreted into the blood stream, the lower is the thyroid's iodine content. Siebert and Linton (1935) observed that discharge may be into the lumen as well as discharge of thyroid hormone into the blood stream. Under the former condition greater activity would increase the colloid storage and thus also the iodine content of the whole gland. In the latter case, a loss of colloid occurs with a decreased iodine content of the gland.

The occurrence of increased thyroid hormone discharge into the blood stream accompanied by increased hyperplasia of the cellular constituents has probably led to the observation by several authors (King, 1940; Bauman, 1896; Marine, 1935b) that the percentage iodine content of the thyroid is inversely proportional to the weight of the gland. When there is accumulation of colloid within the follicular lumina, if this process is carried on for a considerable length of time, there results an increased size and weight of the gland. In this condition, the iodine content varies in direct proportion to thyroid weight, since the weight increase is primarily due to colloid storage. This condition has probably led various authors (King, 1940; Elmer, 1938) to observe that iodine content of the thyroid gland parallels its weight.

THYROID WEIGHT

It is apparent that thyroid weight is increased by both colloid accumulation and increased epithelial constituents. However, in the former instance, the "hormonal" blood iodine and B.M.R. are decreased. In the latter instance "hormonal" blood iodine and B.M.R. are increased. The use of thyroid weight changes as an index of thyroid activity is further complicated by the fact that thyroid weight changes may be due to alterations in vascularity and stroma besides changes in the amount of colloid and size and number of acinar cells (Keating et al., 1945). This is essentially true in mammals. In the fowl, thyroid weight changes may be a more useful index of thyroid activity for the following reasons: Cruickshank (1929) noted that iodine content of the thyroid of the chicken varied with the season, being high in the late winter months and low in the summer months. The thyroid weights of the fowl (Galpin, 1938; Cruickshank, 1929) varied with the season in the same manner, that is, large thyroids in the winter months and smaller glands in the summer months. The iodine content, therefore, parallels the thyroid weight. Since the greatest thyroid activity can be assumed to occur in the winter months, the thyroid weight in the fowl apparently indicates thyroid activity to an approximate degree. Since the iodine content parallels the thyroid weight, the fowl thyroid apparently stores only limited colloid in contrast to greater storage in the thyroids of mammals.

EPITHELIAL CELL APPEARANCE AND MITOTIC ACTIVITY

The comparative height of the follicular epithelium is apparently a fairly reliable index of thyroid activity, flat cells indicating low activity, columnar cells indicating high activity. Since thyroid secretion may result in colloid accumulation as well as increased thyroid hormone output into the blood stream, increased mitotic activity of the epithelial thyroid cells may indicate greater secretory activity, but which of the secretory phases is being increased cannot be determined by observation of this criterion alone (Salter, 1940b). Studies of the iodine content of the thyroid or changes in B.M.R. in conjunction with mitotic changes would seem to be necessary to evaluate the meaning of the changes.

BLOOD IODINE

Determination of the changes of total blood iodine seems of doubtful value as an index of thyroid activity since the values are influenced by the iodine intake and iodine intake above necessary requirements does not influence the metabolic rate (Curtis and Fertman, 1943). However, blood iodine concentration and basal metabolism are correlated, according to Salter (1940a), if analytical technique is good, if exogenous iodine intake is low and a steady physiological state is attained before analysis is made. This indicates, therefore, that under certain conditions the concentration of blood iodine varies with changes in thyroid activity. Recently Salter and McKay (1944) have been able to determine the "hormonal" iodine, which they believe to be a reliable index of thyroid activity.

CONCENTRATION OF THYROTROPHIC HORMONE OF THE ANTERIOR PITUITARY

Since the thyrotrophic hormone of the anterior pituitary is apparently the primary regulating factor of thyroid activity, it is natural to assume that increased thyrotrophin concentration indicates increased thyroid secretion into the blood stream and lowered thyrotrophin concentration would indicate the opposite condition. Methods of assay for the quantity of thyrotrophic factor are available (Bergman and Turner, 1939; Smelser, 1937). Controversial results on the quantities of the thyrotrophic hormone concentration in the anterior pituitary under certain experimental conditions indicate that these determinations may be of questionable value as an index of thyroid activity. On the other hand, differences in results especially after thyroidectomy may be due to poor physical condition of the thyroidectomized animal since feed intake is reduced following thyroidectomy. Poor nutrition in itself results in low thyrotrophin output by the anterior pituitary. Gordon, Goldsmith and Charipper (1945) found decreased amounts of thyrotrophin in the pituitary of rats fed thiourea and sulfadiazine, indicating the possibility that under certain conditions rapid release of thyrotrophic hormone by the anterior pituitary results in a lowered concentration in this gland.

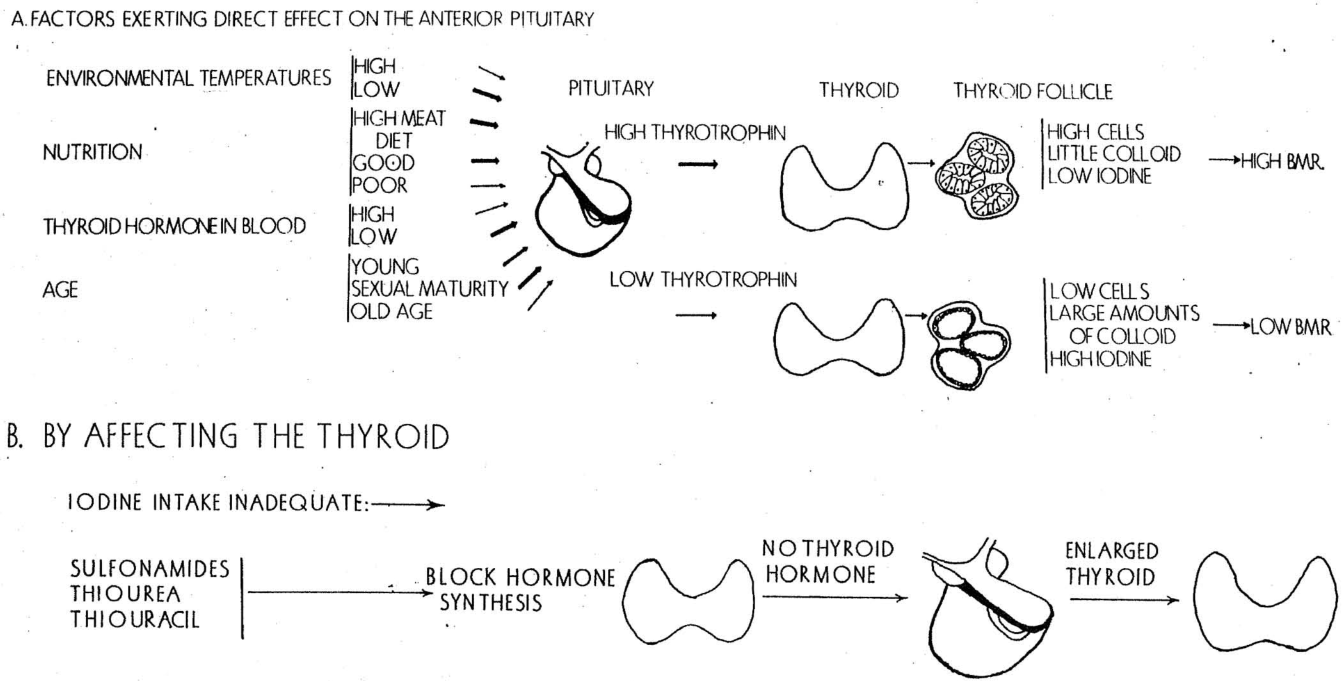
In general the criteria used in studying the changes in thyroid activity are meaningful only if the significance and underlying causes bringing about the phenomenon studied are understood.

THE EFFECT OF ENVIRONMENT ON THYROID ACTIVITY

Modes by Which Thyroid Activity Is Altered

Effect on the Release of Thyrotrophin by the Anterior Pituitary.— Most effects on thyroid activity are imposed on it through the mediation of the anterior pituitary by way of its thyrotrophic hormone. Among these factors are temperature; high temperature depressing the release of thyrotrophin and therefore the thyroid hormone output by the thyroid, low temperature increasing the output of thyrotrophin with a resulting high thyroid hormone output (Ring, 1939; MacGregor and Loh, 1941; Wolf and Greep, 1937 and others). The level of nutrition also influences thyroid activity. A poor nutritive condition depresses thyroid activity in comparison to a good nutritive condition (Mulinos and Pomerantz, 1940; Stephens, 1940). This action is brought about by way of the thyrotrophic hormone. Apparently the inhibitory effect of thyroid hormone on the activity of the thyroid is mediated by way of the anterior pituitary (Salter, 1940b, Astwood et al., 1943 and others.) It is probable that most environmental influences on thyroid activity are mediated by way of the anterior pituitary.

FACTORS AFFECTING THYROID HORMONE SECRETION RATE



Direct Effect on the Thyroid.—Various goitrogenic agents probably exert their effect directly on the thyroid as described in another section (see Goitrogenic Agents). However, the enlargement of the thyroid itself is due to increased thyrotrophic stimulation from the anterior pituitary since hypophysectomy prevents the goitrogenic effect.

A relative deficiency of iodine results in a goitrogenic effect by limiting the thyroid hormone output and therefore lowering the thyroid hormone concentration in the blood. Thus the release of thyrotrophin is stimulated, resulting in a goitrous gland if the iodine deficiency persists.

Direct Effect on Tissue Metabolism.—There are other agents and substances which apparently affect thyroid activity by way of the anterior pituitary but exert their effect directly on the body tissues, thus appearing to have an antagonistic effect on preformed thyroid hormone. Most striking of such compounds is paraxanthine, which appears to be normally present in the blood and acts as a balancing factor on thyroxine in regulating the B.M.R. (Carter et al., 1943). However, no goitrogenic effect of this substance has been reported. According to Sherwood (1938), estrogenic substances affect thyroid function by exerting a direct thyroid hormone antagonism or a direct effect on the tissues so they are less stimulated by thyroid hormone. This conclusion was reached since estrogenic injections depressed the B.M.R. in ovariectomized and thyroidectomized rats fed thyroid as compared to the B.M.R. in similar animals fed thyroid but not receiving estrogen injections. The action of cyanides on thyroid function appears to be exerted by affecting the tissues (Marine, Bauman, Spence and Cipra, 1932). The action of cyanides, however, might be a direct effect on the thyroid in inhibiting thyroid hormone synthesis as suggested by Rawson et al. (1944a) for the action of potassium thiocyanate or possibly a direct effect on the anterior pituitary itself.

However, the well known effect of thyroid hormone in decreasing the lethal effect of acetonitrile would appear to argue for a direct tissue effect.

EFFECT OF SEASON AND TEMPERATURE

Many studies have been made to determine changes in thyroid activity as indicated by the varied criteria described in the preceding section. That there are changes in the thyroid associated with season has been established. Seidell and Fenger (1913) showed that the thyroids of mammals (sheep, hogs and cattle) had a maximum iodine content in the late summer and a minimum iodine content in the late winter. Kendall and Simonsen (1928) confirmed and extended these observations by showing that a higher thyroxine-like fraction is contained in the thyroids of mammals in midsummer as compared to that in February. Orr and Leitch (1929) and Curtis and Fertman (1943) believe that this variation in thyroid iodine is due to seasonal variation in iodine content of the feed. Salter (1940b) suggested that the more likely explanation is that it may be due to a greater secretion of thyroid hormone into the blood stream in cold weather, thereby reducing the colloid storage and decreasing the thyroid iodine content in the winter. Evidence for this explanation, he believes, is indicated by the fact that increased thyroid iodine appears before iodine rich feeds are available.

In the fowl an opposite relationship apparently exists. The higher thyroid iodine content occurs in the winter months and the lower content in the summer months (Cruickshank, 1929). Seasonal variation in thyroid weight in the fowl parallels the thyroid iodine content, being greater in the winter months and smaller in the summer months (Galpin, 1938). The opposite relation of thyroid iodine content with season in mammals and the fowl can be explained on the assumption that little storage of colloid takes place in the fowl thyroid during the summer such as apparently occurs in the thyroid of the mammal. Winchester (1940) determined the thyrotrophic content of hen's pituitaries and found the highest thyrotrophic hormone content during the month of February with a decreased content at following periods. These studies indicate that thyroid activity is greater in the winter months than in the summer months.

Probably seasonal variation is in a large measure due to differences in temperature; however, this has not always satisfactorily explained the situation. Reineke and Turner (unpublished) found an apparent seasonal change in thyroid activity of chicks even when temperature was fairly well controlled. Elmer (1938), discussing seasonal variations of the thyroid, stated that ultra violet light may be responsible in part for seasonal variation. It has been established that day length, that is, the period of light per day received, influences gonad function. If gonad function affects thyroid function, part of the seasonal variation of the thyroid may be indirectly due to variation in day length with season.

That low temperature stimulates the thyroid to increased activity has been established. Low temperature increased the metabolic rate (Ring, 1939) and resulted in thyroids showing high columnar epithelial cells in an active secretory state (Wolf and Greep, 1937). Dempsey and Astwood (1943) found the thyroid glands of rats produced thyroid hormone at a rate of 9.5 γ thyroxine per day when kept at a temperature of 1° C. At a temperature of 35° C. the daily secretion rate was 1.7 γ thyroxine. Also, relatively high temperatures decreased the B.M.R. (MacGregor and Loh, 1941).

Effect of Age and Increasing Body Weight

Human.—Certain changes in thyroid function are associated with increasing age and body weight. Windle (1940) presented evidence showing that functional activity appears to start at a relatively early stage of fetal development in the human. Bits of thyroid gland from a 3 months old human fetus accelerated development of toad larvae, indicating some thyroid activity. Iodine has been found in the fetal thyroid of the human as early as the sixth month and the amount increases toward the end of prenatal life, but is low compared to the adult gland perhaps because of a difference in the amount of colloid stored. Orr and Leitch (1929) stated that the thyroid is poor in iodine content in early fetal life but increases in later fetal development. During post-natal development of the human these authors believed that the relative iodine content of the thyroid is not affected by age from youth to old age. Elmer (1938) gives the following variations in total thyroid iodine with increasing age in man: up to 1 year, 0.9 mg.; from 1 to 10 years, 2.0 mg.; from 10 to 20 years, 8.7 mgs.; from 20 to 60 years, 5.5 mg.; and over 60 years, 7.9 mg., showing in general an increase with age until old age. However, it is probable that the relative thyroid iodine content varies little.

Thyroid weight in man apparently increases in direct proportion with increasing body weight (Brody, unpublished; see Table I). Hertzler (1941) described the changes in the thyroid histology of the human associated with age. The fetal thyroid at 6 months has some well formed follicles and many epithelial cell masses without lumina. At birth the gland weighs approximately 2.5 grams and contains an increased number of true follicles. At 14 years of age the thyroid weighs approximately 10 grams, while at 28 years of age it weighs an average of 25 grams, showing the greatest weight increase after puberty. The secreting cells in the young adult are higher, exhibiting greater activity than in old age when they are flat and appear inactive. There is also an increase in fibrous tissue of the thyroid in old age. Cooper (1925) believed that the human thyroid after maturity decreases in activity with advancing age. In man thyroid activity apparently begins in early fetal life, progressively increases, with a maximum activity after puberty and a possible decrease in activity in old age.

Experimental Animals.—Studies by Gorbman and Evans (1941) indicated that beginning function of the rat thyroid occurs late in fetal development as definite radio-active iodine accumulation can be demonstrated in 18 to 19 day old fetal thyroids.

In a histological study of the mouse thyroid, Smith and Starkey (1940) found that in the newborn animal there appeared to be a high plane of thyroid activity. From 15 to 27 days of age there is an increased storage of colloid in the gland. At sexual maturity (30-32 days of age) there is an apparent increased activity as indicated by high epithelial cells and reduced colloid content. After 38 days of age colloid storage is regained and from then on the thyroid shows greater and greater storage. In old age (220 days) areas of

TABLE I. RELATION BETWEEN THYROID WEIGHT AND BODY WEIGHT

Species	Equation	Units of Y	Units of X
Man (1)	$Y=.1985X^{1.08}$	gms.	kgs.
Horses over 1 year (2)	$Y=.024X^{1.17}$	gms.	kgs.
Growing goats (7)	$Y=174.6X^{0.70}$	mgs.	kgs.
New Zealand White rabbits (3)	$Y=.156X^{0.88}$	mgs.	gms.
Fetal and new-born cats (2)	$Y=.162X^{0.77}$	gms.	kgs.
Guinea pigs (females) (4)	$Y=.366X^{0.83}$	mgs.	gms.
Guinea pigs (males) (4)	$Y=.598X^{0.73}$	mgs.	gms.
Rats (growing) (2)	$Y=.120X^{0.85}$	gms.	kgs.
Growing Leghorn fowl (2)	$Y=.131X^{1.20}$	gms.	kgs.
Brown Leghorn fowl (f.) (5)	$Y=.0157X^{1.36}$	mgs.	gms.
Brown Leghorn fowl (m.) (5)	$Y=.0391X^{1.25}$	mgs.	gms.
Brown Leghorn fowl (capon) (5)	$Y=.0256X^{1.16}$	mgs.	gms.
White Leghorn fowl (f.) (6)	$Y=.0682X^{1.07}$	mgs.	gms.
White Leghorn fowl (m.) (6)	$Y=.095X^{1.03}$	mgs.	gms.
White Leghorn fowl (m.) (7)	$Y=.158X^{0.88}$	mgs.	gms.
White Plymouth Rock fowl (f.) (7)	$Y=.062X^{0.99}$	mgs.	gms.
White Plymouth Rock fowl (m) (7)	$Y=.035X^{1.05}$	mgs.	gms.

(1) Brody (unpublished)
(2) Brody and Kibler (1941)
(3) Kibler, Bergman and Turner (1943)
(4) Mixner, Bergman and Turner (1943)
(5) Juhn and Mitchell (1929) (Calculated by present authors).
(6) Latimer (1924) (Calculated by present authors.)
(7) Author's data

cystic degeneration appear, indicating decreased thyroid activity. Hunt (1944), working with the female rat, found a progressive decrease in mitotic activity of the thyroid cells with increasing age up to 200 days. Thus these studies indicate that thyroid activity decreases in old age.

In studying variations in the thyrotrophin content of rat pituitaries, Turner and Cupps (1939) found a higher concentration in the pituitaries of both the male and female at the period of most rapid growth with a subsequent decline. Thus there appears to be in-

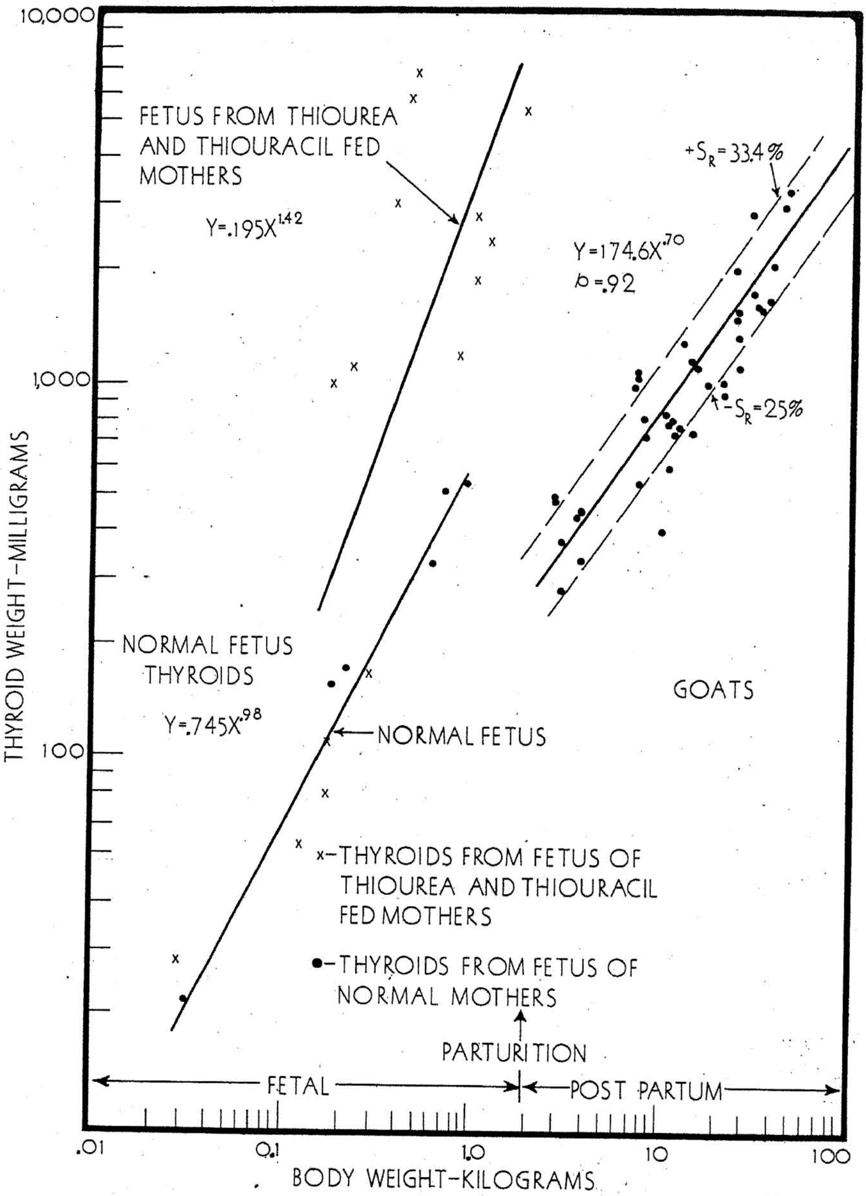


Fig. 1.—Relation of thyroid weight to body weight in fetal and growing goats.

creased thyroid activity at the beginning of sexual maturity. The thyroid weight of rats, rabbits and guinea pigs increases with increasing body weight but the ratio of thyroid weight to body weight decreases as body weight increases (Brody and Kibler, 1941; Kibler, Bergman and Turner, 1943; Mixner, Bergman and Turner, 1943, Table I). Lauson, Golden and Sevringhaus (1942) have recorded data on the thyroid weights of rats with increasing age and body weight and apparently the thyroid weight increases less rapidly proportionally than body weight increases.

Domestic Animals. Review.—Iodine has been found in the fetal thyroid of cattle, sheep and swine at an early stage of development (Windle, 1940), the thyroid becoming active at about the time its structure begins to resemble that of the adult, which presumably is before mid-term. Spielman, Petersen and Fitch (1944) noted that myxoedematous cows, when pregnant past mid-term, tend to become non-myxoedematous presumably because of the thyroid hormone produced by the fetus. Reineke and Turner (1941) found that a young thyroidectomized goat resumed growth in late pregnancy probably because of the thyroid hormone produced by the fetus. Thus there appears to be activity of the thyroid in this class of animals during fetal development. Pfeiffer (1931) found that maximum thyroid iodine content occurs in cattle during the fifth to sixth year of life or about the time of attainment of maturity.

Thyroid weight increases with increasing body weight are expressed by exponential equations for horses in Table I. Extensive data on thyroid weights of various species of animals are given by Krizenecky (1932) and by Crile (1941). In all cases there is increased thyroid weight with body weight increase, indicating possibly an increased thyroid hormone production as body weight increases. However, this increase may be relatively less per unit of body weight since thyroid weight per unit of body weight is less in mature animals.

Relation of thyroid weight to body weight in growing goats. Experimental. As the weights of thyroids from goats serving as controls were available on animals of increasing body weight, it seemed desirable to determine the relation between thyroid weight and body weight. To our own data, additional data obtained by Reineke, Bergman and Turner (1941) were added. The relationship was determined by fitting the exponential equation $Y = ax^b$ to the data since the data plotted on logarithmic paper indicated a straight line relationship (Fig. 1; Tables II and III). The relationship of thyroid weight to body weight for the limited number of fetuses is expressed by the equation $Y = 0.745X^{0.98}$, where Y is the thyroid weight in milligrams and X is the body weight in grams. This indicates that fetal goats weighing 50 grams (50 days fetal age) have a thyroid weight of approximately 34 milligrams; fetuses weighing

800 grams (135 days fetal age) have a thyroid weight of approximately 501 milligrams. Thus thyroid weight of the fetus appears to increase in direct proportion to body weight, the proportionate growth rate being faster than in postnatal life.

TABLE II. THYROID WEIGHT OF THE GOAT

Body weight	Thyroid weight (actual)	Thyroid weight /100 gms. body weight	Calculated (1) thyroid weight	Body Weight	Thyroid weight (actual)	Thyroid weight /100 gms. body weight	Calculated (1) thyroid weight
kg.	mgm.	mgm.	mgm.	kg.	mgm.	mgm.	mgm.
2.68	480	17.9	348*	21.34	1043	4.9	1455**
2.72	500	18.4	351*	21.57	934	4.3	1470*
2.95	375	12.7	364*	24.97	1447	5.8	1627*
3.00*	280	9.3	374	24.97	1968	7.9	1627*
3.77	438	11.6	439*	25.42	1449	5.7	1644*
3.81	455	11.9	441*	25.88	1320	5.1	1666**
3.81	335	8.8	441*	26.56	1105	4.2	1697*
7.72	546 (2)	7.1	718*	29.06	1382	4.5	1807*
7.95	803 (2)	10.1	731*	29.51	2819	9.6	1824*
8.17	715	8.6	750*	30.42	1709	5.6	1862*
8.40	735	8.8	764*	32.69	1618	4.9	1958*
8.63	539	6.2	776*	32.69	1564	4.8	1958*
10.00	710	7.1	861*	36.32	2189	6.0	2105*
10.22	395 (2)	3.9	873*	38.14	1620	4.2	2174*
10.40	839 (2)	8.1	879*	39.50	2059	5.2	2234*
10.44	581 (2)	5.6	885*	42.45	3378	8.0	2344**
10.49	796 (2)	7.6	891*	44.04	2975	6.8	2404*
10.53	784 (2)	7.4	894*	47.67	3304	6.9	2545**
10.90	720	6.6	914*	54.48	4300	7.9	2790**
11.12	753 (2)	6.8	927*	54.71	4190	7.7	2798*
12.26	1293 (2)	10.5	989*				
12.71	812	6.4	1016*				
13.62	738	5.4	1067*				
13.62	1132 (2)	8.3	1067*				
13.85	1106 (2)	8.0	1078*				
17.25	968	5.6	1259*				

(1) calculated from equation $Y=174.6X^{0.70}$
(2) Reineke, Bergman and Turner (1941)
* non-pregnant
** pregnant

TABLE III. THYROID WEIGHT OF THE FETAL GOAT

Body weight of fetus	Thyroid weight of fetus	Thyroid weight /100 grams body weight	Mother's body weight	Mother's thyroid weight	Mother's thyroid weight /100 gms. body weight
gm.	mgm.	mgm.	kgs.	mgm.	mgm.
33.8 (f.)	22.2	65.7	47.7	3304	6.9
38.3 (f.)	21.8	57.0	47.7	3304	6.9
177.4 (f.)	157.8	89.0	42.4	3378	8.0
218.9 (m.)	170.8	78.0	42.4	3378	8.0
660.0 (f.)	335.0	50.8	53.8	4300	8.0
769.0 (f.)	510.0	66.3	53.8	4300	8.0
958.0 (m.)	550.8	57.5	53.8	4300	8.0

The relationship of thyroid weight to body weight in female goats in postnatal life is expressed by the equation $Y = 174.6X^{0.70}$, where Y is thyroid weight in milligrams and X is body weight in kilograms. Thus in postnatal life the ratio of thyroid weight to body weight decreases with increasing body weight and presumably with increasing age until maturity is reached. The exponent $b = 0.70$ compares with the b exponent value of 0.73 for the male guinea pig to 1.17 for the horse as tabulated in Table I. According to the equation, a female goat weighing 5 kg. has a thyroid weight of approximately 540 mg.; one weighing 20 kg. has a thyroid weight of approximately 1400 mg.; and one weighing 50 kg. a thyroid weight of 2650 mg. The fact that there is considerable variability is indicated by the $+S_R$ value of 33 per cent and a $-S_R$ value of 25 per cent.

It can be seen from Fig. 1 that there is a drop in thyroid weight

TABLE IV. THYROID WEIGHT OF THE FOWL

Age of fowl	No. of chicks	Body weight	Thyroid weight (actual)	Thyroid weight /100 gms. body weight	Calculated* Thyroid weight	Calculated* Thyroid weight /100 gms. body weight
		gm.	mgm.	mgm.	mgm.	mgm.
White Plymouth Rock (Females)						
5	18	245	10.93	4.4	14.2*	5.80
7	10	309	19.52	6.3	17.9*	5.79
9	11	529	39.5	7.5	30.3*	5.73
11	10	824	50.86	6.2	47.0*	5.70
13	12	977	47.0	4.8	55.6*	5.69
18	15	1345	81.5	6.1	76.2*	5.67
22	15	1750	119.8	6.8	98.9*	5.65
26	14	1851	105.2	5.9	104.5*	5.65
*Calculated from the equation $Y=.062X^{0.99}$						
White Plymouth Rock (Males)						
5	9	238	9.76	4.1	11.1**	4.66
7	9	379	18.95	5.0	18.1**	4.78
9	9	573	42.25	7.4	28.0**	4.89
11	7	806	35.6	4.4	40.1**	4.98
13	6	1072	44.5	4.2	54.1**	5.05
18	5	1456	60.8	4.2	74.6**	5.12
22	7	2173	125.4	5.8	113.8**	5.24
26	6	2545	152.0	6.0	134.3**	5.28
**Calculated from the equation $Y=.035X^{1.05}$						
White Leghorn (Males)						
2	30	72.7	7.8	10.7	6.8***	9.45
4	10	163.6	15.3	9.3	14.0***	8.56
6	14	346.0	33.7	9.7	26.9***	7.77
7	12	467.3	32.1	6.8	35.1***	7.51
9	9	643.0	51.1	7.9	46.5***	7.23
12	9	1072.0	64.7	6.0	72.8***	6.79
20	7	1266.0	75.1	5.9	84.3***	6.66
24	10	1444.0	100.3	6.9	94.6***	6.55
27	12	1563.0	134.8	8.6	101.4***	6.49

***Calculated from the equation $Y=.158X^{0.88}$

of the goat at about the time of parturition. Data are not available to determine the exact stage at which this temporary involution of the thyroid takes place.

Relation of thyroid weight to body weight in growing fowls. Review. Some data are available on thyroid size in domestic fowls of varying ages and body weights. Brody and Kibler (1941) gathered data from certain sources on thyroid weights in growing White Leghorn chickens. Calculating the relationship existing between thyroid weight and body weight the equation $Y = 0.131X^{1.20}$ was determined as best fitting the available data. Thyroid weight data in the Brown Leghorn fowl of both sexes varying in body weight are given by Juhn

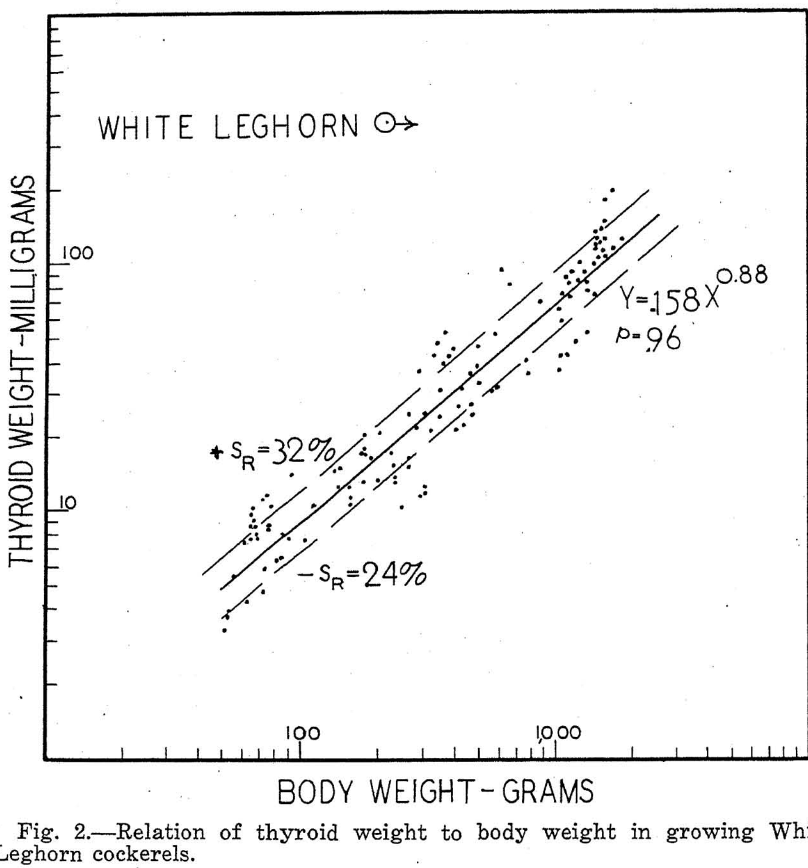


Fig. 2.—Relation of thyroid weight to body weight in growing White Leghorn cockerels.

and Mitchell (1929). Fitting the equation $Y = aX^b$ to these data gives the following relationships: for the female Brown Leghorn, $Y = 0.0157X^{1.36}$; for the male $Y = 0.0391X^{1.25}$. Analyzing the data of Latimer (1924) on the White Leghorn fowl, the equation representing the relation of thyroid weight to body weight for the female is $Y = 0.068X^{1.07}$, for the male $Y = 0.095^{1.03}$. In all the equations Y represents the thyroid weight in milligrams and X the body weight in grams.

Experimental: White Leghorn cockerels. A study of the weight changes with increasing body weight was made on White Leghorn cockerels and White Plymouth Rock cockerels and pullets from 2 to 27 weeks of age. The equation representing the relationship between thyroid weight and body weight in the White Leghorn cockerels varying from 2 to 27 weeks of age is $Y = 0.158X^{0.88}$ (Fig. 2; Table IV), where Y is the thyroid weight in milligrams and X is the body weight in grams. Thus the average thyroid weight of the White Leghorn cockerel weighing 200 grams is about 17 mgs.; those weighing 1000 grams is 68.5 mgs.; and those weighing 1500 grams is 98.0 mgs. The variability of thyroid weight with increasing body weight is $+S_R$ equals 32 per cent and $-S_R$ equals 24 per cent.

White Plymouth Rock cockerels. Determining the same relationship for White Plymouth Rock cockerels varying in age from 5 to 26 weeks, the equation was found to be $Y = 0.035X^{1.05}$ (Fig. 3; Table IV), where Y is the thyroid weight in milligrams and X the body weight in grams. Thus the average thyroid weight of the cockerel of this breed weighing 200 grams is 10 mgs.; those weighing 1000 grams is 50 mgs.; and those weighing 1500 grams is 77 mgs. The variability is about the same as that for the thyroid weight of White Leghorn cockerels, $+S_R$ being 34 per cent and $-S_R$ being 25.4 per cent. Comparing the two breeds, the White Leghorn cockerels have a larger thyroid size than the White Plymouth Rock cockerels when of the same body weight. This relationship between the two breeds will not be true on an age basis since the White Plymouth Rock chickens after about 8 weeks of age have a greater body weight than White Leghorns of the same age.

White Plymouth Rock pullets: Studying the relation of thyroid weight to body weight for White Plymouth Rock Pullets the equation representing the relationship is $Y = 0.062X^{0.99}$ (Fig. 4; Table IV), where Y and X are in the same units as in the above equations. The average thyroid weight of pullets of the White Plymouth Rock breed weighing 200 grams is 11.6 mgs.; of pullets weighing 1000 grams is 57 mgs.; and of pullets weighing 1500 grams is 85 mgs. Thus the female of this breed has a larger average thyroid weight than the male, which is apparently true also for the White Leghorn breed (Latimer's data). The variability of thyroid weight in the White Plymouth Rock pullet is considerably more than in the male of either the White Plymouth Rock or the White Leghorn breeds. The variability value is $+S_R$ equals 45 per cent and $-S_R$ equals 31 per cent. The average thyroid weight of White Plymouth Rock pullets compared with that of the White Leghorn cockerels is smaller on a body weight basis. The foregoing relationships may not hold true

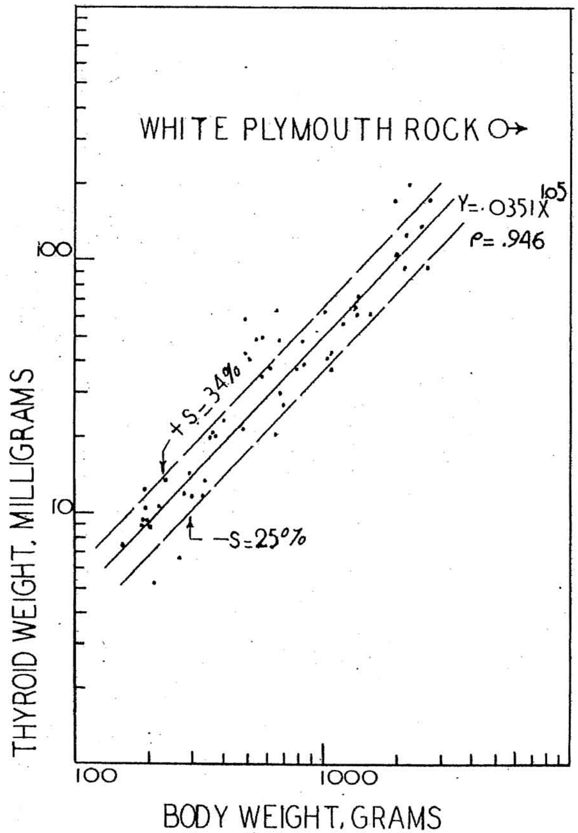


Fig. 3.—Relation of thyroid weight to body weight in growing White Plymouth Rock cockerels.

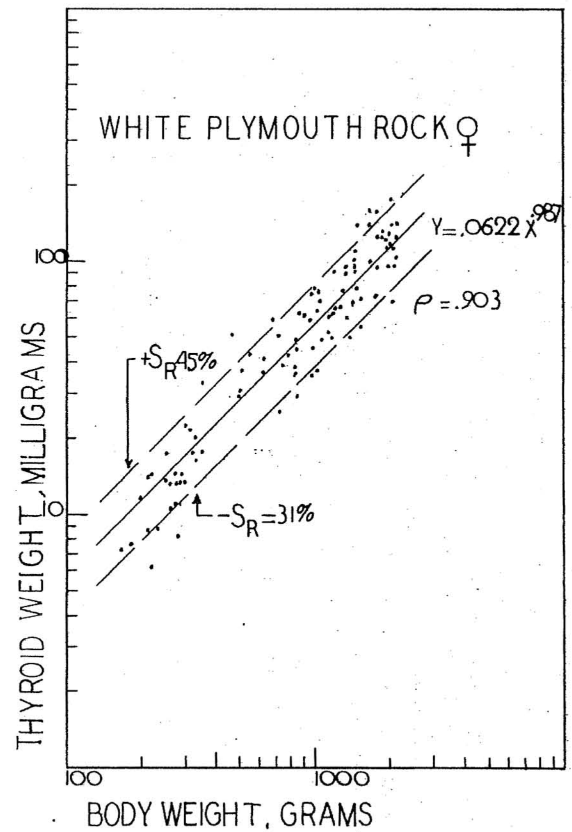


Fig. 4.—Relation of thyroid weight to body weight in growing White Plymouth Rock pullets.

for all ages and body weights as there are deviations from the average line in all cases. Latimer (1924) also showed that the ratio of thyroid weight to body weight fluctuates with increasing age and body weight. The reasons for these fluctuations is unknown. The value of the "b" exponent in these data is somewhat lower than the "b" exponent value in data presented by other authors. This may be due in part to the fact that the data of other investigators extend over age and body weight ranges differing from our data. The age range of the data of Juhn and Mitchell (1929) is from 25 to 47 weeks, the body weight ranging from 700 to 1400 grams. This is a rather low body weight for the age of these chickens. Latimer's data extend over a wider range than our data, the age ranging from 0 to 43 weeks, body weight from 33 to 2100 grams. The data obtained by Latimer covered White Leghorns raised in Minnesota, a somewhat endemic goitrous section.

The results of these studies seem to indicate an increase in thyroid hormone output with increasing age from the time of the beginning function of the thyroid up until maturity if thyroid weight is taken as a criterion of thyroid function.

EFFECT OF DIET AND GOITROGENIC AGENTS

Diet.—The nature and relative amount of food eaten is known to affect thyroid activity. Some natural foods may produce a slight enlargement of the thyroid and many other substances when ingested have a profound effect on the thyroid weight.

The effect of vitamins or avitaminosis on the thyroid is more or less obscure as indicated by the review of Drill (1943). Inanition has a profound effect on thyroid function. Poor nutrition results in small, atrophic, relatively inactive thyroid (Mulinos and Pomerantz, 1940; Stephens, 1940). Breneman (1941), working with chicks, noted that limited diet resulted in decreased thyroid weight.

Goitrogenic Agents.—The ingestion of many substances cause a marked hypertrophy of the thyroid and are therefore considered to be goitrogenic. Marine (1935b) stated that whenever the amount of thyroid tissue becomes insufficient to supply adequate thyroid secretion, whether from reduced iodine intake or from increased demands for the hormone or following partial thyroidectomy, compensatory hyperplasia occurs. This hyperplasia is mediated by way of the thyrotrophic hormone of the anterior pituitary. Probably the best known goitrogenic agent is a deficiency of iodine, leading to endemic goiter. This condition occurs in certain geographical areas where the soils are lacking in iodine. This relationship was first observed by Chatin (1853) and later by Baumann (1896). More recent observations indicate that the requirement for iodine may be relative, many factors influencing the iodine need.

Goitrogenic effects prevented by increased iodine intake. Investigations have shown that many agents have a goitrogenic effect on the thyroid. Watson (1906) observed that prolonged feeding of raw meat to healthy fowls had a goitrogenic effect. Marine and Lenhart (1909) observed that pig's liver was the most potent goitrogenic agent of all meat products. Hunt (1911) also noted that guinea pigs fed liver had more resistance to acetonitrile than those on a normal diet. McCarrison (1917) confirmed St. Lager's work of 1867 that high fat diets induce thyroid enlargement. Recently Remington and Harris (1943) reported the enlargement due to high fat diet is due to edema of the gland and not true hyperplasia. Burget (1917) also observed that rats fed lean beef and especially liver had thyroids 10 per cent larger than those of rats on a normal diet. In addition he noted that rats kept under unhygienic conditions develop thyroid hypertrophy. Marine (1935b) mentioned the hypertrophic effect of toxemias on the thyroid. Cole and Womack (1929) report a goitrogenic effect of certain toxemias associated with a loss of colloid and iodine of the thyroid. These changes of the thyroid can all be prevented by the administration of an increased amount of iodine.

Orr (1931) and Stott (1932) suggest that there may be a goitrogenic effect of calcium intake. Thompson (1933) observed that increased lime, calcium chloride or calcium carbonate intake in conjunction with a diet low in iodine produced goiters in rats. Hellwig (1940) confirmed this observation and expressed the opinion that iodine deficiency is a relative matter, other factors influencing the relative adequacy of iodine intake. Sharpless et al. (1943b) reported that an increase in serum calcium caused no goitrogenic effect. However, along with vitamin D, calcium chloride acts as a goitrogenic agent. It was suggested that chloride causes a loss of iodine which is followed by an increase in thyroid weight when an excess of calcium is absorbed. Although chloride fed in excess decreases the iodine content of the thyroid without calcium, this did not change the thyroid weight in itself (Sharpless et al., 1943a).

Arsenic in the diet in amounts of 0.02 per cent significantly increased thyroid weight and the iodine concentration of the thyroid was decreased (Sharpless and Metzger, 1941). It was calculated that 0.02 per cent arsenic in the diet more than doubled the iodine requirement. The theory advanced for the action of arsenic was that it may have a retarding action on the oxidative-reduction systems of the body and thus increase the requirement for more thyroid hormone.

Sharpless (1938) observed that a soybean diet produced goiters in rats. The pituitaries of these rats showed a decreased proportion of acidophiles and an increased proportion of basophiles with colloid-like vacuoles similar to the picture after thyroidectomy (Sharpless and Hopson, 1940). It was suggested (Sharpless, Pearsons and Prato, 1939) that the goitrogenic effect may be due to a cyanide in soybeans which stimulates the thyroid indirectly by depressing the oxidative systems of the body.

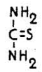
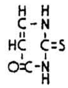
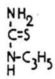
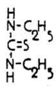
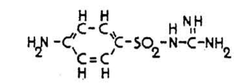
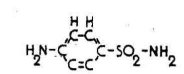
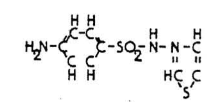
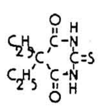
In 1928, Chesney, Clawson and Webster reported that feeding of cabbage leaves to rabbits caused thyroid enlargement, increasing the size of the thyroid over ten times that of normal. This goitrogenic effect could be prevented by increasing the iodine content of the feed (Webster, 1932). It was suggested that the goitrogenic substance is probably a cyanide and its amount in cabbage is increased by irradiation. The variation of light due to seasonal change influenced the degree of the goitrogenic effect.

Marine et al. (1932) found that thyroid hyperplasia frequently develops in prepuberal rabbits fed alfalfa hay and oats and that the goitrogenic effect varied with the content of methyl cyanide of both alfalfa hay and cabbage diets. Traces of iodine entirely prevented the thyroid enlargement. Hertz and Means (1942), citing evidence of the fact that goiters have been produced in experimental animals with sodium cyanide, potassium cyanide and methylcyanide, report the development of goiter in a human that had been treated over a period of a year with potassium thiocyanate for a hypertensive condition. The metabolic rate of this patient was reduced to —17 per cent. After cyanate therapy was withheld for a month, the thyroid size and B.M.R. returned to normal. Further evidence of the effect of thiocyanates is reported by Rawson, Hertz and Means (1943). Rawson, Tannheimer and Peacock (1944a), studying the goitrogenic effect of thiocyanates further in rats, found that hyperplasia of the thyroid with thiocyanate was accompanied by increased uptake of radio-active iodine compared to controls, whereas thiouracil induced hypertrophy was accompanied by lowered intake of iodine. This indicated to these authors that block of thyroid hormone production by thiouracil takes place by obstruction to the uptake of iodine by the thyroid. Thiocyanate blocks production of thyroid hormone after the iodine is collected by the gland.

Of the agents mentioned thus far (aside from thiouracil), the goitrogenic effect can be prevented by administration of increased iodine.

Goitrogenic effects not prevented by increased iodine and nature of their action. Recently certain compounds have been found to exert a goitrogenic effect that is not prevented by even relatively large intake of iodine. MacKenzie, MacKenzie and McCollum (1941) observed that rats on a diet containing sulfaguanidine had markedly enlarged thyroids compared with those of controls. Histologically the thyroids showed marked hyperplasia, the epithelium was distinctly columnar and was so increased and invaginated as to nearly extinguish the lumen. Richter and Clisby (1941) observed that administering phenylthiourea caused thyroid hypertrophy. Kennedy and Purves (1941) had observed a goitrogenic effect of a diet containing Brassica seed which was only partially prevented by iodine administration. In a further search for the active goitrogenic principle in Brassica seed, Kennedy (1942) found that allylthiourea caused marked hypertrophy of the thyroid with almost complete loss of colloid. MacKenzie and MacKenzie (1943), in extending their studies, found that many of the sulfonamides had this goitrogenic

GOITEROGENIC COMPOUNDS

COMPOUND	STRUCTURAL FORMULA	OPTIMUM LEVEL FOR THYROID HYPERTROPHY	TOXICITY
THIOUREA		RAT .10% IN WATER CHICK 0.05%-0.075% IN FEED SHEEP 1GM. PER 100 LBS BODY WEIGHT COW 0.5GM PER 100 LBS BODY WEIGHT (A PR.) GOAT 1GM PER 100 LBS BODY WEIGHT	NON-TOXIC NON-TOXIC TOXIC AT 0.1% NON-TOXIC SLIGHTLY TOXIC TOXIC
THIOURACIL		RAT 0.1% IN FEED CHICK 0.1% IN FEED GOAT 3GM. PER 100 LBS BODY WEIGHT	NON-TOXIC NON-TOXIC NON-TOXIC
ALLYLTHIOUREA		RAT 0.1% IN WATER	TOXIC
DIETHYLTHIOUREA		RAT 0.1% IN WATER	TOXIC
SULFAGUANIDINE		RAT 5.0% IN FEED MICE 1-2% IN FEED CHICKENS-3% -NO THYROID ENLARGEMENT	TOXIC AT 3% TOXIC
SULFANILAMIDE		RAT 2% IN FEED	TOXIC
SULFATHIAZOLE		RAT 1% IN FEED	NON-TOXIC
5 5-DIETHYL-2-THIOBARBITURIC ACID		RAT 0.02% OR LESS IN FEED	NON-TOXIC

effect on the thyroid. Diethyl thiourea and allylthiourea were goitrogenic, while acetyl thiourea and thioacetamide were not. Negative results were obtained with sulfonal, cystine, cysteine and thiamine hydrochloride. Gordon, Goldsmith and Charipper (1944) also noted this effect with para-aminobenzoic acid. Astwood, Sullivan, Bissell and Tyslowitz (1943) verified the goitrogenic action of sulfaguanidine, other sulfonamides and of thiourea and Astwood (1943) made a detailed report of the goitrogenic action of many compounds. He found that the most active compounds were substances having the thioureylene radical (NH.CS.NH). Thiourea itself was found to be very active and most of the derivatives are also although they are more toxic than thiourea itself. 2-thiouracil is active and of low toxicity. Of the aniline derivatives, 13 were found to exert goitrogenic effects including para-aminobenzoic acid, sulfanilamide, sulfaguanidine and sulfathiazole.

Most of the experimental animals used in these studies were rats. Mixner, Reineke and Turner (1944) showed that the thyroids of chicks were also markedly enlarged by treatment with thiourea or thiouracil.

All authors investigating the effects of these goitrogenic agents report that the thyroid's histological picture indicates hyperactivity, that is, much increased acinar tissue with increased height of the epithelial cells, greatly reduced colloid, increased size and number of follicles and hyperemia (MacKenzies, 1943; Astwood et al., 1943; Gordon, Goldsmith and Charipper, 1944).

Although the enlargement and histological picture of the thyroid induced by the drugs indicate a hyperactive gland, studies of their indirect effects on physiologic processes indicate hypofunction of the thyroid. The basal metabolic rate in rats fed the drugs decreases with continued administration, 5 to 7 days of feeding resulting in a drop of 10 per cent in the B.M.R., 10 to 14 days in a 20 per cent drop compared to that of control animals (MacKenzie and MacKenzie, 1943). Astwood et al. (1943) noted no drop in the B.M.R. for the first 7 to 14 days treatment, but a drop of 30 per cent in the B.M.R. was evident on the 27th day of treatment in the rat. The decrease was more marked in a cold environment. Prolonged feeding of sulfonamides (MacKenzie and MacKenzie, 1944) reduced the B.M.R. to thyroidectomy levels and withdrawal of the drug resulted in a return to normal B.M.R. levels. Reineke, Mixner and Turner (1945) observed a depression of 23.7 per cent in the B.M.R. of rats receiving 0.1 per cent thiouracil in the drinking water. Himsworth (1944), in treating thyrotoxicosis in man with thiouracil, observed that the B.M.R. fell from an average of +39.7 before treatment to +8.6 with a dosage of 0.2 grams five times a day. Similar results have been observed by Sloan and Shorr (1944) and others. Hughes (1944) showed that administration of thiouracil to young rats resulted in cretinism with the growth rate very similar to that of thyroidectomized rats. Hughes and Astwood (1944) observed also that thiouracil inhibited the metamorphosis of tadpoles.

A study of changes in pituitary histology associated with administration of aniline and thiourea derivatives also indicates hypofunction of the thyroid. There is a decrease in the number of acidophiles, vacuolation and increase in number and size of the basophiles similar to the changes in the pituitary following thyroidectomy (MacKenzie and MacKenzie, 1943; Griesbach, 1941; Kennedy, 1942; Gordon, Goldsmith and Charipper, 1944). These changes are thought to be associated with an increase in the output of thyrotrophic hormone by the anterior pituitary similar to the increase after thyroidectomy. Gordon, Goldsmith and Charipper (1945) found that there are increased amounts of thyrotrophic hormone in the blood serum and hypophysis after thyroidectomy, but that the feeding of thiourea and sulfadiazine to rats for 14 or 45 days increased the amount of thyrotrophin in the blood sera and pituitary glands. They interpreted these results to mean that thiourea and similar drugs, by depressing the formation of the thyroid hormone, cause an increased release of thyrotrophin into the blood where it appears in reduced amounts because of its increased utilization by the enlarged thyroid.

Much study has been devoted to the altered iodine metabolism induced by administration of these drugs, especially with radio-active iodine. That increasing the intake of iodine does not prevent the effect of these goitrogenic drugs is amply proven. MacKenzie and MacKenzie (1943) added 0.1 per cent NaI 5 days before sulfaguanidine treatment and concluded that iodine had no protective effect. Astwood et al. (1943) stated that the addition of several thousand times the minimal daily requirement of iodine exerted no preventive effect on the action of these drugs. Bauman, Metzger and Marine (1944) concluded also that even when large quantities of iodine were given, there was no prevention of the action of thiourea and this iodine was promptly excreted in the urine.

The iodine content of the thyroid was reduced in animals fed thiourea. Rawson, Evans, Means, Peacock, Lerman and Cortell (1944) found that patients given thiouracil as a pre-operative treatment for thyroidectomy, when given doses of radio-active iodine, excreted most of this iodine in the urine, very little of this iodine being found in the thyroid. Very little thyroid hormone activity could be demonstrated to be contained in the gland of these thiouracil treated patients. Keston, Goldsmith, Gordon and Charipper (1944) found that when radio-active iodine was administered to rats fed thiourea, 48 hours after the injection of the iodine, no appreciable amounts could be found in their thyroids, whereas the thyroids of normal rats not receiving any thiourea contained considerable quantities of radio-active iodine chiefly in organic combination. Experiments on the thyroids *in vitro* incubated with thiourea showed no formation of organically bound radio-active iodine, while those of normal controls contained such iodine. Larson, Keating, Peacock and Rawson (1945a) found that the thyroids of chicks receiving thiouracil had a lowered ability to collect injected radio-active iodine compared to the controls. When the drug was removed the capacity to collect iodine returned to the thyroid and was increased above that of controls similar to the ability of thyroids under stimulation of thyrotrophin. Rawson, Tannheimer and Peacock (1944a) showed that the ability of rat thyroids to collect radio-active iodine was diminished by thiouracil administration but that glands made goitrous by potassium thiocyanate collected a greater proportion of injected radio-active iodine than did the controls. Larson, Keating, Peacock and Rawson (1945b) showed that radio-active iodine collection by the thyroid of the chick was inhibited to a maximum degree one hour after administration of thiouracil. This inhibition occurred also in glands made hyperplastic by the injection of thyrotrophic hormone. This inhibitory effect of thiouracil practically disappeared within 24 hours after the discontinuance of thiouracil administration. Franklin and Chaikoff (1944) and Schachner, Franklin and Chaikoff (1944) found that sulfanilamide inhibited the *in vitro* conversion of radio-active iodine to thyroxine and diiodotyrosine but had little effect on the iodine-concentrating capacity of the thyroid tissue. Later Franklin, Lerner and Chaikoff (1944) found that thiouracil limited the capacity of the rat thyroid to collect radio-active iodine.

Astwood and Bissell (1944) found that the administration of thiouracil to rats was followed by almost complete disappearance of iodine from the thyroids in 5 days. Withdrawal of the drug caused a return to normal accumulation of thyroid iodine. This was confirmed by Salter and McKay (1944). That the goitrogenic effects of these drugs is mediated by way of the pituitary is shown by the fact that removal of the pituitary prevents the occurrence of the effect in thiouracil or thiorea treated animals (Astwood et al., 1943; Griesbach, Kennedy and Purves, 1941). The thyroids were atrophic but filled with colloid upon hypophysectomy. More detailed evidence that the thyroid hypertrophy is mediated by way of the thyrotrophic hormone is given by Larson, Keating, Peacock and Rawson (1945a). They compared the effect of thiouracil administration with the injection of thyrotrophic hormone on the anatomic and iodine collecting properties of the chick thyroid. The histology of the thyroid following administration of thiouracil was found to be identical with that following thyrotrophin injection, with the exception that there is a 5 day lag before the effects are produced with thiouracil, while the thyrotrophin effect is immediate. Although the glands showed greatly diminished capacity to collect radio-active iodine with thiouracil treatment, when the treatment was discontinued the hyperplastic gland acquired a capacity to collect radio-active iodine in larger quantities than the controls and similar to the capacity of thyroids made hyperplastic with thyrotrophic hormone. These investigations suggested that the primary action of thiouracil may be to inhibit the collection of iodine or thiouracil may act on iodines so as to prevent the thyroid from combining them in organic synthesis (Larson, Keating, Peacock and Rawson, 1945b).

Further studies on the action of thiourea and thiouracil were carried out by Dempsey (1944), who presented evidence that peroxidase is present in the normal thyroid cell and that it tends to disappear when thiouracil is administered. He concluded that thiouracil may act by inhibiting such an enzyme system, thus preventing the condensation of diiodotyrosine into thyroxine. Bauman, Metzger and Marine (1944), by giving slightly more iodine than enough to combine with measured quantities of injected thiourea, found that no preventive effect on thyroid enlargement resulted. They concluded, therefore, that thiourea does not combine with iodine in the body and that it prevents thyroglobulin formation even in the presence of excessive quantities of iodine.

The exact mechanism of action of these goitrogenic drugs has not been definitely determined, but most authors believe it is due to interference in an enzyme system that is involved in the synthesis of the thyroid hormone. The inhibition of hormone formation is responsible for the thyroidectomy-like effects and the lack of thyroid hormone results in increased release of thyrotrophic hormone causing thyroid hyperplasia (MacKenzie and MacKenzie, 1943; Astwood et al., 1943; Rawson, Evans, Means, Peacock, Lerman and Cortell, 1944; Martin, 1943; and others).

Although excessive intake of iodine has been repeatedly shown not to prevent the goitrogenic effect of these drugs, the injection of preformed thyroxine does prevent the thyroid hypertrophy when given during the time of treatment with the drugs (Astwood et al., 1943; MacKenzie and MacKenzie, 1943). Furthermore, these authors find that the calorigenic effect of the injected thyroxine is not interfered with by administration of thiouracil or thiourea, indicating that the drugs do not inhibit the action of thyroxine per se.

Studying these relationships, Dempsey and Astwood (1943) conceived the idea of using thiouracil treatment simultaneously with thyroxine injections as a method of determining the thyroid's secretion rate.

Thiouracil and thiourea—experimental. (1) Effect of thiouracil on the thyroid weight of the fowl: Mixner, Reineke and Turner (1944) determined the effect of increasing levels of thiourea and thiouracil in the drinking water on the thyroid weight of White Plymouth Rock chicks and found that 0.1 per cent of thiouracil or thiourea caused maximum thyroid enlargement, the maximum effect being produced when the drug was administered for 12 to 14 days.

In the experiments to be reported, thiouracil or thiourea was administered by mixing the drug with the feed. Varying amounts of

TABLE V. EFFECT OF THIOURACIL AND THIOUREA IN FEED ON THYROID WEIGHT OF WHITE LEGHORNS

Treatment	Sex	No. of Chicks	Age of chicks at end of experiment	Time drug was fed	Body weight	Thyroid weight	Thyroid Weight /100 grm. body weight
			weeks	weeks	gms.	mgs.	mgs.
Control	M	30	2	0	73	7.8	10.7
0.1% Thiouracil	M	19	2	2	63	22.3	35.6
0.15% "	M	13	2	2	65.2	14.4	22.1
0.20% "	M	10	2	2	63.8	19.9	31.7
0.40% "	M	15	2	2	66.2	13.3	20.1
0.80% "	M	10	2	2	64.7	12.1	18.7
Control	M	9	9	0	647.0	51.1	7.9
0.10% Thiouracil	M	10	9	2	681.9	165.7	24.3
0.60% "	M	6	9	2	678.9	118.8	17.5
Control	M	9	12	0	1078.0	64.7	6.0
0.10% Thiouracil	M	9	12	2	1005.0	204.0	20.2
0.20% "	M	10	12	2	1022.0	216.6	21.2
0.40% "	M	9	12	2	1082.0	239.2	22.1
0.10% " first 4 wks. 0.2% " last 4 wks.	M	5	8	8	419.0	500.4	119.4
0.1% " first 4 wks. 0.20% " next 2 wks. 0.4% " last 2 wks.	M	5	8	8	423.9	504.0	118.9
0.1% " first 4 wks. 0.2% " next 2 wks. 0.6% " last 2 wks.	M	5	8	8	343.4	327.0	95.2
Controls	M	4	20	20	1450	174.9	11.9
"	F	3	20	20	1170	109.8	9.0
0.2% Thiouracil	M	11	20	20	1363	2598.0	190.6
"	F	11	20	20	1080	1948.6	180.4
0.1% Thourea	M	1	20	20	820	324.0	39.5
"	F	7	20	20	785	185.5	23.6

thiouracil were added to the ration fed to one day old White Leghorn cockerels. Thiouracil was fed at 0.1, 0.15, 0.2, 0.4 and 0.8 per cent levels in the feed for a 2-week period. The results obtained (Table V) show that the 0.1 per cent level induced maximum thyroid enlargement. The higher dosage levels resulted in no greater thyroid enlargement and the 0.4 and 0.8 per cent levels probably had a depressing effect on the enlargement. Feeding thiouracil for 2 weeks at a 0.6 per cent level in the ration resulted in less thyroid enlargement than the 0.1 per cent level in chicks 9 weeks old when killed. Very little difference in thyroid enlargement was noted between 0.1, 0.2 or 0.4 per cent levels fed to the chicks when 12 weeks of age. Thus in all chicks of various ages the addition of 0.1 per cent thiouracil to the ration resulted in maximum thyroid enlargement.

Astwood, Bissell and Hughes (1944) found that increased enlargement of the thyroid occurred with continued treatment with the thiouracil. In our experiments, White Leghorn cockerels receiving from 0.1 to 0.4 per cent thiouracil in the feed for a period of 8 weeks showed from three to five times the thyroid enlargement induced by feeding the drug for a 2 week period. Increasing the level to 0.6 per cent for 8 weeks resulted in less thyroid enlargement than the lower levels. Feeding .2% thiouracil in the ration to White Leghorns of both sexes from the time of hatching to 20 weeks of age resulted in thyroid enlargement approximately 20 times that of

TABLE VI. EFFECT OF THIOURACIL IN FEED ON THYROID WEIGHT OF WHITE PLYMOUTH ROCK CHICKENS

Thiouracil in feed	Sex	No. of chicks	Age at beginning of Exp.	Length of time of thiouracil feeding	Body weight	Average thyroid weight	Thyroid weight /100 gm. body weight
%			days	days	gm.	mgs.	mgs.
.1	m.	10	28	14	357.0	98.9	27.7
.1	f.	6	28	14	367.4	116.1	31.6
.2	m.	7	28	14	368.1	76.2	20.7
.2	f.	9	28	14	368.7	119.1	32.3
.4	m.	6	28	14	372.0	78.5	21.1
.4	f.	8	28	14	374.3	110.8	29.6
.6	m.	10	28	14	376.2	61.7	16.4
.6	f.	6	28	14	314.1	60.0	19.1
.1	m.	12	28	10	342.8	40.8	11.9
.1	f.	8	28	10	305.0	36.6	12.0
.1	m.	20	28	15	353.9	81.4	23.0
.1	f.	14	28	15	375.6	98.4	26.2
.1	m.	10	28	21	388.9	94.9	24.4
.1	f.	10	28	21	333.6	82.4	24.7
.1	m.	13	28	26	537.7	172.6	32.1
.1	f.	7	28	26	505.9	180.6	35.7
.1	m.	11	28	30	566.1	302.3	53.4
.1	f.	8	28	30	554.5	303.3	54.7
.1	m.	7	28	60	825.0	410.0	49.7
.1	f.	11	28	60	928.4	584.0	62.9
.1	m.	9	56	14	788.7	104.9	13.3
.1	f.	8	56	14	693.7	111.0	16.0
.2	m.	11	56	15	953.2	146.8	15.4
.2	f.	6	56	15	647.6	93.9	14.5
.4	m.	8	56	14	882.9	77.7	8.8
.4	f.	10	56	14	687.1	69.4	10.1
.6	m.	10	56	14	846.6	87.2	10.3
.6	f.	11	56	14	691.0	99.5	14.4
.1	m.	4	56	7	792.7	54.7	6.9
.1	f.	4	56	7	646.8	60.8	9.4
.1	m.	3	56	12	889.0	80.9	9.1
.1	f.	4	56	12	676.6	136.0	20.1
1	m.	6	56	21	877.8	197.5	22.5
.1	f.	12	56	21	761.1	205.5	27.0
.1	f.	8	56	24	763.7	212.3	27.8

the controls (Table V) and greater than that for an 8 week period of treatment. Thus increasing the time of feeding the drug markedly increased thyroid enlargement.

Similar experiments with the White Plymouth Rock chick of both sexes showed similar results (Table VI) except that for a definite dosage level the White Plymouth Rock cockerels required three weeks' treatment with thiouracil to attain thyroid enlargement as great per 100 grams body weight as that attained by the White Leghorn cockerel with 2 weeks treatment (Fig. 5). No comparisons are available between the two breeds for the females. In general the

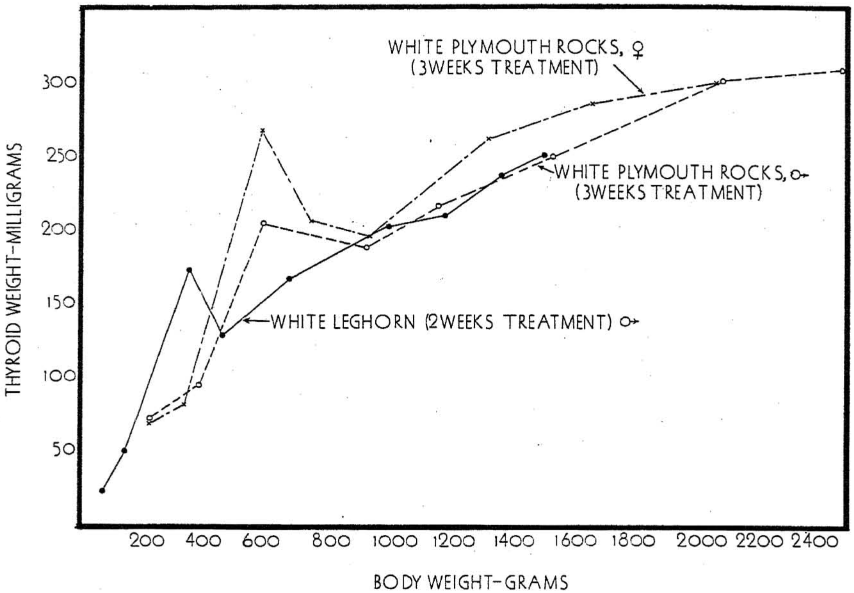


Fig. 5.—Effect of thiouracil on thyroid weight with increasing body weight in the fowl.

results obtained with the White Plymouth Rock breed show that for both sexes maximum thyroid enlargement is attained when 0.1 per cent thiouracil is contained in the ration. At a 0.6 per cent level thiouracil-induced enlargement was less. Extending the time of feeding the 0.1 per cent level of thiouracil increased the thyroid weight further. Those chicks receiving 0.1 per cent thiouracil in the ration for 60 days showed from two to three times the enlargement attained when receiving the drug for 14 days (Fig. 6). The older chicks tend to attain less thyroid enlargement per 100 grams body weight than younger chicks. In practically all cases, the females attained greater enlargement per 100 grams body weight than the males.

(2) Effect of thiouracil on glands other than the thyroid. To determine the effect of thiouracil feeding on the size of glands in the fowl other than the thyroid, the weights of the heart, adrenals, liver, thymus, ovary and testes were recorded from fifteen-week old Barred Plymouth Rock chickens that had received .1% thiouracil in their ration for 3 weeks. The weights of these same glands were recorded from comparable chickens not receiving thiouracil but the

same basal ration. Statistical analysis of the data obtained on the different gland weights of the two groups indicated that the liver and kidney are of significantly greater weight in chickens receiving .1% thiouracil for 3 weeks. The liver weight from chickens receiving .1% thiouracil averaged 32.5 gms. for 45 individuals, and 26.6

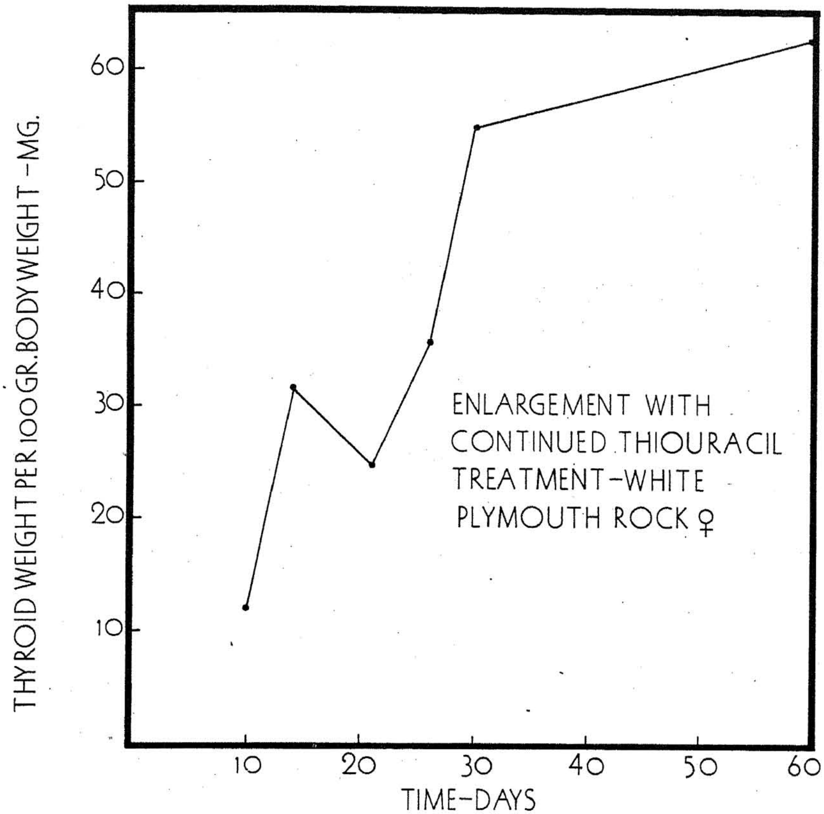


Fig. 6.—Effect of length of time of feeding thiouracil on thyroid weight in the fowl.

gms. for 57 individuals receiving the basal ration. The kidney weight from the 45 chickens receiving thiouracil in the ration averaged 10.2 gms. while the kidney from the 57 chickens receiving the basal ration averaged 8.4 gms. The body weights of the two groups compared were approximately the same. There was no apparent significant difference between the adrenal, thymus, heart, ovary or testes weights of the Barred Plymouth Rock chickens receiving thiouracil for 3 weeks and those not receiving thiouracil. The variability of the testes weights among individuals within the same group was very great and with the small number of individuals available for comparison of testes weight, it was not possible to detect any difference. However, a study of differences in the testes weight of 23 normal White Leghorn cockerels 12 weeks of age and at a body weight of about 1050 gm. were compared with 28 individuals receiving thiouracil for 2 weeks. To our own data for the testes weights of 12 week old White Leghorn chickens receiving no thiouracil, data from Parker, McKenzie and Kempster (1942) and from Jones and Lamoreux (1942) were added. Their data were similar to ours with respect to body weight and testes weight of White Leghorns at 12 weeks of age. In comparing the testes weight of White Leghorns receiving thiouracil for 2 weeks with the testes weight of normal White Leghorns it was found that feeding thiouracil definitely depresses the testes weight. The 23 White Leghorn cockerels at 12 weeks of age not receiving thiouracil had a testes weight averaging 3.848 gms. and were much larger than in the 15 week old Barred Plymouth Rock cockerels. The 28 White Leghorn cockerels 12 weeks of age receiving thiouracil in the ration for 2 weeks had a testes weight averaging 1.624 gms. Statistical analysis showed this difference in testes weights of the two groups to be significant although the standard deviation was quite high in both groups.

(3) Effect of thiourea on the thyroid weight of the fowl: Increasing levels of thiourea in the ration fed to White Plymouth Rock chicks begun a day or two after hatching and continued for 3 weeks showed that maximum enlargement of the male chick's thyroid was induced when from 0.05 to 0.075 per cent was fed in the ration. The thyroid weight obtained was 17 to 19 mg. per 100 grams body weight. This is less than that obtained in the male with 0.1 per cent thiouracil in the feed with chicks of the same breed, age and period of treatment. In the female maximum enlargement was induced by a level of 0.075 per cent thiourea in the feed. The thyroid weight was 25.9 mg. per 100 grams body weight and about the same as that obtained in the female treated with 0.1 per cent thiouracil. Increasing levels of thiourea in the ration fed White Leghorn chicks begun a day after hatching and continued for 3 weeks showed that maximum thyroid enlargement was induced in both sexes at a level of .05 to .075% (Table X(d)). The enlargement of the male thyroid was about 28 mg. per 100 grams body weight, the female thyroid enlargement was about 35 mg. per 100 grams body weight. Thyroid enlargement with similar White Leghorn chicks using thiouracil at a .075% level resulted in an average thyroid enlargement of both sexes of 57 mg. per 100 grams body weight. This was considerably higher than obtained in other experiments with thiouracil. This may have been due to a difference in the goitrogenic effect of the particular sample of thiouracil used since in all cases where this particular sample was used greater goitrogenic effect resulted.

(4) Effect of thiouracil and thiourea on the thyroid weight of the goat: In the absence of data relative to the goitrogenic effect

of thiouracil or thiourea on the thyroid of the goat, varying dosages of thiourea were administered both orally and by subcutaneous injection in the younger animals (Table VII). In older animals the effect of oral administration of both thiourea and thiouracil was

TABLE VII. THE EFFECT OF THIOUREA AND THIOURACIL ON THE THYROID WEIGHT OF GOATS
(Administered once daily for 30 days)
- THIOUREA -

Dosage	Method of administration	No. of goats	Sex	Body weight average	Thyroid weight average	Thyroid wt. /100 gms. body wt.	Condition	
mgm.				kg.	mgm.	mgm.		
360	oral	7	m. & f.	10.2	2620	25.69	non-preg.	
720	oral	3	m. & f.	11.9	2262	19.00	non-preg.	
180	subcut.	3	m. & f.	7.1	595	8.38	non-preg.	
360	subcut.	5	m. & f.	7.4	981	13.26	non-preg.	
540	subcut.	2	m. & f.	7.9	1053	13.32	non-preg.	
720	subcut.	2	f.	6.1	498	8.16	non-preg.	
750	oral	1	f.	21.3	2973	13.96	non-preg.	
1500	oral	1	f.	20.4	2220	10.88	non-preg.	
1500	oral	2	f.	31.3	4189	13.38	non-preg.	
1500	oral	2	f.	43.8	4991	11.39	preg.	
1000	oral	1	f.	53.6	9000	16.79	preg.	
gm.					- THIOURACIL -			Stage of pregnancy days
5.0	oral	1	f.	23.2	2468	10.64	non-preg.	
5.0	oral	1	f.	43.1	2398	5.56	preg.	140
5.0	oral	1	f.	45.4	7200	15.86	preg.	40
4.0	oral	1	f.	35.9	2110	5.88	preg.	40
4.0	oral	1	f.	37.7	4150	11.00	preg.	145
3.0	oral	1	f.	35.2	2256	6.41	preg.	150
3.0	oral	1	f.	35.9	2326	6.48	preg.	145
3.0	oral	1	f.	44.3	4100	9.26	preg.	50
3.0	oral	1	f.	48.1	3142	6.53	preg.	75
2.0	oral	1	f.	39.5	4365	11.05	preg.	145
1.5	oral	1	f.	21.3	1236	5.80	preg.	50
1.5	oral	1	f.	34.5	2120	6.14	preg.	145
1.5	oral	1	f.	58.6	6094	10.40	preg.	75
1.5	oral	3	f.	17.3	1295	7.50	non-preg.	
1.0	oral	2	f.	22.2	2216	10.00	non-preg.	
1.0	oral	1	f.	23.2	1431	6.17	preg.	70
1.0	oral	1	f.	34.3	1980	5.77	preg.	80
1.0	oral	2	f.	35.9	4461	12.43	non-preg.	

determined. With all animals the administration of the drug was made once per day for a 30 day period. Oral administration was made by capsule.

In young goats approximately 2 months of age when killed, thiourea when given orally at the rate of 360 mg. per day induced maximum thyroid enlargement. The average thyroid weight induced was 116.4 mg. per pound body weight or 25.7 mg. per 100 grams body weight, which is about 3.5 times normal thyroid weight. Administration of 720 mg. thiourea daily produced an average thyroid weight of 88.0 mg. per pound body weight, or 19.0 mg. per 100 grams body weight. Subcutaneous injection of thiourea in water solution produced less goitrogenic effect than oral administration. Maximum enlargement attained with subcutaneous injection was 60 mg. per pound body weight or 13 mg. per 100 grams body weight, with a dosage level of from 360 to 540 mg. per day. Smaller and larger dosages induced less enlargement. The difference in the rate of loss of thiourea from the body with daily administration by the two routes may account for the difference in the degree of enlargement attained.

In older and larger goats, response of the thyroid to thiouracil and thiourea was extremely variable as can be seen in Table VII. Thiourea was more effective than thiouracil in inducing thyroid enlargement. In general about 1 gram of thiourea per 100 pounds body weight gave the greatest goitrogenic effect, inducing a thyroid weight 2.5 times that of controls. Results with thiouracil were extremely variable. A dosage of 1 gram thiouracil per day varied from no effect to enlargement twice that of normal. A dosage of 5 grams thiouracil per day, on the average, induced a thyroid weight not quite twice that of the thyroid weight of control animals.

In milking goats weighing an average of 95 pounds, 1.5 grams of thiourea per day administered orally caused marked reduction in milk yield (about 45 per cent) in 14 days of treatment. Oral administration of 3.0 grams thiouracil per day for 14 days resulted in a reduction of 35 per cent in milk yield. In both cases milk yield could be brought back to normal by the injection of proper doses of thyroxine, indicating that the milk yield reduction was caused by the anti-thyroidal effects of the drugs rather than as a toxic effect separate from the anti-thyroid effect.

TABLE VIII. THYROID WEIGHTS OF GOAT FETUSES FROM MOTHERS TREATED WITH THIOUREA AND THIOURACIL

Body weight of fetus	Fetal thyroid weight	Fetal thyroid wt. /100 gm. body weight	Body weight of mother	Thyroid weight of mother	Thyroid wt. /100 gms. body weight (mother)	Daily treatment for mother
gm.	mgm.	mgm.	kg.	mgm.	mgm.	
25.6	30.0	117.2	21.3	1236	5.80	1.5 gm. thiouracil
121.4	58.4	48.1	23.2	1431	6.17	1.0 gm. "
172.0	116.0	67.4	34.3	1980	5.77	1.0 gm. "
181.7	80.9	44.5	34.3	1980	5.77	1.0 gm. "
186.0	1000.2	537.7	48.6	6100	12.55	1.5 gm. thiourea
220.0	1112.0	505.4	48.6	6100	12.55	1.5 gm. "
257.0	160.8	62.6	58.6	6094	10.40	1.5 gm. thiouracil
382.2	3000.0	784.9	53.6	9000	16.79	1.0 gm. thiourea
427.0	5800.0	1358.3	53.6	9000	16.79	1.0 gm. "
451.2	6800.0	1507.0	53.6	9000	16.79	1.0 gm. "
1003.3	2800.0	279.1	34.5	2120	6.14	1.5 gm. thiouracil
1024.5	1866.0	182.1	34.5	2120	6.14	1.5 gm. "
1185.0	2372.0	200.2	39.5	4365	11.05	2.0 gm. "
1935.0	5000.0	258.4	35.2	2256	6.41	3.0 gm. "

Since several of the experimental goats were pregnant there was opportunity to study the effect of thiouracil or thiourea on the thyroid weight of the fetus (Fig. 1, Table VIII). Before the fetus attained a body weight of about 160 grams (75 days fetal age or mid-term), little or no effect on the weight of the fetal thyroid was observed when the mother received thiouracil or thiourea. After mid-term the fetal thyroid was greatly enlarged when the mother received thiouracil.

While the average thyroid weight of the fetus after mid-term from mothers receiving no treatment was 63 mg. per 100 grams body weight, the average thyroid weight per 100 grams body weight of the fetus from mothers receiving thiouracil was 624.0 mg., practically ten times the normal thyroid size. Whether this enlargement is brought about by the drug passing into the fetal circulation and affecting the fetal thyroid directly or whether the hyperplasia is brought about by an increased thyrotrophic hormone circulating in the blood stream of the mother and passing into the fetal circulation was not determined. However, that the drug passes into the fetal blood stream and affects the fetus' own thyroid and thyrotrophic hormone release is indicated by the work of Tobin (1941). He found that the fetal thyroids of rats were not stimulated by thyrotrophic hormone injection into the mother. Apparently the thyrotrophic hormone does not pass through the placental barrier. Goldsmith, Gordon and Charipper (1944) noted enlarged thyroids of rats 4 to 24 days after birth from mothers treated with thiourea, but were uncertain of the effect on the thyroid of pre-partum treatment.

The fact that the fetal thyroid is not affected before mid-term but is affected after mid-term may be an indication that the pituitary-thyroid secretions begin in the fetus at about that time.

(5) Effect of thiouracil and thiourea in cattle: The goitrogenic effect of thiouracil or thiourea administered orally once or twice daily to the calf (2 months of age) is not very great (Table IX). No difference between the two drugs could be detected with regard to the goitrogenic effect in the calf. The average thyroid enlargement obtained with a dosage level of from 1.0 to 3.0 grams of thiourea or thiouracil per day was 1.5 times that of the normal thyroid weight.

In lactating cows, about 5 grams thiourea per day per 1000 pounds body weight administered orally from 8 to 12 days reduced milk production between 20 and 35 per cent, the smaller percentage reduction occurring with 8 days treatment and the larger reduction with 12 days treatment.

Comparison of thiourea and thiouracil and variability of their goitrogenic effect. (1) Anti-thyroid properties: Apparently there is a difference between the degree of goitrogenicity of thiourea and thiouracil. In general, thiourea is more goitrogenic than thiouracil per unit weight of the drugs in the ruminants. This relationship holds for the goitrogenic effect of the drugs on the goat thyroid and for the anti-thyroid effect in lactating goats and cows. In the chick the dosage of thiourea required to bring about its maximum goitrogenicity is about half that required of thiouracil to bring about its maximum effect. In the goat the required dosage of thiourea for a definite goitrogenic effect is probably less than one-third that of the thiouracil requirement. A comparison of the anti-thyroid effect of the two drugs with lactating goats indicates that twice the dosage

TABLE IX. EFFECT OF THIOUREA OR THIOURACIL ON THYROID WEIGHT OF THE MALE CALF

Calf No.	Treatment	Length of treatment	Age of calf when killed	Body weight	Thyroid weight	Thyroid weight /100 lbs. body weight
		days	days	lbs.	gm.	gm.
46 H	none		110	250	10.800	4.3
67 H	none		60	164	6.600	4.0
470 G	2.0 g. thiourea - once daily	30	60	122	12.200	10.0
750 H	2.0 g. " " "	30	60	187	13.300	7.1
*	3.5 g. " " "	30	60	152	11.300	7.6
37 H	1.0 g. " " "	30	60	182	10.800	5.9
24 H	1.0 g. " " "	30	60	115	7.700	6.7
8 H	1.0 g. " 2x per day	30	60	139	8.400	6.0
2 H	1.0 g. " " " "	30	60	177	8.100	4.6
9 H	1.0 g. " " " "	30	60	132	11.000	8.3
475 G	3.0 g. thiouracil - once daily	30	60	128	16.475	12.9
472 G	1.0 g. "	30	60	151	7.125	4.7
61 H	1.0 g. "	30	60	210	9.700	4.6
54 H	3.0 g. "	30	60	180	10.680	6.0
477 G	1.0 g. "	30	60	161	8.400	5.2
11 H	1.0 g. thiourea + 1000 γ thyroxine	30	60	160	8.100	5.1
12 H	1.0 g. " - 2x per day + 1500 γ d,l-thyroxine	30	60	113	5.000	4.4

G = Guernsey
H = Holstein
* = Heifer

or more of thiouracil compared with thiourea was required to depress milk production 30 to 40 per cent.

A definite comparison of the anti-thyroid potency between the two drugs on goats cannot be made with any degree of accuracy because of the great variability of response among individual goats to different levels of intake of the drugs. Also, the toxic effects of thiourea on the animal influence the response of the thyroid to the drug. This is because when the animal is in poor physical condition the thyrotrophic hormone release is low.

Part of the difference of the goitrogenic effect of the two drugs is probably due to the difference in the drug per se. Part of the difference in goitrogenic effect between species and the variability of results may be due to differences in absorption from the alimentary tract and differences in the rate of loss of the drugs from the body. Williams, Kay and Jandorf (1944) and Williams (1944), in studies on the absorption, distribution and excretion of thiouracil in rats, found that on oral ingestion 15 per cent of the drug was destroyed in the alimentary tract and about 33 per cent was excreted in the urine. They assumed, therefore, that about 50 per cent of the drug is broken down in the body tissues. When thiouracil was injected intravenously, rapid destruction took place; more than 65 per cent was broken down within 3 hours and only traces were found after

24 hours. In the case of a ruminant such as the goat or cow, it is possible that much of the drug is destroyed in the alimentary tract. It is also possible that administering the drug once daily does not maintain a sufficiently high level of the drug in the blood to consistently bring about a great degree of thyroid hyperplasia.

TABLE X (a). THE EFFECT OF THIOURACIL ON THE THYROID WEIGHT OF THE FOWL
(0.1% thiouracil in feed)

No. of chicks	Sex	Body weight	Thyroid weight	Thyroid weight /100 gms. body weight	Age when killed
		gm.	mgm.	mgm.	weeks
	White Leghorns (treated 14 days)				
19	Male	62.7	22.3	35.6	2
10	"	138.0	49.6	35.9	4
10	"	352.2	172.7	49.1	6
12	"	463.9	128.8	27.7	7
10	"	682.0	165.7	24.3	9
9	"	1009.0	204.0	20.2	12
8	"	1192.0	209.5	19.0	20
10	"	1371.0	236.5	17.3	24
7	"	1517.0	250.5	16.5	27
	White Plymouth Rocks (treated 21 days)				
9	Male	222.0	68.0	30.6	5
15	Female	223.0	67.1	30.1	5
10	Male	338.0	94.9	24.4	7
10	Female	334.0	82.4	24.7	7
9	Male	603.0	200.4	33.2	9
8	Female	599.0	269.0	44.9	9
5	Male	941.0	187.6	19.9	11
12	Female	760.0	205.5	27.0	11
8	Male	1154.0	216.5	18.8	13
7	Female	949.0	195.0	20.5	13
7	Male	1540.0	249.8	16.2	18
12	Female	1333.0	262.8	19.7	18
6	Male	2094.0	301.4	14.4	22
12	Female	1677.0	286.4	17.1	22
6	Male	2523.0	308.0	12.2	26
10	Female	2073.0	298.6	14.4	26

Differences in thyroid enlargement between animals of different ages of the same species may be considered to be due to differences in thyrotrophin release by the pituitary. For example, the enlargement of the thyroid of young chicks following thiouracil treatment is greater per 100 grams body weight than in older chicks. Also, the enlargement of the thyroid of the fetal goat is much greater than in older animals. If this is the correct interpretation of the results observed, the degree of thyroid enlargement with standard thiouracil treatment may be used as a criterion of the magnitude of release of thyrotrophin under normal and experimental conditions. The difference in thiouracil-induced thyroid enlargement between sexes is probably due to differences in the extent of thyrotrophin

TABLE X (b). THE EFFECT OF THIOURACIL ON THE THYROID WEIGHT OF THE FOWL. (14 days treatment with 0.1% thiouracil in drinking water)

Breed	Sex	Age	No. of chicks	Body weight	Thyroid weight	Thyroid wt. /100 gms. body wt.
		weeks		gm.	mgm.	mg.
Buff Minorcas*	Male	2	11	82.0	42.7	52.1
	Female	2	9	79.0	55.1	69.8
White Leghorn*	Male	2	13	93.0	35.2	37.8
	Female	2	12	91.0	62.6	68.8
White Leghorn-N.H. Red Cross*	Male	2	8	92.0	51.2	55.6
	Female	2	11	103.0	59.6	57.9
New Hampshire Red*	Male	2	10	90.0	30.0	33.3
	Female	2	7	88.0	38.3	43.5
Buff Orpington*	Male	2	7	100.0	26.9	26.9
	Female	2	9	91.0	42.8	47.0
Barred Plymouth Rock*	Male	2	12	81.0	24.4	30.1
	Female	2	12	81.0	28.7	35.4
White Plymouth Rock*	Male	2	8	86.0	26.9	31.3
	Female	2	10	93.0	29.2	31.4
White Wyandotte*	Male	2	12	89.0	26.9	30.2
	Female	2	13	91.0	30.4	33.4
S. C. Rhode Island Red*	Male	2	9	91.0	24.5	26.9
	Female	2	12	77.0	23.6	30.7
Black Giants	Male	2	20	76.0	12.3	16.2
	Female	2	15	79.5	17.6	22.2

*Data from Mixner, Reineke and Turner (1944)

TABLE X (c). THE EFFECT OF THIOURACIL ON THE THYROID WEIGHT OF THE FOWL. (21 days treatment with 0.1% thiouracil in feed)

Breed	Sex	Dosage of stilbestrol* in feed	Length of treatment with stilbestrol	Age when killed	No. of chicks	Body weight	Thyroid weight	Thyroid wt. /100 gms. body weight
		%	weeks	weeks		gm.	mgm.	mgm.
White Plymouth Rock	M.	None		5	14	226.0	93.9	41.6
	F.	"		5	9	215.0	128.6	59.8
White Plymouth Rock	M.	0.01	5	5	14	250.0	151.6	60.6
	F.	0.01	5	5	10	226.4	200.9	88.7
White Plymouth Rock	M.	0.02	5	5	14	237.4	76.3	32.1
	F.	0.02	5	5	9	235.5	128.1	54.4
White Plymouth Rock	M.	0.04	5	5	12	229.8	84.5	36.8
	F.	0.04	5	5	11	220.0	139.4	63.3
Barred Plymouth Rock	F.	0.01	9	15	10	1305.0	243.4	26.3
Barred Plymouth Rock	F.	None		15	17	1245.0	404.8	32.5
Barred Plymouth Rock (capons)	M.	None		15	5	1513.0	675.0	44.6
Barred Plymouth Rock (normal)	M	None		15	9	1587.0	391.4	24.7

*Dimethyl ether of diethylstilbestrol

release stimulated by a lack of circulating thyroid hormone. Differences in the enlargement of the thyroid between different breeds of chicks (Mixner, Reineke and Turner, 1944) probably occur for the same reason. The feeding of dimethyl ether of diethylstilbestrol at a level of 0.01 per cent in the feed in conjunction with 0.1 per cent thiouracil increased the goitrogenic effect of thiouracil by about 1.5 times that of thiouracil alone in 5 week old White Plymouth Rock chicks. In 15 week old Barred Plymouth Rocks the same level of

Table X (d)

Effect on thyroid weight of chickens fed thiourea from hatching time to 3 wks. of age.

Treatment	Sex	No. of chicks	Body weight	Thyroid weight	Thyroid weight /100 gms. body weight
			gm.	mgm.	mgm.
White Rock					
Controls	F	15	131.2	15.0	11.4
Controls	M	12	123.4	12.7	10.3
0.05% Thiourea	F	15	104.4	20.6	19.7
0.05% Thiourea	M	16	119.5	22.5	18.8
0.075%Thiourea	F	18	104.7	27.1	25.9
0.075% Thiourea	M	13	120.5	20.5	17.0
0.10% Thiourea	F	20	82.4	16.0	25.9
0.1% Thiourea	M	10	88.6	21.4	18.0
White Leghorns					
.006% Thiourea	F	9	134.6	24.4	18.1
"	M	13	143.8	25.9	18.0
.0125% Thiourea	F	13	127.2	21.2	16.6
"	M	11	143.9	29.8	20.7
.018% Thiourea	F	7	134.0	26.7	19.9
"	M	13	144.1	23.2	16.1
.025% Thiourea	F	18	127.2	29.3	23.0
"	M	7	140.0	26.6	19.0
.0375% Thiourea	F	20	116.4	29.1	25.0
"	M	3	156.6	33.4	21.3
.05% Thiourea	F	15	124.6	43.5	34.9
"	M	7	131.1	37.0	28.2
.075% Thiourea	F	18	117.7	44.4	37.7
"	M	6	117.7	33.1	28.1

stilbestrol in the feed resulted in a decrease in the goitrogenic effect of thiouracil. Castration of male Barred Plymouth Rocks at 6 to 7 weeks of age resulted in increasing the goitrogenic effect, following thiouracil treatment for 3 weeks, when the capons were killed at 15 weeks of age.

For a comparison of results of the effect of thiouracil on thyroid enlargement with age, breed and certain experimental conditions, see Tables X(a), X(b) and X(c).

Superimposed on these general relationships is the influence of variations in temperature. The temperature under the conditions of these experiments averaged about 80° F. Probably season of the year had some effect on variability of the thyroid response to thiouracil administration. The general physical condition of the animals probably influenced the degree of response also.

(2) Toxic effects: Thiourea was much more toxic than thiouracil at similar dosage levels for the chicken, goat and lactating cow.

In the chicken 0.6 per cent thiouracil in the ration was essentially non-toxic. Mixner, Reineke and Turner (1944) reported that thiourea was toxic to young chicks when administered at 0.6 and 0.8 per cent levels in the drinking water. Reineke and Turner (1945b) reported 100 per cent mortality in young chicks in from 2 to 4 weeks treatment with 0.5 and 1.0 per cent thiourea. Treatment with thiourea began at hatching time of the chicks.

In our experiments, dosages of 0.15 per cent thiourea or above in the feed were toxic. All chicks started on thiourea treatment at hatching time, died within 3 weeks on a 0.4 per cent level. At a 0.3 per cent level all chicks died in 24 days; at a 0.2 per cent level all died in 37 days; and at a 0.15 per cent level all died in eighty-seven days. A 0.1 per cent level resulted in a smaller body weight, the body weight of this group being about two-thirds that of the controls at 3 weeks of age. Mortality rate in the chicks fed 0.1 per cent thiourea in the ration was not greater than that in controls at 3 weeks of age.

Lambs weighing about 20 pounds tolerated 360 mgs. thiourea per day orally and continued to tolerate a thiourea dosage of about 1.5 grams per day per 100 pounds body weight for over a year. Their growth rate, however, was retarded since they averaged 60 pounds body weight at one year of age. A dosage of thiourea of over 2.0 grams per day per 100 pounds body weight to lambs was definitely toxic, resulting in death with about 1 or 2 weeks of treatment.

Young goats weighing about 20 pounds tolerated 360 mgs. thiourea per day orally by capsule fairly well. However, this dose was toxic to some individuals. Larger goats weighing 50 to 100 pounds tolerated 0.75 grams thiourea per day orally fairly well although it was toxic to some of those of the lighter weights. Dosages greater than 0.75 grams were definitely toxic when given for a period of 30 days. Although most goats weighing 75 to 100 pounds were affected by the toxic properties of thiourea when administered 0.75 gm. or more per day within a 10 to 14 day period of treatment, some of the lactating goats showed little evidence of the effects of toxicity when given 1.5 grams per day for 14 days. However, this particular group of lactating goats had been treated in previous experiments with lower than 0.75 gm. thiourea per day for a period of two weeks and may have developed increased resistance to the toxicity of thiourea.

This observation is in agreement with the observation of Griesbach, Kennedy, and Purves (1944) where they observed that rats which have survived a first dose of thiourea developed an immunity to thiourea administration. The frequent occurrence of toxic effect of thiourea seems to eliminate its use for practical experimentation since later experiments with lactating goats showed toxic effects on administration of 0.75 gm. per day.

Thiouracil given in dosages as large as 5 grams per day to goats weighing somewhat less than 100 pounds was well tolerated.

No toxic effects of thiourea were noted in 150 pound calves when given in amounts as large as 3 grams per day orally for 30 days. In cows even 5 grams thiourea per day per 1000 pounds live weight was toxic as indicated by the fact that cows so treated went off feed and digestive disturbances were induced. Administration of 12 grams thiouracil per day to lactating cows caused no toxic effects.

The toxic effect of thiourea was usually manifested by digestive disturbances. The animals, if continued on treatment, became emaciated and some died within 10 days of treatment. In goats the pleural cavity showed edema. Toxicity of thiourea has been reported by Astwood (1943a) and Himsworth (1944). Apparently these drugs (sulfonamides, thiourea and thiouracil) may occasionally cause an agranulocytosis in humans treated with them. Goldsmith, Gordon, Finkelstein and Charipper (1944) noted that thiourea feeding to rats resulted in a neutrophilic granulocytopenia which could be prevented by administration of solubilized liver or folic acid. This agrees with the findings of other investigators. Whether this condition was induced by thiourea in our goats was not determined.

It is possible that the relatively large amounts of thiourea given once a day showed a toxic effect because of temporarily induced high thiourea concentration in the blood immediately after administration. It is possible that if the same amount were mixed in the feed, the toxicity would not be as great since absorption from the alimentary tract into the blood might be slower. Griesbach, Kennedy and Purves (1944) reported the sudden appearance of thiourea toxicity in rats. They believed this toxic effect to be independent of its antithyroid effect. Our observations agree with this conclusion since goats receiving thiourea plus a considerable dosage of thyroxine exhibited toxicity symptoms as readily as those receiving thiourea alone. Griesbach, Kennedy and Purves (1944) found that by pretreating rats with potassium iodide the toxicity was prevented. Goats in our experiments did not respond similarly to potassium iodide administration.

Administration of either thiourea or thiouracil at low environmental temperature sometimes resulted in death of the treated animal. To illustrate, chicks 4 weeks of age receiving 0.1 per cent thiouracil alone in the feed and kept in a room at 82° F. were accidentally subjected to a temperature of about 70° F. overnight and the following 2 days about 40 per cent died, whereas similar control chicks did not show any ill effects from this exposure. Goats suddenly subjected to below freezing temperatures while being treated with thiouracil or thiourea died suddenly after appearing to be in good physical condition prior to death. This effect is believed to be due to the fact that the drugs, by preventing thyroid hormone synthesis, interfere with the heat regulatory mechanism. As a result they cannot withstand low temperatures. This is suggested by the work of Le Blond and Gross (1943) with rats. Thyroidectomized rats, when exposed to cold after becoming accustomed to a warm environment, died suddenly when apparently in good physical condition.

QUANTITATIVE DETERMINATION OF THE THYROID SECRETION RATE

Review of Literature

Determinations of the secretory activity of the thyroid in terms of crystalline thyroxine have been made for several animal species. Most of these determinations are based on replacement therapy in myxoedematous humans or thyroidectomized animals. In nearly all of these determinations the crystalline thyroxine used was probably d, l-thyroxine since the usual methods of preparation yield the racemized product. Reineke and Turner (1945a) have shown that l-thyroxine is twice as active physiologically as d, l-thyroxine. Since the form of thyroxine produced by the thyroid is the l-form (Harington and Salter, 1930), the determinations made probably represent twice the actual l-thyroxine produced by the thyroid.

Although crystalline l-thyroxine may not be the true thyroid hormone (Harington, 1933), values of the thyroid secretion rate can be obtained that may be expressed in equivalent amounts of a definite standard, namely, crystalline d, l or l-thyroxine. When crystalline thyroxine is referred to it may be assumed that it refers to the racemic form unless the l-form is specifically mentioned.

Boothby and Baldes (1925) calculated that the daily thyroxine requirement in the human is somewhere near 0.4 mg. per day. This calculation was based on determination of the total thyroxine present in the body and the percentage rate of loss of thyroxine per day. Thompson et al. (1935) found that from 0.25 to 0.35 mg. of thyroxine must be injected daily in order to maintain a normal basal metabolic rate in myxoedematous patients at bed rest. Eppinger and Salter (1935) obtained values of about 0.33 mg. of thyroxine as the rate of thyroid hormone secretion in the human. Elmer (1938), reviewing results by various authors, observed that on the basis of the optimum iodine requirements on the one hand and the minimum requirement of thyroid hormone in myxoedema on the other, the thyroid of the human secretes about 0.33 mg. of thyroxine (about 200 gamma thyroxine iodine) in 24 hours. Salter and McKay (1944) determined the concentration of protein bound iodine in blood serum or plasma using this determination as an index of net thyroid function. Determination of the "hormonal" iodine for hyperthyroid patients ranged from 10.5 to 12.2 micrograms per cent. A myxoedematous patient had a concentration of 1.4 microgram per cent while the blood of a normal woman had a value of 6.6 microgram per cent.

Working with thyroidectomized rats, Evans et al. (1939) found that injection of 0.005 mg. of crystalline thyroxine resulted in a return to normal basal metabolic rate and also normal growth. To maintain normal heart rate in thyroidectomized rats, Fishburne and Cunningham (1938) found that 0.04 mg. of thyroxine per day was required. Folley (1938) found that milk production in rats was lowered by thyroidectomy but that administration of 0.25 mg. thyroxine every fourth day after operation or 0.1 mg. injected daily caused no apparent improvement in lactation performance as indicated by the growth curve of the litters of such treated mothers. Dempsey and Astwood (1943), using the thyroid weight response to varying dosages of thyroxine as an index of normal equilibrium between the thyrotrophic and thyroid hormones in thiouracil treated rats, found that the thyroid's secretion rate at normal temperatures (25° C.) was 5.2 micrograms l-thyroxine per day. Reineke, Mixner and Turner (1945) determined that the thyroid of the rat weighing about 140 grams and kept at a temperature of about 25° C. secreted at a rate of 4.8 micrograms d, l-thyroxine daily from the thyroid response of thiouracil treated animals when administered thyroxine in graded dosages. The return of the B.M.R. to normal with increasing dosages of thyroxine in thiouracil treated animals indicated a similar thyroxine secretion rate (4.75 micrograms daily). Using the "hormonal" iodine as net thyroid function, Salter and McKay (1944) found that the blood of the rat had a concentration of 3.9 micrograms per cent.

The thyroid of a rat weighing about 150 grams, therefore, appears to secrete about 5 micrograms thyroid hormone per day in terms of crystalline d, l-thyroxine under room temperature conditions.

One mg. of thyroxine injected daily into thyroidectomized goats maintained about normal heart rate and milk production in some individuals, while others required a somewhat higher dosage to maintain a similar level (Ralston et al., 1940). Thus individual variability in the thyroid's secretion rate is indicated.

Winchester (1940) reported that injections of 0.93 mg. of thyroxine per kilogram to 0.73 power of body weight per week in laying hens increased egg production of thyroidectomized fowls to 40 per cent of normal. Twice this dosage increased egg production to 60 per cent of normal. This would indicate that the laying hen's thyroid secretes more than 0.35 mg. thyroxine per day. Accepting the thyroxine secretion rate of man to be about 0.33 mg. and that of a 150 gram rat at 0.005 mg. per day, it is probable that 0.35 mg. per day for the laying fowl is too high. The high value obtained by Winchester may be because of the fact that thyroxine injections were made only twice per week. Experiments carried on in this laboratory to determine the effect of thyroprotein on egg production of White Leghorn and New Hampshire Red hens show that 0.022 per cent thyroprotein in the feed results in increased egg production with no detrimental effect to the hen. This amount of thyroprotein, on the basis of its effectiveness in bringing about control thyroid size in thiouracil fed chicks, would be the equivalent of 72 micrograms d, l-thyroxine per hen per day for the White Leghorns and 58 micrograms d, l-thyroxine equivalent per hen per day for the New Hampshire Reds. Considering these hen's thyroids to be practically inactive due to high thyroid hormone in the blood, these amounts of d, l-thyroxine may be considered to be somewhat above the normal amount of thyroxine secreted since the physiological process of egg production was above normal.

EXPERIMENTAL

The Determination of Thyroxine Secretion Rate in the Fowl.

Methods and Materials. Because of the availability of chicks and their adaptability to experimental work and since chick thyroids were found to be made goitrous with thiourea or thiouracil treatment (Mixner, Reineke and Turner, 1944), initial studies of the effect of increasing age and body weight on the thyroxine secretion rate were conducted with the domestic fowl. Also there are breeds of chickens varying in mature body weight, fattening ability, conformation, laying ability and temperament and thus there is opportunity to study the differences in thyroxine secretion rate between breeds of animals varying in such characteristics. Because of the important role the thyroid hormone plays in animal physiology, it is important to study the variation in thyroid activity between breeds varying in these characteristics to determine the relation of the degree of thyroid activity to fattening ability, conformation, growth rate and other functions.

The method of determining the rate of thyroxine secretion in the fowl is essentially the same as that used by Dempsey and Astwood (1943) with rats and that used by Mixner, Reineke and Turner (1944) with chicks. The method involves the use of a goitrogenic agent such as thiourea or thiouracil that has a thyroidectomy effect and results in thyroid enlargement. By the subcutaneous injection of graded dosages of thyroxine, the weight of the thyroid is reduced in thiouracil- or thiourea-treated animals and the weight reduction in general is proportional to the thyroxine dosage. Thus the establishment of a normal thyroxine-thyrotrophin equilibrium by injection of thyroxine in thiouracil-treated animals results in a thyroid weight equal to that in control animals. The quantity of thyroxine required to bring about this result is considered to be an estimate of the normal thyroid secretion rate by the glands of a particular group of animals.

In all determinations with chickens, 0.1 per cent thiouracil in the ration was used as the goitrogenic agent. In experiments with the White Leghorn breed the assay period was 2 weeks. In experiments with the White Plymouth Rock and Barred Plymouth Rock breeds the assay period was extended to 3 weeks, because the heavier breeds required a longer time to attain sufficient thyroid enlargement in order to make an accurate thyroxine secretion rate determination under all conditions of the experiments.

The White Leghorn and Barred Plymouth Rock chickens were reared and kept in a basement animal room with limited diffused daylight. The White Plymouth Rock chickens were purchased from a local poultryman who kept the birds until a day or two before the assay was started for each age group. The temperature of the animal room was controlled by thermostat and varied from 82 to 90° F. For the determinations made in the summer the temperature ranged from 84 to 90° F. and for those made in the winter the temperature was nearer 82° F. Lights were kept on from 7:30 A. M. to 9:00 P. M. for the White Leghorn groups and the 5 to 13 week old groups of White Plymouth Rocks. For the White Plymouth Rock groups from 18 to 26 weeks of age and for the Barred Plymouth Rocks the lights were kept on from 7:30 A. M. to 5:00 P. M. The determinations on White Leghorn cockerels started in March and ended in November. The first determination for the White Plymouth Rocks was started in June and the final determination was finished in November. Determinations for the Barred Rocks were made in January and February.

The same basal ration was fed to all breeds and ages of chickens in these experiments and was made up of the following ingredients:

Yellow corn meal	45	parts	by	weight
Shorts	15	"	"	"
Soybean oil meal	15	"	"	"
Alfalfa meal	10	"	"	"
Meat scraps (50% protein)	7	"	"	"
Bran	5	"	"	"
Bonemeal	0.5	"	"	"
Common salt	1	"	"	"
Cod liver oil	0.25	"	"	"

(400 A.O.A.C. units Vitamin D/gm.)

Each group of chickens for a particular assay was divided into sub-groups of as nearly uniform weight as possible. One sub-group received the basal ration alone. The second sub-group received the basal ration plus 0.1 per cent thiouracil. The other sub-groups received the basal ration plus 0.1 per cent thiouracil and d, l-thyroxine administered subcutaneously in increasing amounts. The solution of thyroxine was prepared so that 0.1 ml. contained the daily dosage.

The method of plotting the data obtained on thyroid weights in the different sub-groups is illustrated in Fig. 7, which is the data obtained with White Leghorn cockerels 4 weeks of age when killed.

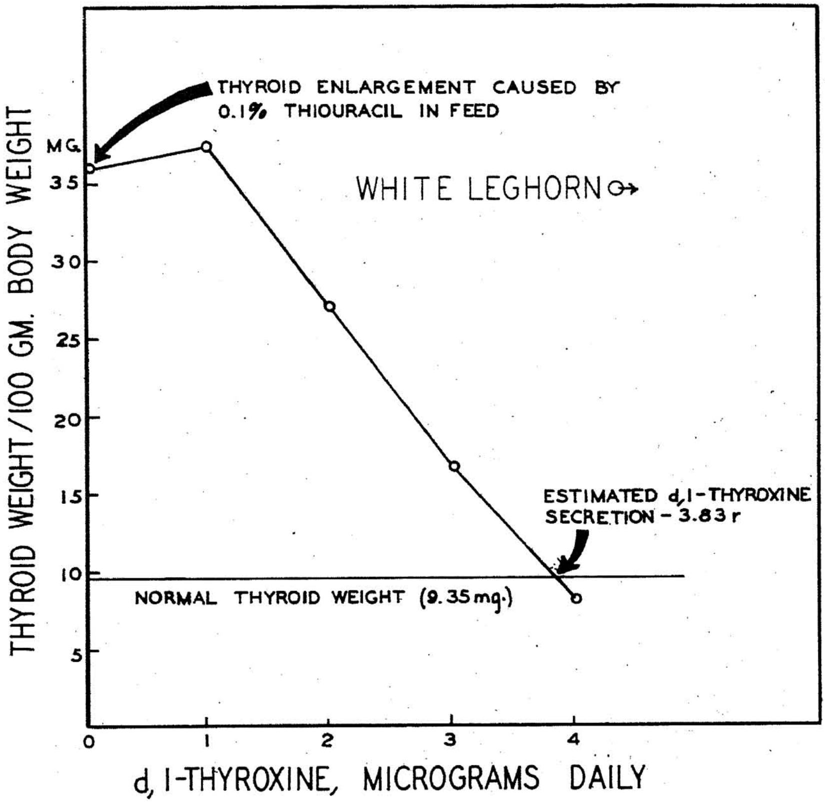


Fig. 7,—Method of plotting data to determine thyroxine secretion rate of the fowl. (Data for 4-week-old White Leghorn cockerels.)

Crystalline d, l-thyroxine was used in these experiments and all determinations are expressed in terms of micrograms of d, l-thyroxine. Since the thyroxine produced by the thyroid is in the l-form, and this form is twice as active physiologically as the d, l-form (Reineke and Turner, 1945a) all determinations should be halved to obtain the equivalent amount produced by the thyroid.

TABLE XI. THYROXINE SECRETION RATE OF GROWING WHITE LEGHORN COCKERELS

Sub-group treatment	Group I - 2 weeks of age				Group II. - 4 weeks of age				Group III - 6 weeks of age			
	No. of chicks	Body wt. gm.	Average thyroid weight mgs.	Thyroid wt. /100 gm. Body wt. mg.	No. of chicks	Body wt. gm.	Average thyroid weight mgs.	Thyroid wt. /100 gm. body wt. mg.	No. of chicks	Body wt. gm.	Average thyroid weight mgs.	Thyroid wt. /100 gm. body wt. mg.
Normal controls no thiouracil no thyroxine	30	72.7	7.8	10.7	10	163.6	15.3	9.35	14	346.0	33.7	9.7
0.1% thiouracil* no thyroxine	19	62.7	22.3	35.6	10	138.0	49.6	35.9	10	352.2	172.7	49.1
0.1% thiouracil* + 1γ d,1-thyroxine	27	73.7	17.0	23.1	10	157.0	58.8	37.4				
0.1% thiouracil* + 2γ d,1-thyroxine	21	72.6	7.4	10.2	13	141.6	38.5	27.2				
0.1% thiouracil* + 3γ d,1-thyroxine	19	72.4	4.6	6.4	12	149.0	25.0	16.7	15	346.0	111.7	32.2
0.1% thiouracil* + 4γ d,1-thyroxine	17	67.0	3.9	5.8	15	144.4	11.7	8.1	12	352.1	145.5	41.3
0.1% thiouracil* + 5γ d,1-thyroxine									15	316.6	63.5	20.1
0.1% thiouracil* + 6γ d,1-thyroxine									13	354.3	74.4	21.0

*thiouracil administered in feed

Group I: Ave. body weight = 70.7 gm. d,1-thyroxine per chick = 1.94 γ daily

d,1-thyroxine /100 gm. chick = 2.76 γ daily

Group II: Ave. body weight = 148.3 gm. d,1-thyroxine per chick = 3.83 γ daily

d,1-thyroxine /100 gm. chick = 258γ daily

Group III: Ave. body weight = 343.4 gm. d,1-thyroine per chick = 7.55 γ daily

d,1-thyroxine /100 gm. chick = 2.20 γ daily

TABLE XI. Cont'd. THYROXINE SECRETION RATE OF GROWING WHITE LEGHORN COCKERELS

Sub-group treatment	Group IV - 7 weeks of age				Group V - 9 weeks of age				Group VI - 12 weeks of age			
	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.
		gm.	mgs.	mg.		gm.	mgs.	mg.		gm.	mgs.	mg.
Normal controls no thiouracil no thyroxine	12	467.3	32.1	6.8	9	643.0	51.1	7.9	9	1072.0	64.7	6.0
0.1% thiouracil* no thyroxine	12	463.9	128.8	27.7	10	682.0	165.7	24.3	9	1009.0	204.0	20.2
0.1% thiouracil* + 4 γd,1-thyroxine	12	448.4	109.1	24.3	9	662.0	150.9	22.8				
0.1% thiouracil* + 8 γd,1-thyroxine	11	489.8	80.8	16.4	10	688.0	108.8	15.8				
0.1% thiouracil* + 10 γ d,1-thyroxine									10	1000.5	134.6	13.5
0.1% thiouracil* + 12 γ d,1-thyroxine	12	470.6	23.8	5.1	10	660.0	79.7	12.1				
0.1% thiouracil* + 15 γd,1-thyroxine									10	1016.0	69.7	6.8
0.1% thiouracil* + 16 γd,1-thyroxine	12	478.7	22.7	4.7	10	643.0	34.0	5.3				
0.1% thiouracil* + 20 γd,1-thyroxine									9	969.0	44.4	4.6
0.1% thiouracil* + 25γ d,1-thyroxine									9	995.2	35.4	3.6
	Ave. body weight = 469.7 gm. d,1-thyroxine per chick = 11.35γ daily				Ave. body weight = 663.0 gm. d,1-thyroxine per chick = 14.4 γ daily				Ave. body weight = 1008.0 gm. d,1-thyroxine per chick = 16.5γ daily			
*thiouracil administered in feed	d,1-thyroxine /100 gm. body weight chick = 2.42γdaily				d,1-thyroxine /100 gm. body weight chick = 2.17γdaily				d,1-thyroxine /100 gm. body weight chick = 1.64γdaily			

TABLE XI. Cont'd. THYROXINE SECRETION RATE OF GROWING WHITE LEGHORN COCKERELS

Sub-group treatment	Group VII - 20 weeks of age				Group VIII - 24 weeks of age				Group IX - 27 weeks of age			
	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.	No. of chicks	Body wt.	Average thyroid weight	Thyroid wt. /100 gm. body wt.
		gm.	mgs.	mg.		gm.	mgs.	mg.		gm.	mgs.	mg.
Normal controls	7	1266	75.11	5.9	10	1444	100.3	6.9	12	1563	134.8	8.6
0.1% thiouracil alone	8	1192	209.5	19.0	10	1371	236.5	17.3	7	1517	250.5	16.5
0.1% thiouracil* + 15 γ thyroxine	8	1127	66.6	5.9	9	1405	153.3	10.9				
0.1% thiouracil* + 20 γ thyroxine	7	1177	70.9	6.0	10	1421	116.2	8.2	8	1529	182.5	11.9
0.1% thiouracil* + 25 γ thyroxine					9	1493	102.4	6.9	7	1532	130.8	8.5
0.1% thiouracil* + 30 γ thyroxine	10	1206	43.5	3.6					9	1524	111.7	7.3

*thiouracil in feed

Group VII:
Ave. body weight = 1193 gm.
Thyroxine secretion rate per chick = 20.0 γ daily

Thyroxine secretion /100 gm. body wt. = 1.68 γ daily

Group VIII:
Ave. body weight = 1426 gm.
Thyroxine secretion rate per chick = 23.5 γ daily

Thyroxine secretion /100 gm. body wt. = 1.65 γ daily

Group IX:
Ave. body weight = 1536
Thyroxine secretion rate per chick = 25 γ daily

Thyroxine secretion /100 gm. body wt. = 1.63 γ daily

White Leghorn cockerels. The rate of thyroxine secretion by the thyroid of White Leghorn cockerels of increasing age groups from 2 to 27 weeks of age was determined.

As would be expected, the average daily thyroxine secretion per chick increased with increasing age and body weight (Table XI). At the end of 2 weeks when the chicks weighed 70.7 grams, 1.95 micrograms of d, l-thyroxine were required to restore the thyroids to their normal weight. At 4 weeks, when the chicks weighed 148.3 grams, the thyroxine requirement was 3.83 micrograms daily. The amount of thyroxine needed to return the thyroids of the thiouracil-treated chicks to a normal weight increased regularly until at 27 weeks and at an average body weight of 1536 grams, 25 micrograms of thyroxine were required, indicating an average daily secretion of an equivalent amount of hormone. It was apparent from an examination of the data on the individual chicks that within the same age group under each dosage of thyroxine, the larger chicks possessed larger thyroids than the smaller chicks, indicating that at any given age the larger chicks were secreting more thyroxine than the smaller. Whether the greater body weight was due to the more

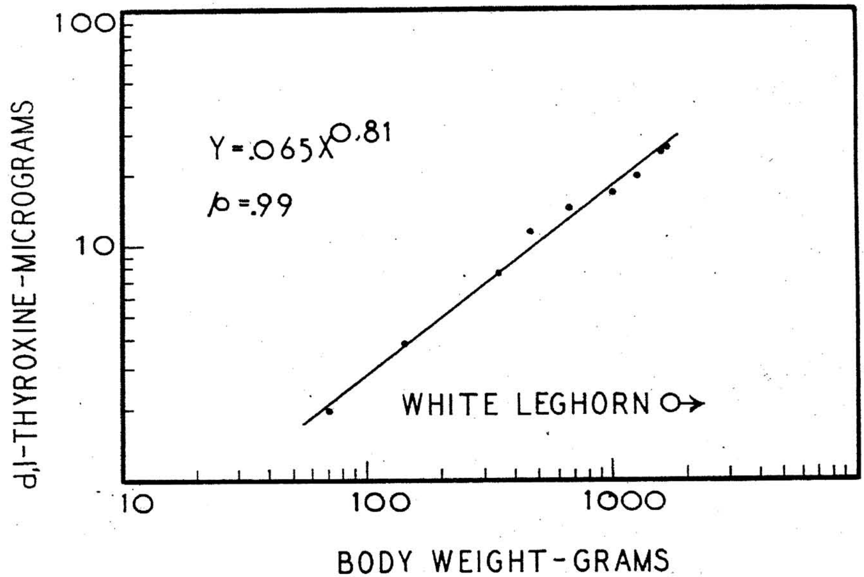


Fig. 8.—Relation of thyroxine secretion rate to body weight of White Leghorn cockerels.

active thyroids of such chicks or the greater thyroxine production was due to their greater size per se is an interesting problem requiring further study.

In the previous section a study was presented indicating the relationship between increasing body weight with age and thyroid weight. It seemed of interest to determine similarly the relation between increase in body weight with age and thyroxine secretion. The equation representing this relationship is Y (d, l-thyroxine) $= 0.065X^{0.81}$ (body weight, with increasing age), covering the period from 2 to 27 weeks of age (Fig. 8). It should be understood that this equation applies only to the environmental conditions described. The exponent 0.81 of the equation indicates that the rate of secretion of thyroxine increases less rapidly, proportionally, than body weight increases, but approximately at the same rate as the thyroid gland weight. It should be noted that the last three groups of Leghorns on which determinations were made were relatively underweight and since the thyroid activity is related to body weight and nutritional condition of the animal, the later three determinations are probably low for the age of the animals and may possibly be somewhat low for the body weight represented.

White Plymouth Rock chickens. To determine the thyroid activity in a breed of chickens differing in mature size, rate of growth, body conformation, temperament and possible laying ability compared to the White Leghorn breed, thyroxine secretion rate determinations were made with the White Plymouth Rock breed varying in age from 5 to 26 weeks. Determinations were made on both sexes of this breed to obtain a sex comparison. It was thought that in breeds varying in constitution as much as these two breeds the difference in their rates of thyroxine secretion would be indicative of the role that the thyroid plays in influencing breed characteristics. The amount of d, l-thyroxine required to cause the thyroid weights of thiouracil-fed chickens of both sexes to return to the thyroid weight of control chicks increased with increasing age and body weight of the chick (Table XII). The females showed a somewhat higher rate of thyroid activity per chick than did the males up until 11 weeks of age. At 13 weeks of age the thyroid secretion rate in the males was somewhat higher than in the females and this difference became greater with increasing age up to 26 weeks. At 26 weeks of age the thyroid secretion rate for males was 35 γ per chick daily, while that for females was 29 γ per chick daily. Since the males increased in body weight much faster with increasing age than did the females, the secretion rate per 100 grams body weight was always higher for the females than for the males. However, at 26 weeks of age both sexes tend to attain similar secretion rates on a body weight basis, the males secreting the equivalent of 1.44 γ d, l-thyroxine per 100 grams body weight and the females 1.50 γ d, l-thyroxine per 100 grams body weight.

The average relationship of thyroid activity in terms of d, l-thyroxine to increasing body weight may be expressed by the same equation used in the study of the similar relationship in White Leghorn cockerels to body weight. The equation representing the relationship for the males is $Y = 0.053X^{0.80}$ (Fig. 9). In the equation, Y equals thyroid secretion rate expressed in micrograms of d, l-thyroxine, whereas X equals body weight in grams. The relationship determined for White Leghorn cockerels was $Y = 0.55X^{0.81}$. A com-

TABLE XII. THYROXINE SECRETION RATE OF GROWING WHITE PLYMOUTH ROCK CHICKENS

Group I 2-5 weeks of age

Treatment Sub-group	MALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.	FEMALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.
Normal controls	9	238	9.76	4.10	18	245	10.93	4.46
0.1% thiouracil alone	9	222	68.00	30.63	15	223	67.1	30.08
0.1% thiouracil 3 γ thyroxine	14	235	35.80	15.23	13	228	43.1	18.90
0.1% thiouracil 4 γ thyroxine	8	208	14.37	6.91	15	244	20.4	8.36

Males:
Ave. body weight = 277 gm.
d,1-thyroxine per chick daily = 4.52 γ
d,1-thyroxine /100 gm. body wt. = 1.98 γ

Females:
Ave. body weight = 236 gm.
d,1-thyroxine per chick daily = 4.75 γ
d,1-thyroxine /100 gm. body wt. = 2.01 γ

Group II 4-7 weeks of age

Treatment Sub-group	MALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.	FEMALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.
Normal controls	9	379	18.95	5.0	10	309	19.52	6.3
0.1% thiouracil alone	10	388	94.9	24.4	10	334	82.40	24.7
0.1% thiouracil 4 γ thyroxine	10	427	96.61	22.6	8	412	93.0	22.6
0.1% thiouracil 6 γ thyroxine	12	470	67.15	14.3	5	400	65.46	16.4
0.1% thiouracil 8 γ thyroxine	12	378	19.20	5.1	8	380	33.66	8.9

Males:
Ave. body weight = 410 gm.
d,1-thyroxine per chick daily = 8.1 γ
d,1-thyroxine /100 gm. body wt. = 1.98 γ

Females:
Ave. body weight = 360 gm.
d,1-thyroxine per chick daily = 8.75 γ
d,1-thyroxine /100 gm. body wt. = 2.43 γ

Group III 6-9 weeks of age

Treatment Sub-group	MALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.	FEMALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.
Normal controls	9	573	42.25	7.4	11	529	39.5	7.5
0.1% thiouracil alone	9	603	200.4	33.2	8	599	269.0	44.9
0.1% thiouracil 6 γ thyroxine	9	664	124.2	18.7	10	597	234.2	39.2
0.1% thiouracil 10 γ thyroxine	11	638	116.7	18.2	7	591	102.4	17.3
0.1% thiouracil 14 γ thyroxine	8	549	20.9	3.8	10	533	38.0	7.1

Males:
Ave. body wieght = 608 gm.
d,1-thyroxine per chick daily = 13.0 γ
d,1-thyroxine /100 gm. body wt. = 2.14 γ

Females:
Ave. body weight = 566 gm.
d,1-thyroxine per chick daily = 13.7 γ
d,1-thyroxine /100 gm. body wt. = 2.42 γ

Group IV 8-11 weeks of age

Treatment Sub-group	MALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.	FEMALES No. of chicks	Body wt. gm.	Average thyroid weight mg.	Thyroid wt. /100 gms. body wt. mgs.
Normal controls	7	806	35.6	4.4	10	824	50.86	6.2
0.1% thiouracil alone	5	941	187.6	19.9	12	760	205.5	27.0
0.1% thiouracil 12.5 γ thyroxine	11	775	41.9	5.4	8	717	59.5	8.3
0.1% thiouracil 17.5 γ thyroxine	12	869	15.6	1.8	7	800	20.7	2.6

Males:
Ave. body weight = 837 gm.
d,1-thyroxine per chick daily = 13.0 γ
d,1-thyroxine /100 gm. body wt. = 1.55 γ

Females:
Ave. body weight = 776 gm.
d,1-thyroxine per chick daily = 14.3 γ
d,1-thyroxine /100 gm. body wt. = 1.84 γ

TABLE XII. Cont'd. THYROXINE SECRETION RATE OF GROWING WHITE PLYMOUTH ROCK CHICKENS

Treatment sub-group	MALES No. of chicks	MALES Body wt. gm.	MALES Average thyroid weight mg.	MALES Thyroid wt. /100 gms. body wt. mgs.	Treatment	FEMALES No. of chicks	FEMALES Body wt. gm.	FEMALES Average thyroid weight mg.	FEMALES Thyroid wt. /100 gms. body wt. mgs.
Group V 10-13 weeks of age									
Normal controls	6	1072	44.5	4.2		12	977	47.0	4.8
0.1% thiouracil	8	1154	216.5	18.8		7	949	195.0	20.5
0.1% thiouracil 12.5γ thyroxine	13	1147	121.6	10.6		5	964	154.3	16.0
0.1% thiouracil 17.5γ thyroxine	8	1270	92.4	7.3		10	994	58.4	5.9

Males: Ave. body weight = 1164 gm.
d,1-thyroxine per chick daily = 18.8 γ
d,1-thyroxine /100 gm. body wt. = 1.62γ

Females: Ave. body weight = 974 gm.
d,1-thyroxine per chick daily = 18.0γ
d,1-thyroxine /100 gm. body wt. 1.84γ

Treatment sub-group	MALES No. of chicks	MALES Body wt. gm.	MALES Average thyroid weight mg.	MALES Thyroid wt. /100 gms. body wt. mgs.	Treatment	FEMALES No. of chicks	FEMALES Body wt. gm.	FEMALES Average thyroid weight mg.	FEMALES Thyroid wt. /100 gms. body wt. mgs.
Group VI 15-18 weeks of age									
Normal controls	5	1456	60.8	4.2		15	1345	81.5	6.1
0.1% thiouracil	7	1540	249.8	16.2		12	1333	262.8	19.7
0.1% thiouracil 15γ thyroxine	7	1505	128.6	8.5		13	1386	151.4	10.9
0.1% thiouracil 20γ thyroxine	8	1468	85.0	5.8		12.	1355	88.9	6.6
0.1% thiouracil 30γ thyroxine	9	1526	45.5	3.0		11	1382	48.2	3.5

Males: Ave. body weight = 1502 gm.
d,1-thyroxine per chick daily = 23.0γ
d,1-thyroxine /100 gm. body wt. = 1.53γ

Females: Ave. body weight = 1360 gm.
d,1-thyroxine per chick daily = 21.5γ
d,1-thyroxine /100 gm. body wt. = 1.58γ

Treatment sub-group	MALES No. of chicks	MALES Body wt. gm.	MALES Average thyroid weight mg.	MALES Thyroid wt. /100 gms. body wt. mgs.	Treatment	FEMALES No. of chicks	FEMALES Body wt. gm.	FEMALES Average thyroid weight mg.	FEMALES Thyroid wt. /100 gms. body wt. mgs.
Group VII 19-22 weeks of age									
Normal controls	7	2173	125.4	5.8		15	1750	119.8	6.8
0.1% thiouracil alone	6	2094	301.4	14.4		12	1677	286.4	17.1
0.1% thiouracil 17.5γ thyroxine	4	2260	226.4	10.0	14.5γ thyroxine	15	1598	162.8	10.2
0.1% thiouracil 25γ thyroxine	6	1858	159.4	8.6	21γ thyroxine	14	1583	158.4	10.0
0.1% thiouracil 29γ thyroxine	5	1996	121.9	6.1	27.2 γ thyroxine	12	1567	95.0	6.1

Males: Ave. body weight = 2069 gm.
d,1-thyroxine per chick daily = 29.5γ
d,1-thyroxine /100 gm. body wt. = 1.43γ

Females: Ave. body weight = 1637 gm.
d,1-thyroxine per chick daily = 26.0γ
d,1-thyroxine /100 gm. body wt. = 1.59γ

Treatment sub-group	MALES No. of chicks	MALES Body wt. gm.	MALES Average thyroid weight mg.	MALES Thyroid wt. /100 gms. body wt. mgs.	Treatment	FEMALES No. of chicks	FEMALES Body wt. gm.	FEMALES Average thyroid weight mg.	FEMALES Thyroid wt. /100 gms. body wt. mgs.
Group VIII 23-26 weeks of age									
Normal controls	6	2545	152.0	6.0		14	1851	105.2	5.9
0.1% thiouracil	6	2523	308	12.2		10	2073	298.6	14.4
0.1% thiouracil 25.5γ thyroxine	6	2305	168	7.3	22.5γ thyroxine	14	1909	134.0	7.0
0.1% thiouracil 30γ thyroxine	8	2357	253.4	10.7	25γ thyroxine	7	1910	130.0	6.8
0.1% thiouracil 37γ thyroxine	6	2524	163.6	6.5	29γ thyroxine	14	1941	117.1	6.0

Males: Ave. body weight = 2427 gm.
d,1-thyroxine per chick daily = 35 γ
d,1-thyroxine /100 gm. body wt. = 1.44γ

Females: Ave. body weight = 1931 gm.
d,1-thyroxine per chick daily = 29γ
d,1-thyroxine per 100 gm. body wt. = 1.50γ

parison of the average secretion rate of the thyroid of the White Leghorn male with that of the White Plymouth Rock male indicates that the rate of the White Leghorn is higher than that of the White Plymouth Rock on a body weight basis (Fig. 10). There is an average difference of 10 per cent in the thyroxine secretion rate of the two breeds of chickens. A greater number of determinations is necessary before the difference can be shown to be statistically significant.

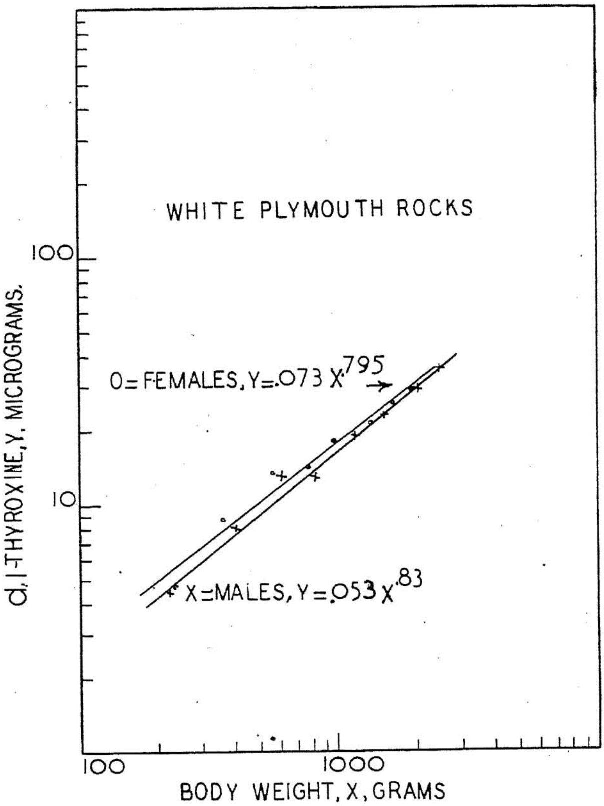


Fig. 9.—Relation of thyroxine secretion rate to body weight of White Plymouth Rock chickens of both sexes.

It should be recalled that the determinations for the White Leghorns were made from March to June and those on the White Plymouth Rocks from June to November, with, however, environmental conditions controlled as previously outlined.

On an age basis, the White Leghorn's thyroid secretion rate is higher when the growth rate of the two breeds is approximately the same. When the growth rate of the White Leghorn falls below that of the White Plymouth Rock, which occurs after about the 10th to 12th week, then the thyroxine secretion rate of the two breeds on an age basis tends to become the same. At an older age when the growth rate of the White Leghorn is definitely below that of the

White Plymouth Rock, the White Leghorn's actual secretion rate, on an age basis, is lower than that of the White Plymouth Rock.

Essentially this same relationship holds when the two sexes of the White Plymouth Rock are compared as to thyroxine secretion rate. In the young chicks, the female which is growing at about the same rate as the male shows a somewhat higher secretion rate. However, when the growth rate of the female becomes definitely slower than in the male, the secretion rate, on an age basis, is also definitely lower. Whether the degree of thyroxine secretion rate is a causative factor in growth rate cannot be determined from this study.

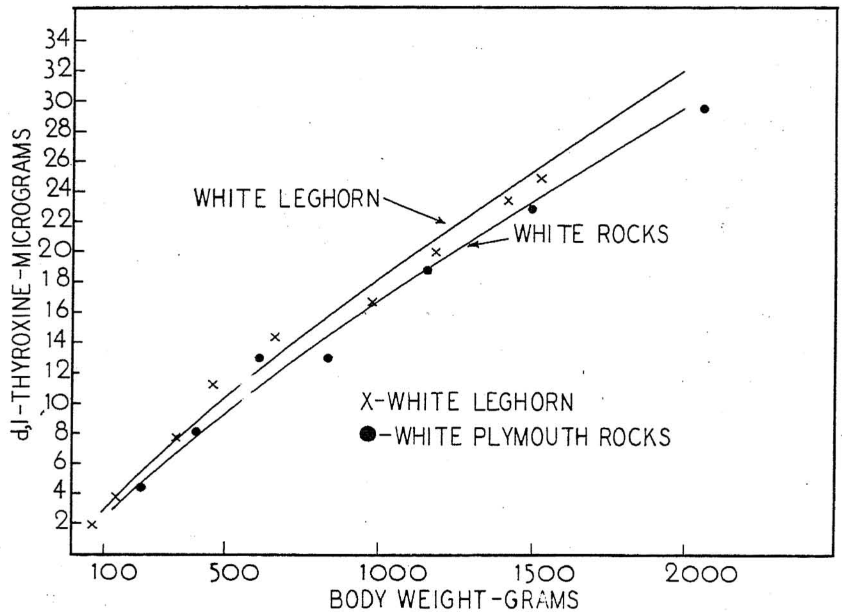


Fig. 10.—Comparison of the thyroxine secretion rates of the White Leghorn and White Plymouth Rock cockerels.

It would be of interest to compare the thyroid secretion rate of fast growing chicks with those of slower growing chicks of the same age, breed and sex. However, these determinations were made with groups of chicks and the secretion rate of each individual was not determined. From the rather wide variation of thyroid response between individuals within the same age group, on the same dosage of thyroxine, it is apparent that there is considerable variation in the thyroxine secretion rate between individuals.

When thyroid responses of the same age group are compared on a basis of return to control thyroid size with increasing dosage of thyroxine, some individual's thyroids returned to control size on a much lower thyroxine dosage than other individuals. When compared on this basis, the chicks having the higher rate of thyroxine secretion appeared to be approximately 30 per cent above that of the average of the group and there were chicks having an apparent 25 per cent lower thyroxine secretion than the average of the group. Usually the larger chicks had the higher rate and the smaller ones the lower rate.

No tendency toward an increase in the rate of thyroid secretion is apparent in the pullets beginning to lay in the 26 week old group of the White Plymouth Rock breed.

Growth rate and thyroxine secretion rate increases follow parallel trends up until the age of 12 or 13 weeks in the White Plymouth Rock male and this same parallelism of trend is present in the White

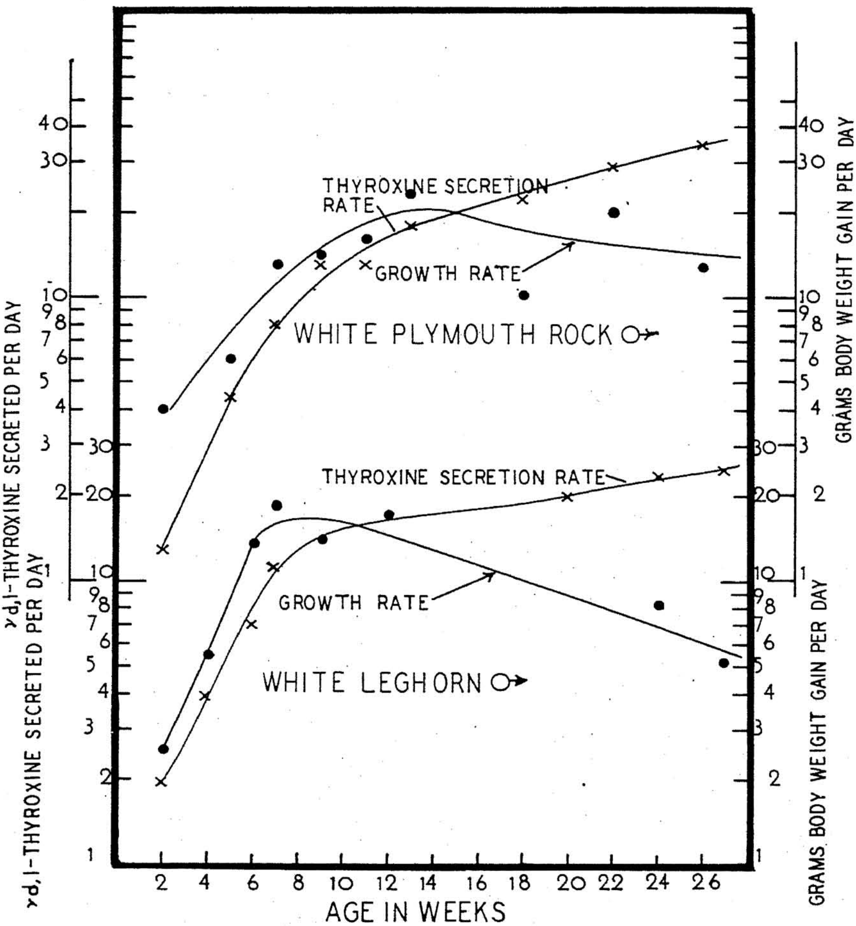


Fig. 11.—Comparison of the proportionate growth rates and thyroxine secretion rates of White Leghorn and White Plymouth Rock cockerels.

Leghorn male up until the age of 7 or 8 weeks (Fig. 11). After these ages the proportionate growth rate falls off rapidly while the thyroxine secretion rate continues to increase but not as fast as in the young chicks.

It is well known that the onset of sexuality in the White Leghorn male occurs at an earlier age than in White Plymouth Rock males. Growth rate may be adversely affected by sexual maturity.

On a body weight basis covering the period studied in comparing the thyroxine secretion rate of males and females of the White Plymouth Rock breed, it appears that the thyroid of the female is more active than that of the male, secreting at a higher rate per 100 grams body weight. A sex difference in thyroid function has been indicated by many studies on size, histology and iodine content of the thyroids in the two sexes. Riddle (1929) found that the thyroid of the female common pigeon was larger than that of the male. Marza and Blinov (1936) noted a higher secretory activity in the female pigeon compared to the male according to histological examination.

Effect of castration. The effects of castration on thyroid activity are somewhat controversial. Chouke, Friedman and Loeb (1930) observed no significant difference in the mitotic activity of the thyroids of castrated and non-castrated guinea pigs. Zalesky (1935), working with the 13-lined ground squirrel, observed that castration had no effect on the thyroid either grossly or microscopically. A decreased rate of metabolism has been observed after castration in the rat (Sherwood, Savage and Hall, 1933). In the fowl metabolism was decreased up to a maximum of 13.5 per cent and the effect extended at least over several months (Mitchell, Card and Haines, 1927).

Castration lowered the thyrotrophic hormone content of the pituitary in cattle (Bates et al., 1935; Reese and Turner, 1937) and in rats (Turner and Cupps, 1940).

To determine the effect of castration in the male fowl, two groups of Barred Rock males were assayed for the thyroxine secretion rate of their thyroids, one group being castrated for a period of 8 weeks, the second group being normal cockerels. Both groups were of the same age and the treatment was the same. The chickens were 15 weeks of age when killed. Results obtained on thyroid weights with various treatments involved in the assay are shown in Table XIII. The variability of thyroid weights in each sub-group is expressed by the ± values, this being the calculated standard error of the mean (Snedecor, 1940). The rate of thyroxine secretion of the castrated males of this breed was 25 γ d, l-thyroxine.

The normal cockerel's thyroid secreted 30 γ d, l-thyroxine per day. Considering the values of the standard error of the mean for the thyroid weights of the sub-groups near thyroid size of the controls, it is probable that this difference in secretion rate is significant and that castration lowers the thyroxine secretion rate. In this experiment the thyroxine secretion rate was lowered about 16 per cent. The difference cannot be assigned to differences in body weight as the body weights were very nearly the same.

TABLE XIII. THYROXINE SECRETION RATE OF BARRED PLYMOUTH ROCK COCKERELS AND CAPONS*

	Cockerels - 15 weeks of age				Capons - 15 weeks of age			
Treatment Sub-group	No. of chicks	Body weight	Thyroid weight	Thyroid wt. /100 gms. body weight	No. of chicks	Body weight	Thyroid weight	Thyroid wt. /100 gms. body weight
		gm.	mgm.	mgm.		gm.	mgm.	mgm.
Controls	11	1446	103.2	7.1 ± 0.6	8	1458	68.6	4.7 ± 0.80
0.1% thiouracil alone	9	1587	391.4	24.7 ± 3.3	5	1513	675.0	44.6 ± 10.80
0.1% thiouracil 10γ d,1-thyroxine	9	1463	280.5	19.2 ± 2.0	7	1503	408.2	27.2 ± 5.40
0.1% thiouracil 16γ d,1-thyroxine	10	1491	219.0	14.7 ± 1.6	14	1432	170.4	11.9 ± 1.20
0.1% thiouracil 22γ d,1-thyroxine	7	1400	153.0	10.9 ± 1.2	13	1402	88.4	6.3 ± 0.60
0.1% thiouracil 28γ d,1-thyroxine	9	1470	128.0	8.7 ± 0.7	8	1552	67.3	4.3 ± 0.53

Ave. body weight = 1478 gms.
d,1-thyroxine per chick per day = 30 γ
d,1-thyroxine/100 gms. body wt. = 2.03 γ

Ave. body weight + 1463 gms.
d,1-thyroxine per chick per day = 25 γ
d,1-thyroxine/100 gms. body wt. = 1.69 γ

*Caponized at 6-7 weeks of age

It can be observed from Table XIII that the thyroid weight of the castrated males was considerably less than for normal cockerels. This is in agreement with the data by Juhn and Mitchell (1929) on castrated Brown Leghorns. The castrated male attained much greater thyroid hypertrophy with thiouracil treatment than did the normal cockerel. This is surprising in view of the findings that the thyrotrophic hormone content of the pituitary decreases with castration. Also surprising is the fact that increased hypertrophy occurs with thiouracil feeding but the thyroid weight of the untreated castrate is lower than for the normal cockerels. This indicates a situation where increased thyrotrophin has little effect in stimulating thyroid activity which is contrary to the normal situation generally believed to exist.

Effect of estrogens. Sex hormones have an apparent effect on thyroid function. Sherwood, Wilson and Boneta (1937) observed that ovariectomized rats given theelin had a decreased B.M.R. Sherwood (1938) believed this effect was a direct effect on the tissues. Farbman (1944) reported that the administration of large doses of estrogen to humans seemed to have an inhibitory effect on the thyroid. Emmens (1938) showed that the fowl thyroid is depressed by estrogen. Kunde et al. (1930) reported that estrogen administration resulted in marked hyperplasia of the thyroid in the dog. It has been shown that rapid fattening is produced in male chickens by the administration of estrogens (Zondek and Marx, 1939; Lorenz, 1943). Since the lowering of thyroid activity in fowls by feeding of thiouracil for 2 to 4 weeks resulted in more rapid fattening than normal (Kempster and Turner, 1945), it was thought possible that estrogens may affect the thyroid thus resulting indirectly in improved fat deposition.

Other experimental work has shown that the dimethyl ether of diethylstilbestrol is one of the most potent orally administered estrogens in the fowl insofar as oviduct stimulation is concerned (Jaap and Thayer, 1944). It was, therefore, decided to use this compound to determine the effect of estrogens on thyroid activity in the fowl. To determine the level of dimethyl ether of diethylstilbestrol in the feed that would induce a significant effect on the thyroid, if there was such an effect, varied levels of the compound in the feed were administered to chicks also receiving 0.1 per cent thiouracil in the feed. Levels of 0.01, 0.02 and 0.04 per cent of dimethyl ether of diethylstilbestrol were fed to White Plymouth Rock chicks from hatching time to 5 weeks of age. During the last 3 weeks 0.1 per cent thiouracil was added to the ration. A control group receiving no dimethyl ether of diethylstilbestrol but 0.1 per cent thiouracil was included in the experiment.

The results obtained showed that when fed at a 0.01 per cent level in the ration for 5 weeks dimethyl ether of diethylstilbestrol in conjunction with 0.1 per cent thiouracil in the diet for 3 weeks induced greater thyroid enlargement than did 0.1 per cent thiouracil alone in both males and females. The increased enlargement was approximately 1.5 times that obtained with 0.1 per cent thiouracil alone. Levels of 0.02 and 0.04 per cent had no effect on thyroid enlargement over that induced by 0.1 per cent thiouracil (Table X(c)). These observations indicate that the release of thyrotrophin of the anterior pituitary is stimulated by administration of estrogens at the proper level.

Since the feeding of dimethyl ether of diethylstilbestrol at a 0.01 per cent level in the feed appeared to increase the goitrogenic effect of thiouracil in chicks, it was decided to use this level in determining the effect of estrogen on thyroid activity. This dosage was fed to one group of Barred Plymouth Rock pullets for a period of 8 weeks. This group was assayed for their thyroxine secretion rate, the assay ending when the pullets were 15 weeks of age. A second group of Barred Rock pullets of the same age but receiving no stilbestrol were assayed for their thyroxine secretion rate during the same period. The results are shown in Table XIV. The thyroxine secretion rate of the pullets fed a ration without stilbestrol showed a thyroxine secretion rate of 23 γ per day. The pullets fed 0.01 per cent stilbestrol in the ration showed a secretion rate of 21.3 γ thyroxine. When the variability within the two groups is considered, this difference of 1.7 γ is probably not significant since the difference between thyroid weights of the sub-groups receiving 0.1 per cent thiouracil plus 22 γ thyroxine is not significantly different.

TABLE XIV. COMPARISON OF THYROXINE SECRETION RATE OF NORMAL AND DIETHYLSTIBESTROL* FED BARRED ROCK PULLETS (15 weeks of age)

Treatment sub-group	Normal pullets: No. of chicks	Body weight	Thyroid weight	Thyroid weight /100 gm. body weight	Diethylstibestrol-fed* pullets (0.01% in ration): No. of chicks	Body weight	Thyroid weight	Thyroid wt. /100 gm. body wt.
		gm.	mgm.	mgm.		gm.	mgm.	mgm.
Normal controls	23	1233	85.5	6.9	10	1316	86.9	6.6
0.1% thiouracil alone	17	1245	404.8	32.5	10	1305	343.4	26.3
0.1% thiouracil 10γ thyroxine	9	1329	328.5	24.7	11	1127	206.6	18.3
0.1% thiouracil 16γ thyroxine	22	1270	168.9	13.3	9	1258	122.9	9.8
0.1% thiouracil 22γ thyroxine	18	1237	96.1	7.8	7	1068	64.5	6.2

Ave. body weight = 1255 gm.
d,1-thyroxine per chick per day = 23γ
d,1-thyroxine/100 gm. body wt. = 1.83γ

Ave. body weight = 1221 gm.
d,1-thyroxine per chick per day = 21.3γ
d,1-thyroxine/100 gm. body wt. = 1.74γ

*dimethyl ether of diethylstibestrol fed 8 weeks prior to end of experiment.

Although the 0.01 per cent level of feeding dimethyl ether of diethylstilbestrol to 5 week old chicks increased the goitrogenic effect of thiouracil on the thyroid and stimulated oviduct growth, this level in 15 week old chicks had no such effect on either the thyrotrophin or thyroxine secretion rates. Undoubtedly the optimum dosage level for thyroid response is different at 15 weeks of age.

Effect of thyroprotein on goitrous thyroids induced by thiouracil. Since thyroid enlargement due to thiouracil treatment can be brought back to control size with thyroxine administration, it was thought that it would be of interest to determine the amount of thyroprotein (thyroidally active material) that is required to bring thiouracil-induced thyroid enlargement back to control size. Therefore experiments were carried out in which 0.1 per cent thiouracil alone was included in the ration of one sub-group. Another sub-group received the basal ration only. Other sub-groups received 0.1 per cent thiouracil and increasing percentages of thyroprotein in the ration. Groups of White Plymouth Rocks of both sexes killed at 8, 11 and 13 weeks of age showed that 0.009 per cent of thyroprotein in the feed brought about control thyroid weight. The same percentage in the ration had the same effect on the thyroid weights of 27 week old White Leghorn males. Thus the amount of food eaten with increasing age and body weight appeared to increase so that the same percentage level of thyroprotein in the feed in conjunction with thiouracil brought about thyroid size comparable to that of the controls in all ages of chickens studied.

The thyroprotein used in this study contained 2.7 per cent thyroxine as determined by a modification of the Blau (1935) method for thyroxine determination of desiccated thyroid powder (Reineke et al., unpublished).

The Effect of Thyroprotein with Thiouracil and Thiourea on Body Weight of the White Leghorn.—The growth rate of mice has been increased significantly by administration of an optimum level of thyroprotein feeding (Koger & Turner, 1943). The feeding of various levels of thyroprotein to growing chickens up to 12 weeks of age has been reported by Parker (1943) and Irwin, Reineke and Turner (1943). It was therefore thought desirable to determine the effect of varying levels of thyroprotein on the growth of chicks whose thyroid secretion had been inhibited by thiouracil. Since previous studies showed that growth rate appears to be depressed with the approach of sexual maturity (Fig. 11) all chickens used in this experiment were castrated at 6 to 8 weeks of age. All groups were under similar conditions with respect to basal ration, temperature, age, etc. Previous work has indicated that about .009% thyroprotein maintains a normal thyroid size in chickens of all ages studied that are fed thiouracil. Amounts of thyroprotein fed varied below and above the normal requirement.

One group of White Leghorns of both sexes received the basal ration alone and served as a control group. A second group received 0.1% thiourea alone. A third group received .2% thiouracil alone. A fourth group received 0.1% thiouracil plus 0.005% thyroprotein in the feed. A fifth group received 0.1% thiouracil plus 0.01% thyroprotein. A sixth group received 0.01% thyroprotein plus 0.1% thiouracil until 9 weeks of age, then .015% thyroprotein from 9 weeks to 20 weeks of age. A seventh group received 0.01% thyroprotein plus 0.1% thiouracil until 9 weeks of age, then the thyroprotein level was increased to 0.015% until 15 weeks of age, and increased again to 0.02% from 15 weeks to 20 weeks of age. Individual body weights of all groups were taken every 2 weeks. The body weights of the different groups at two week intervals are recorded in Table XIVa. It is apparent that thiouracil and thiourea depressed the body weight attained in both sexes during the experimental period. However, thiourea was much more effective in depressing growth than the thiouracil. This seems to indicate that thiourea is able to more completely block synthesis of the thyroid hormone than is thiouracil since no toxic effect was apparent in the thiourea fed group.

TABLE XIVa - EFFECT OF THIOUREA, THIOURACIL, AND THYROPROTEIN ON GROWTH

Treatment	Group. 1 Controls		Group. 2 .1% Thiourea		Group. 3 .2% Thiouracil		Group. 4 .005% Thyroprotein .1% Thiouracil		Group. 5 .01% Thyroprotein .1% Thiouracil		Group. 6 .015% Thyroprotein .1% Thiouracil		Group. 7 .02% Thyroprotein .1% Thiouracil	
Sex	M	F	M	F	M	F	M	F	M	F	M	F	M	F
No. of Chicks	4	4	1	7	11	11	18	6	14	7	10	17	5	12
Age in weeks	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
2	82	80	80	80	72	72	77	77	83	83	85	85	85	85
4	150	150	130	120	146	146	163	163	144	144	149	149	149	149
6	295	290	225	220	260	260	279	279	346	346	270	270	270	270
8	445	415	390	400	447	353	447	444	425	418	462	400	447	403
10	612	510	470	490	581	484	630	591	628	610	639*	545*	647*	570*
12	785	620	600	570	769	611	801	720	771	735	807	680	790	659
14	1000	750	700	690	990	745	1035	895	995	911	1077	860	972	837
16	1210	910	800	770	1164	875	1217	1019	1194	1088	1273	1001	1089**	974**
18	1370	1070	805	800	1286	988	1388	1125	1339	1186	1405	1116	1245	1073
20	1450	1170	815	780	1363	1080	1458	1225	1380	1242	1455	1186	1385	1128

All chickens castrated at 6 to 8 weeks of age

* Thyroprotein in feed increased from .01 to .015% at 9 weeks of age.

** Thyroprotein in feed increased from .015 to .020% at 15 weeks of age.

With regard to the groups receiving varying amounts of thyroprotein in their feed compared to the controls there is little or no difference in the body weights attained at 20 weeks of age in either sex. From 12 to 15 weeks of age the feeding of the various levels of thyroprotein appears to result in an increased body weight over the controls. However, below normal requirements (.005%) of thyroprotein shows this tendency as well as above normal amounts of thyroprotein (.01 and .015%). This may be due to the fact that less than normal thyroprotein feeding increased fattening compared to the controls and thus body weight increase, while above normal thyroprotein feeding resulted in greater growth and therefore greater body weight at these ages. Casual inspection of the lot fed 0.005% thyroprotein and 0.1% thiouracil indicated a more compact and finished carcass considering that they were Leghorns.

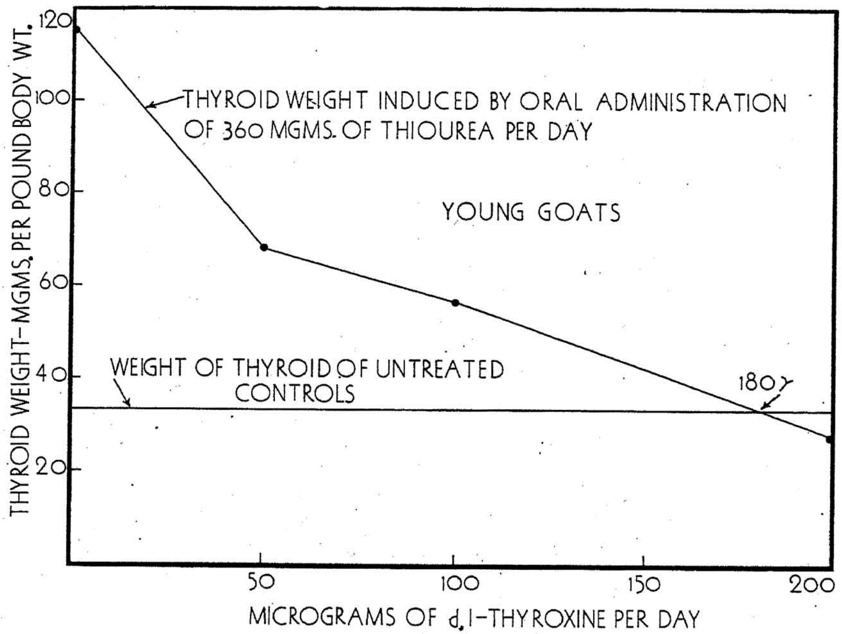


Fig. 12.—Method of plotting thyroid weight data to determine the thyroxine secretion rate in growing goats.

The Determination of the Thyroxine Secretion rate in the Goat.

Growing goats. As our primary interest in the determination of the thyroxine secretion rate was in relation to productive ability of dairy animals, the goat was selected as an experimental animal. The goats used were of mixed breeding of the dairy type. Some were raised in our experimental herd and others were purchased locally.

Determinations of thyroid activity were made on groups of increasing body weight. A group of young goats started on assay at 1 month of age were killed at 2 months, thus the assay period was 30 days in length. The assay period extended over the same period in all age groups. The method of assay was essentially the same as that for the fowl. Certain of the goats of a body weight classification were treated with thiourea or thiouracil for 30 days in amounts described in a preceding section. Other goats received thiourea or thiouracil plus varying dosages of d, l-thyroxine injected subcutaneously. Glands from control goats receiving no treatment but comparable in age and body weight to the other two groups above described were killed and their thyroid weights determined. All of these thyroid weights served as a basis of comparison and were plotted as shown in Fig. 12 to determine the normal thyroxine secretion rate of a group of goats.

Environmental temperature was not controlled except for supplementary heat in the colder weather. Goats included in each thyroxine secretion determination were not all assayed at the same time. Some were assayed in the spring and some in the fall at a time when the temperature range probably fell between 50 and 80° F. Data obtained from goats treated with thiourea and thyroxine in the winter were generally not comparable with the other data and were therefore not included in the determinations.

The values determined for the thyroxine secretion rate in goats of three different body weight classes are shown in Table XV. For the 2 months old goats the thyroxine secretion rate was 180 micrograms d, l-thyroxine daily. This group averaged about 22 pounds in body weight. For the group averaging about 45 pounds body weight the secretion rate was about 640 micrograms d, l-thyroxine per goat daily; for the group averaging 76 pounds body weight the secretion rate was about 930 micrograms d, l-thyroxine per goat daily. Thus in growing goats the thyroid secretion rate increases with increasing body weight (Fig. 13). The increase in thyroxine secretion rate with increase in body weight is rapid in the younger goats and apparently tends to level off as animals reach maturity.

These values of the thyroid secretion rate are not particularly accurate since environmental conditions were not controlled and much variation in body weight and in individuals occurred within a group. Nevertheless they are an indication of the manner in which thyroid activity increases with increasing body weight.

Several animals receiving dosages of thiourea and thyroxine in amounts that caused the thyroid weight to return to normal in other similar animals in warm weather had a much greater thyroid weight than the control when treated in the winter, thus indicating a much higher thyroxine secretion rate in cold weather.

TABLE XV. THYROXINE SECRETION RATE OF GROWING GOATS

Sub-group treatment	No. of goats	Body weight average	Thyroid weight average	Thyroid weight /pound body wt.
Group I (Male and Female) 14-30 lbs. body weight				
		lbs.	mgm.	mgm.
Normal controls	6	21.5	705.0	33.0
Thiourea alone (360 mg/day)	7	22.5	2620.0	116.4
Thiourea (360 mg/day) + d,1-thyroxine (50γ/day)	4	20.8	1368.0	65.7
Thiourea (360 mg/day) + d,1-thyroxine (100γ/day)	2	17.7	1000.5	56.5
Thiourea (360 mg/day) + d,1-thyroxine (200γ/day)	5	23.8	640.8	26.9
Average body weight = 21.8 pounds d,1-thyroxine per goat per day = 180γ				
Group II (Females) 30-60 lbs. body weight				
Normal controls	7	49.3	1219.2	24.7
Thiourea (0.75 to 1.0 gm./day) or Thiouracil (1.0 to 1.5 gm./day)	7	45.4	2078.5	45.8
Thiourea (0.75 to 1.0 gm./day) or Thiouracil (1.0 to 3.0 gm. /day) plus d,1-thyroxine (430 γ /day)	4	41.1	1518.0	37.0
Thiourea (0.75 to 1.0 gm./day) or Thiouracil (1.0 to 3.0 gm. /day) plus d,1-thyroxine (600γ/day)	5	40.4	1094.8	27.0
Average body weight = 45 pounds d,1-thyroxine per goat per day = 640γ				
Group III (Females) 60-90 lbs. body weight				
Normal controls	7	75.0	1734.4	23.1
Thiourea (0.75 to 1.5 gm./day) or Thiouracil (1.0 to 5.0 gm./day)	10	78.6	3625.0	46.1
Thiourea (0.75 to 1.5 gm./day) or Thiouracil (1.0 to 3.0 gm. /day) plus d,1-thyroxine (500γ/day)	2	75.0	4163.0	55.5
Thiourea (0.75 to 1.5 gm./day) or Thiouracil (1.0 to 3.0 gm. /day) plus d,1-thyroxine (700γ/day)	2	77.0	3384.0	43.9
Thiourea (0.75 to 1.5 gm./day) or Thiouracil (1.0 to 3.0 gm. /day) plus d,1-thyroxine (875γ/day)	4	72.0	2038.0	28.3
Average body weight = 76.0 pounds d,1-thyroxine per goat per day = 930γ				

No particular difference could be discerned in thyroid activity in pregnant and non-pregnant animals. If there is any it was overridden by individual variation in these experiments and it would take much more accurately controlled conditions to find any existing difference.

Lactating goats. Determination of the thyroxine secretion rate in lactating animals was made by using the rate of milk production as an index of the amount of thyroid hormone in the blood. As was reported in a preceding section, milk production was depressed from 35 to 45 per cent by oral administration of the proper dosage of thiourea or thiouracil for 2 weeks. The quantity of thyroxine required to bring milk flow back to normal with thiourea or thiouracil treatment was considered the normal thyroxine secretion rate for that animal. Once the dosage of thiourea or thiouracil required to depress milk production, a significant amount was determined, the assay was conducted by feeding the goitrogenic drug in conjunction with enough thyroxine to maintain normal production. This method eliminates the unfavorable influence of the fact that once milk secretion is depressed a greater stimulus is required to bring production back to normal than is required to maintain a normal milk flow.

The goats on which the determinations were made were of mixed breeding of dairy type. One determination was made on six individuals in various stages of lactation and producing from 1.6 to

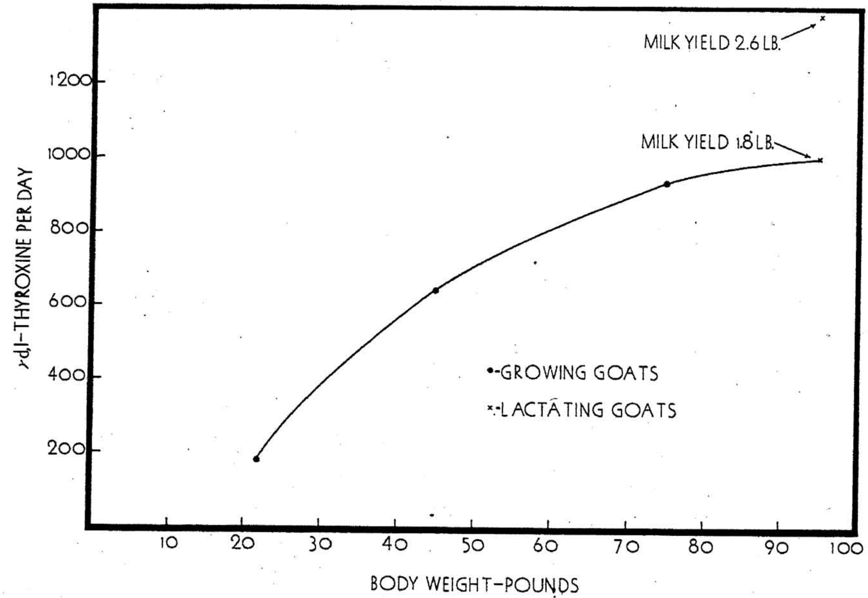


Fig. 13.—Relation of the thyroxine secretion rate to body weight and milk production of the goat.

4.7 pounds of milk per day or an average of 2.6 pounds daily. The amount of thyroxine required for approximately normal production ranged from 1000 to 2000 γ d, l-thyroxine per day. The average rate of thyroxine secretion was 1425 γ d, l-thyroxine. Milk production was not quite brought back to normal in some animals but taking the normal decline with advancing lactation into account, the return was probably near the amount the goats would have been producing normally (Fig. 14). This determination was made in August.

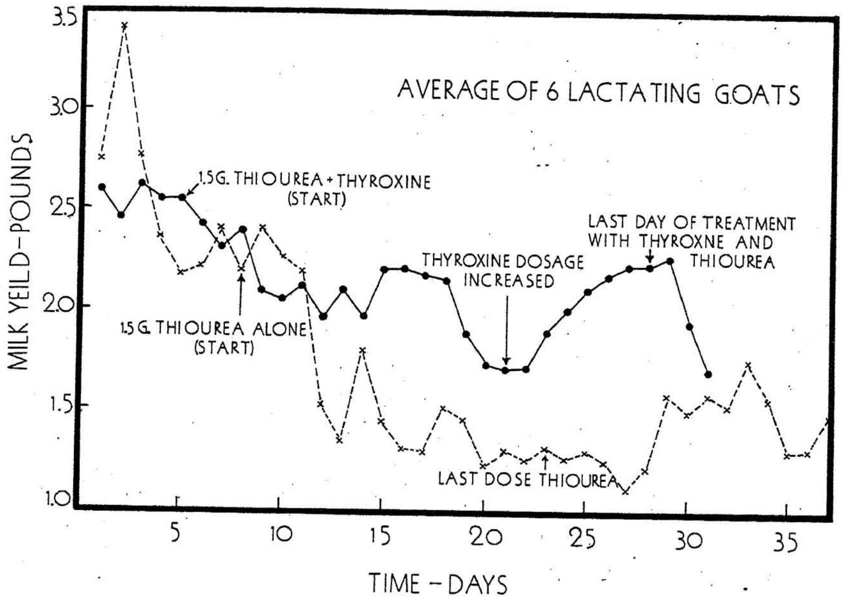


Fig. 14.—Effect of thiourea administration alone and in conjunction with thyroxine (1425 γ d, l-thyroxine) injection on lactation in the goat.

Another determination was made on these same six goats in November (Fig. 15). At this time the milk production varied from 1.2 to 2.8 pounds per day with an average production of 1.8 pounds. The amount of thyroxine required to bring about approximately normal milk production ranged from about 900 to 1200 γ with an average of 1040 γ per day of d, l-thyroxine. Whereas in the experiment carried out in August the goats secreting the most milk showed the higher thyroxine requirement, in the November determination two of the lower producers required the most thyroxine. This is probably due to the fact that in the latter experiment the goats were depressed in milk production before thyroxine was administered and the two low producers being so nearly dry required more stimulus to bring back their production so that the data on these two should probably be discarded. If that is done the average thyroxine secretion rate is about 1000 γ d, l-thyroxine.

The Determination of the Thyroxine Secretion Rate in Dairy Cattle. *Calves.* Dairy calves (mostly Holsteins) were started on thiourea or thiouracil treatment when 1 month of age and treated for 30 days. As reported in a preceding section, slight hypertrophy of the thyroid

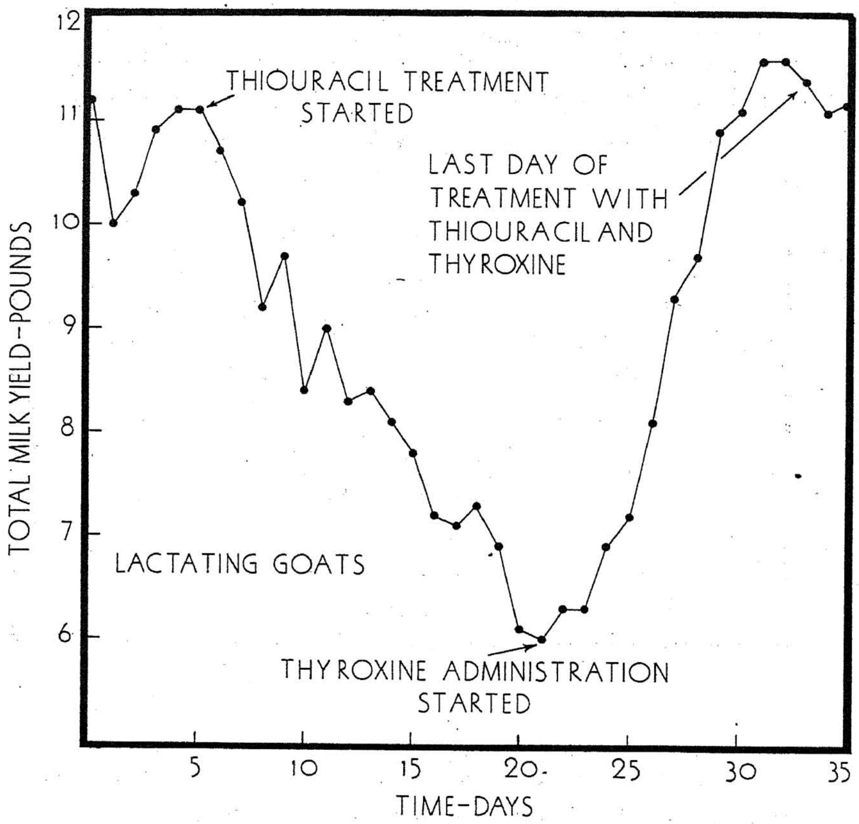


Fig. 15.—Effect of 1000 γ d, l-thyroxine (subcutaneous) on milk yield of goats treated with 3.0 grams thiouracil daily (orally).

was obtained. On 13 calves treated with from 1 to 3 grams thiouracil per day or from 1.0 to 3.5 grams thiourea per day a thyroid weight of 10.41 grams was obtained. These calves had an average body weight of 156.6 pounds. The thyroid weight per 100 pounds of body weight was therefore 6.65 grams. The average thyroid weight of 3 normal calves averaging 179.3 pounds body weight at 2 months of age was 7:53 grams or 4.20 grams per 100 pounds body weight. One calf receiving 1 gram thiouracil plus 1000 γ d, l-thyroxine weighed 160 pounds with a thyroid weight of 8.1 grams or 5.1 grams per 100 pounds body weight. Another calf weighing 113 pounds and treated with 2.0 grams thiourea daily (1.0 grams administered twice per day) plus 1500 γ d, l-thyroxine had a thyroid weight of 5.0 grams or

4.4 grams per 100 pounds body weight. The thyroid weight of the latter calf appears to be about normal, so that 1500 γ d, l-thyroxine would appear to be the normal thyroxine secretion rate. However, the slight goitrogenic effect of the drugs used on the calf thyroid together with the small number of individuals treated with thiourea plus thyroxine indicates the need of further observations.

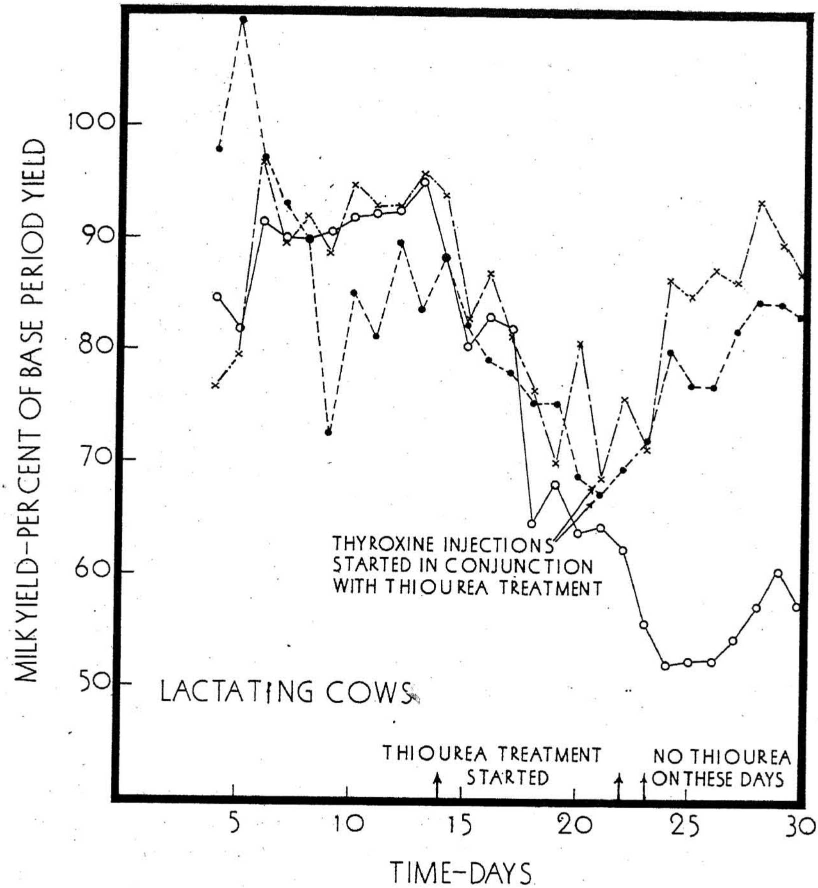


Fig. 16.—Effect of thiourea and thiourea plus 10 mg. d, l-thyroxine on milk yield in cows.

Lactating cows. Determinations of the effect of 10 mg. of d, l-thyroxine injected daily per cow on milk production of 2 cows fed thiourea were made. Thiourea alone depressed milk production about 30 to 35 per cent. A Holstein giving an average of 55 pounds of milk per day fell off in production to 40 pounds per day with administration of 7.0 grams thiourea per day for 8 days. When 10 mg. of thyroxine were injected per day with thiourea administration continued, milk production came back to 48 pounds in 7 days or maintained production at about 87 per cent of normal. A Jersey producing an average of 22 pounds of milk per day was maintained at about a 96 per cent level with injection of 10 mg. of d, l-thyroxine per day when administered in conjunction with thiourea (Fig. 16).

TABLE XVI. THE THYROXINE SECRETION RATE OF VARIOUS ANIMAL SPECIES

Animal	Body weight	Thyroxine secretion rate/day as γ d,1-thyroxine	Thyroxine secretion rate/day /100 gm. body wt. as γ d,1-thyroxine	Temperature conditions
		mgm.	mgm.	Fah.
Rat (1)	139.0 gm.	4.80	3.47	77
White Leghorn cockerels	343.0 gm.	7.55	2.20	82-86
	1536.0 gm.	25.00	1.63	82-86
White Plymouth Rock cockerels	410.0 gm.	8.10	1.98	82-86
	1502.0 gm.	23.00	1.53	82-86
White Plymouth Rock pullets	360.0 gm.	8.75	2.43	82-86
	1637.0 gm.	26.00	1.59	82-86
Growing goats	10.0 kg.	180.00	1.80	50-80 probable range
	20.4 kg.	640.00	3.13	" " " "
	34.5 kg.	930.00	2.70	" " " "
Lactating goats				
(milk yield 1.8 lbs./day)	43.5 kg.	1,000.00	2.29	" " " "
(milk yield 2.6 lbs./day)	43.5 kg.	1,425.00	3.44	" " " "
Calf	72.6 kg.	1,500.00	2.06	" " " "
Cow (lactating)	454.0 kg.	10,000.00	2.20	" " " "

(1) Reineke, Mixner and Turner (1945).

These results indicate that somewhere near 10 mg. of d, l-thyroxine are required to maintain normal milk flow, and that the amount required is variable, probably depending on the intensity of production.

DISCUSSION

In the absence of methods for the quantitative determination of the rate of secretion by the thyroid gland there has been only more or less presumptive evidence that changes occur in thyroid activity under varied conditions. With the introduction of a method to determine quantitatively thyroid activity, new knowledge of the manner in which physiologic function is related to thyroid function can be gained. The experiments reported in this paper show that thyroid activity increases with increase in body weight in the growing fowl and goat.

With continued experience with the assay method and resulting improvement in technique, a knowledge of the manner in which thyroid function changes with certain other physiologic functions such as in pregnancy, lactation, etc., can be attained.

With any new method and in the absence of similar determinations for comparison, there is always the possibility of serious error being introduced by some misunderstood factor involved in the method of assay. However, practically all available evidence on the inter-relationships of thyroid function to hormonal and environmental influences lend support to the fact that the method of assay used in these experiments is a reasonably accurate method of evaluating the thyroid's secretion rate. Evidence previously reviewed shows the thyroidectomy-like effect of the drugs used in the assay. In fact, the drugs have been found to lower the basal metabolism more than thyroidectomy (Dempsey and Astwood, 1943). This may happen since tissues other than the thyroid may be able to synthesize some thyroid hormone (Chapman, 1941). This evidence leads to the conclusion that assay by these drugs would give a more nearly accurate determination of the thyroid hormone production rate of an animal than would replacement therapy in thyroidectomized animals. That administration of the drugs causes cretinism similar to that observed after thyroidectomy, inhibits metamorphosis of tadpoles and results in inhibition of the iodine collecting ability of the thyroid is added evidence of their thyroidectomy effect. Evidence that the enlargement of the thyroid resulting from the administration of the drugs is mediated by way of the thyrotrophic hormone has been reviewed. In addition, Adams and Jensen (1944) and others have shown that injected thyroxine reduces the thyrotrophin potency of the anterior pituitary. Dempsey and Astwood (1943) showed that the goitrogenic effect is inversely proportional to the dosage of thyroxine injected, the larger the thyroxine dosage the less the thyroid hypertrophy induced by the drugs. In normal animals, thyroxine injection in suitable amounts entirely prevents the goitrogenic effect; iodine administration has no effect and l-diiodotyrosine only a very slight effect, if any. The return of thyroid size to normal from thiouracil-induced enlargement with graded dosages of thyroxine followed a response curve similar to that of the return to normal metabolic levels with increasing dosages of thyroxine in thiouracil treated rats (Reineke, Mixner and Turner, 1945).

Dempsey and Astwood (1943) considered the possibility of an error being introduced in the method by the existence of performed thyroid hormone being present in the gland but showed that the degree of error introduced was insignificant with a suitable period of treatment.

An error would be introduced in the method if the goitrogenic drugs partially inhibited the action of preformed thyroxine as paraxanthine may act. Astwood, Sullivan, Bissel and Tyslowitz (1943) showed that the toxic effects of large doses of thyroxine were not affected by simultaneous administration of the goitrogenic drugs. Neither was the calorigenic effect of thyroxine materially affected, thus indicating that these drugs do not interfere with the action of preformed thyroxine.

Evidence by Dietrich and Beutner (1944) indicated that thiouracil and thiourea inhibit to some extent the protective action of the thyroid to the toxicity of acetonitrile in white mice. They therefore concluded that the drugs have an antagonistic effect on preformed thyroid hormone. Their results might also be interpreted on the following basis. Administration of thyroid hormone to animals with intact thyroids would cause an immediate effect of raising the thyroid hormone concentration in the blood and therefore produce increased resistance to acetonitrile toxicity. With administration of thiouracil or thiourea, thyroid hormore production by the animal's own thyroid is prevented, thus lowering the thyroid hormone amount in the blood with resulting lowered resistance to acetonitrile toxicity.

Astwood and Bissell (1944) determined the rate of loss of iodine from the thyroids of rats treated with thiouracil and showed that the initial rate of loss would approximate 3 γ iodine per day. This is equivalent to 4.8 γ thyroxine per day. This figure agrees very well with the previous estimate of thyroxine secretion of 5.2 γ thyroxine per day, the latter figure determined for similar rats using the method under discussion. Also, Evans et al. (1939) found that 5 γ thyroxine administered to thyroidectomized rats resulted in normal growth rate and normal B.M.R.

In our experiments, determination of the thyroxine secretion rate of groups of chicks repeated at different times showed minor variations from the average relationship previously determined to exist between increasing body weight and thyroxine secretion rate. These variations, however, might well be due to variations in environmental conditions such as temperature or to difference between groups of chicks. For example, Barred Plymouth Rock chicks showed a higher thyroxine secretion rate than White Plymouth Rock chicks of similar age. However, the Barred Plymouth Rocks had a somewhat faster growth rate than the White Plymouth Rocks. Also, the determinations on the Barred Rocks were made in February when room temperature ranged nearer 82° F., while determinations of comparable White Plymouth Rocks were made in September when the room temperature ranged nearer the highest limit or 88° F. In other words, thyroid activity appears to be extremely sensitive to environmental conditions and physical well-being of the animal. Therefore, thyroxine secretion determinations should be made under standard conditions and with standardized animals insofar as possible. Also, since thyrotrophic hormone release and thyroid function are influenced by the physical well-being of the animal, the goitrogenic drugs should be non-toxic. This latter consideration together with the inability to control environmental conditions may decrease the accuracy of our thyroxine secretion rate determinations with goats.

The results of our experiments indicate that there is an increasing rate of thyroid hormone secretion with increasing age and body weight in the animals studied. There is more or less similarity of trend in this respect with all animals studied. There are variations in the trend due to sex and breed. The Leghorn breed's thyroid activity is higher on a body weight basis than that of the heavier breeds of fowls when determined under like conditions. The female's thyroid is also more active on a body weight basis than that of the male. However, the effect of body weight overrides these sex or breed effects in that total thyroxine secretion per animal per day is greater in the male Plymouth Rock because the body weight is greater than that for the lighter breeds or females at a definite age. It is possible, however, that the animal with the most active thyroid on a body weight basis is more responsive to environmental influences.

Since in the growing fowl thyroid weight increases in about the same proportion that thyroxine secretion rate increases, the thyroid weight appears to be a fair index of thyroid activity in the fowl. Also, castration reduces thyroid size as well as the thyroxine secretion rate in the growing fowl. After maturity the relative value of thyroid weight as an index of thyroid activity has not been determined.

In the goat, thyroid size increased at a rate of about the 0.7 power of body weight. The thyroxine secretion rate increased much faster so that it is apparent that the close relationship between increasing thyroid weight and thyroxine secretion rate does not exist in this animal.

Comparing the thyroxine secretion rate of the various animals used in these experiments, it is indicated that the fowl, on a 100 gram weight basis, has a lower secretion rate than the goat. The thyroxine secretion rate in the fowl varies from 1.4 to 2.76 γ per 100 grams body weight per day. The goat varied from 1.8 to 3.44 γ per 100 grams body weight per day. These figures, however, may not be comparable since the fowls were in general kept under a higher environmental temperature. Considering the values obtained for cattle to be representative, the thyroxine secretion rate per 100 grams body weight was 2.0 to 2.28 γ per day, which is similar to the values obtained with the goats.

With the extension of the determinations of the thyroxine secretion rate to animals varying in milk production, growth rate, fattening ability and reproductive ability and determining the relationship of the thyroxine secretion rate to productive performance, practical knowledge concerning the methods by which these processes can be enhanced should be obtained. Productive processes that are found to be associated with the above average thyroid function should be enhanced by adding thyroidally active materials to the feed. Productive processes that are found to be associated with below average thyroid function should be enhanced by adding an anti-thyroid compound such as thiourea or thiouracil to the feed. If low breeding performance in certain domestic animals during the summer months was found to be associated with low thyroid function, this seasonal trend could at least be partially eliminated by the administration of thyroidally active materials in the ration.

SUMMARY

1. Determination of the thyroid's secretion rate in equivalent amounts of d, l-thyroxine was made in the growing fowl and growing and lactating goats. Limited investigation of the rate of thyroxine secretion of dairy cattle was made.

2. The rate of secretion by the thyroid of the White Leghorn cockerel ranged from an average of 1.95 micrograms d, l-thyroxine per chick per day at 2 weeks of age (2.76 micrograms per 100 grams body weight) to 25 micrograms d, l-thyroxine per chick per day at 27 weeks of age (1.63 micrograms per 100 grams body weight). The relation of the thyroxine secretion rate to body weight can be expressed by the equation $Y = 0.065X^{0.81}$, where Y equals micrograms d, l-thyroxine and X the body weight in grams).

3. The rate of secretion by the thyroid of White Plymouth Rock chickens ranged from an average of 4.5 micrograms d, l-thyroxine per chick per day at 5 weeks of age (1.98 micrograms per 100 grams body weight) to 35 micrograms d, l-thyroxine per chick per day at 26 weeks of age (1.44 micrograms per 100 grams body weight) in the male. In the female the rate ranged from an average of 4.75 micrograms d, l-thyroxine per chick per day at 5 weeks of age (2.01 micrograms per 100 grams body weight) to 29 micrograms d, l-thyroxine per chick per day at 26 weeks of age (1.50 micrograms per 100 grams body weight). The relation of thyroxine secretion rate to body weight for the male is $Y = 0.053X^{0.83}$, for the female $Y = 0.073X^{0.80}$, where Y equals micrograms d, l-thyroxine and X body weight in grams.

4. The thyroxine secretion rate was higher, on a body weight basis, for the White Leghorn cockerels than for the White Plymouth Rock cockerels, being about 10 per cent higher under the conditions of the experiments. However, since the White Plymouth Rock cockerels attain greater weight at an advanced age, the average thyroxine secretion rate per chick was higher for the White Plymouth Rock cockerels.

5. Castration of the male fowl resulted in a decrease in the thyroxine secretion rate, this decrease being about 16 per cent less than that of normal under the conditions of the experiment.

6. The average rate of secretion by the thyroid of the growing goat was 180 micrograms d, l-thyroxine per goat per day at 2 months of age and at a body weight of 22 pounds. At a body weight of 45 pounds, the average thyroxine secretion rate was approximately 640 micrograms d, l-thyroxine per goat per day and at a body weight of 76 pounds the average rate was about 930 micrograms d, l-thyroxine per goat per day under the conditions of the experiments.

7. Lactating goats (6) producing an average of 2.6 pounds of milk per day had an apparent thyroid secretion rate of 1425 micrograms d, l-thyroxine per animal per day. These same 6 goats, further

advanced in lactation, and producing an average of 1.8 pounds of milk per day, had an apparent average secretion rate of 1000 micrograms d, l-thyroxine per animal per day.

8. One cow whose milk production was depressed by thiourea treatment was brought back to 87 per cent of normal production by the administration of 10 mg. d, l-thyroxine per day. Another cow similarly treated was brought back to 96 per cent of normal production by administration of 10 mg. d, l-thyroxine.

9. The faster growing chicks of a group appeared to have a higher thyroxine secretion rate. However, since the secretion rate increases with increased body weight, the larger chicks may have had a higher secretion rate because of their greater body weight.

10. A study of the relationship of thyroid weight and body weight in the normal fowl showed that this relation can be expressed for the White Leghorn by the equation $Y = 0.158X^{0.88}$; for White Plymouth Rock cockerels by $Y = 0.035X^{1.05}$; for White Plymouth Rock pullets by $Y = 0.062X^{0.99}$, in all equations Y being the thyroid weight in milligrams and X the body weight in grams.

11. A study of the relation of thyroid weight to increasing body weight in the normal goat showed that the relationship was $Y = 174.6X^{0.70}$, where Y equals the thyroid weight in milligrams and X the body weight in kilograms.

12. Differences in thiouracil and thiourea with respect to their ability to induce enlargement of the thyroid and their relative toxic effect were determined. In general, thiourea induced its maximum goitrogenic effect at a lower dosage level than did thiouracil. Per unit weight of the drug, thiourea is much more toxic than thiouracil.

13. Thyroid enlargement induced by the feeding of 0.1 per cent thiouracil in the ration for a period of 2 or 3 weeks was greater per 100 grams body weight in the female than in the male fowl. It was greater in the lighter than in the heavier breeds. It was greater in younger chicks than in older chicks and in castrated chicks than in comparable chicks. It was likewise greater in chicks and goats in good physical and growing condition than in those animals in poor physical condition.

14. In 15 week old White Plymouth Rock chicks the goitrogenic effect of 0.1 per cent thiouracil in the feed was enhanced by the feeding of 0.01 per cent dimethyl ether of diethylstilbestrol in the ration for 5 weeks. This same level of this compound lowered the goitrogenic effect of thiouracil in 15 week old Barred Plymouth Rock pullets. Feeding the 0.01 per cent level of dimethyl ether of diethylstilbestrol for 9 weeks to the 15 week old Barred Plymouth Rock pullets resulted in no apparent change in their thyroxine secretion rate compared to that of similar control pullets.

15. While thyroid enlargement induced by 360 mg. of thiourea administered orally once per day was three to four times the normal

thyroid size in young goats, the goitrogenic effect in older goats was extremely variable with dosages ranging from 0.75 to 1.5 grams thiourea per day or with 1.0 to 5.0 grams thiouracil per day. This indicates the need for a more satisfactory method of administering the drugs to obtain more consistent results.

16. The thyroids of fetal goats past mid-term of intrauterine life were greatly enlarged when the mothers were treated with thiourea or thiouracil. The thyroids of fetal goats prior to mid-term were not affected by such treatment of the mother. If the thyrotrophic hormone of the mother cannot pass the placental barrier as has been indicated to be the case by other experiments, then the stage of development of the fetus when its thyroid is affected may be the time of the beginning of function by the pituitary and thyroid of the fetus.

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