A COMPARISON OF THE EFFECTS OF THREE GOITROGENIC
COMPOUNDS ON GOITER DEVELOPMENT
IN THE NEWLY HATCHED CHICK

by

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STATEMENT BY AUTHOR

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ABSTRACT

The response of White Leghorn chickens to the addition of one of three dietary goitrogenic compounds from hatch to 70 days was investigated. Of the three goitrogens used, thiourea did not cause increases in DNA above that seen in controls although thyroid weight increased. This thought to be due to a secondary toxic effect of thiourea on the chick thyroid which inhibits cellular replication. The other two goitrogens examined caused cellular hyperplasia and increases in DNA amounts beyond that seen in controls. The rapid thyroid growth in methimazole treated birds from 30 to 70 days of age is partially due to follicular colloid accumulation. Growth of the thyroid in PTU fed birds is thought to be due to cellular hyperplasia and hypertrophy since DNA values are greatest in these glands.

Histology of the thyroid glands confirm the above findings with all three goitrogens. The ultrastructural changes associated with the prolonged stimulation of the chick thyroid are: an increase in the amount of primary and secondary lysosomes, microvilli and mitochondria and a dilation of the endoplasmic reticulum and golgi apparatus. It is thought that micropinocytosis is a more important endocytotic process during goiter development in the chick than is phagocytosis.
INTRODUCTION

It has been well established that members of the thioureylene group of antithyroid compounds are among the most potent goitrogenic compounds known. Several of these drugs, most notably, 6-propyl-2-thiouracil (PTU) and 1-methyl-2-mercaptoimidizole (methimazole, MMI), have been widely used for more than three decades in the treatment of hyperthyroidism. Another of these drugs, thiourea, and some of its derivatives, phenylthiourea and allylthiourea, were among the first compounds observed to cause marked thyroid hyperplasia in rats even in the presence of adequate amounts of dietary iodine (Astwood et al. 1943; MacKenzie and MacKenzie 1943).

Events leading to the production of hyperplastic goiter, colloidal goiter and nodular goiter are now well established. MacKenzie and MacKenzie (1943) and later Keston et al. (1944) and Franklin, Chaikoff and Lerner (1944) showed that antithyroid drugs act by inhibiting the synthesis and/or output of thyroxine since the administration of thyroxine to animals placed on thiourea completely suppressed any thyroid hyperplasia. MacKenzie and MacKenzie (1943) and Astwood et al. (1943) hypothesized that the primary route of goiter development involves the thyroid pituitary axis. Thiourea, in this case, blocks the output of thyroxine which results in an increase in thyrotropin secretion. This
increase in thyrotropin secretion results in hypertrophy and hyperplasia of the thyroid and a loss of colloid.

The hypothesis put forward by MacKenzie and MacKenzie suggesting thyroid hyperactivity was questioned by several workers (Baumann, Metzger and Marine 1944) who observed that histologically the thyroid gland appears to be hyperactive following antithyroid drug administration. According to Grasso (1946), the thyroid gland is indeed hyper-secretory following antithyroid treatment but the secretion product has very low hormonal activity.

Although it has been shown that antithyroid drugs inhibit the formation of thyroxine by the thyroid follicle cell (Richards and Ingbar 1959; Laurberg 1978; Davidson et al. 1979), the exact mechanism of inhibition of thyroxine production by the thioureylene antithyroid compounds has not been definitely established. Clearly, the effect of thioureylene compounds is to reduce the amount of circulating thyroxine (Tanabe et al. 1965; Griessen and LeMarchand-Beraud 1973), the reduction in thyroxine resulting in a rapid increase in plasma TSH as measured by radioimmunoassay (Griessen and LeMarchand-Beraud 1973).

**Extrathyroidal Effects of Antithyroid Compounds**

There may be several other ways in which an antithyroid agent may affect the pituitary output of TSH besides inhibiting the output of thyroxine. Recently, Muranyi-Kovacs, Rudai and Arnaud (1979) suggested that thiourea may directly disturb the hypothalamo-pituitary axis since the administration of radiolabelled thiourea results in a greater accumulation of radioactivity in the pituitary than in all other tissues.
examined with the exception of the thyroid. In addition, it was observed by Jolin, Morrealle de Escobar and Escobar del Rey (1970) that there is an extrathyroidal effect of PTU on circulating plasma levels of insulin unrelated to the antithyroid action of the compound. It has been suggested that PTU may impair the activity of the pituitary and pituitary dependent glands in a way similar to that suggest by Muranyi-Kovacs, Rudai and Arnaud (1979) for thiourea.

PTU also has a depressing effect on the extrathyroidal metabolism of thyroxine (Escobar del Rey et al. 1962; Hershman and Van Middlesworth 1962; Morrealle de Escobar and Escobar del Rey 1962) resulting in a reduction of physiologically active hormone. Furthermore, there is an increase in the fecal excretion of iodinated compounds (Tanabe et al. 1965). It may be inferred from these studies that the decrease in deiodination of thyroxine might in itself cause an increase in plasma TSH before the synthesis of thyroxine is impaired (Griessen and LeMarchand-Beraud 1973; Geffner, Azukigawa and Hershman 1975).

Methimazole, on the other hand, has been shown by several investigators to have no effect on the peripheral deiodination of thyroxine (Grossvenor 1962; Herrerra, Morrealle de Escobar and Escobar del Rey 1968; Laurberg 1978). Contrary to these findings, Van Pilsum, Boen and Bans (1973) have suggested an extrathyroidal effect of methimazole. In their studies, methimazole seemed to interfere in some way with the extrathyroidal utilization of thyroxine. Pogorilor, Van Mannen and Sellers (1971) suggest that methimazole may interfere with the binding of thyroxine by specific receptor sites thus blocking the action of the hormone.
Thiourea has no known effect on the extrathyroidal utilization or action of thyroxine in mammals (Escobar del Rey and Morrealle de Escobar 1961; Tanabe et al. 1965) but it has been shown to have a variety of effects on other tissues throughout the body as do PTU and methimazole. It has been noted that thiourea, when administered for extended periods of time, has a carcinogenic effect on the thyroid (Astwood et al. 1943; MacKenzie and MacKenzie 1943). Several derivatives of thiourea have also been shown to be carcinogenic to some tissues. Alpha-napthyl thiourea (ANTU) and other toxic thioureas are known to be very potent rat poisons. In addition to causing pulmonary edema and hemorrhaging, these substances also stimulate the growth of intravenously seeded tumor cells, possibly by decreasing the innate resistance to tumor growth in the lungs (Van den Berek, Kelley and Holland 1978). It is also interesting to note that thiourea, methimazole and PTU have all been implicated in the production of thyroid tumors which have been reported to metastasize to the lungs of rats (Purves and Griesbach 1946; Paschkis, Cantarow and Stasney 1948; Bielchowsky et al. 1949; Jemec 1977).

It is possible that many goitrogens may induce primary tumors in mammals. Ethylene thiourea is one goitrogenic agent which has been termed carcinogenic for both liver and thyroid (Graham et al. 1973). Muranyi-Kovacs, Rudai and Arnaud (1979) have also suggested that thiourea will increase the incidence of intracranial bone tumors in mice.
Effects of TSH on the Thyroid

The elevated thyrotropin levels caused by the administration of a goitrogenic substance has three immediate and long term effects on the thyroid follicle cell: 1) The follicle cell undergoes endocytotic activities whereby thyroglobulin re-enters the follicle cell and is eventually secreted to the blood (Seljelid 1967a; Bjorkman et al. 1974; Ekholm et al. 1975; Ericson and Engstrom 1978). This endocytosis-release process is directly stimulated by TSH and also by epinephrine released from intrathyroidal sympathetic neurons (Melander 1971); 2) The endoplasmic reticulum begins the rapid production of new proteins, primarily thyroglobulin, which are delivered to the follicular lumen via exocytosis (Bjorkman et al. 1974; Bjorkman, Ekholm and Ericson 1978; Pavlovic-Hournac and Delbauffe 1975, 1976); 3) The follicle cell increases the production of RNA and DNA for utilization in extensive cell growth and replication (Wollman and Brietman 1970; Christov, Wollman and Thomas 1973; Yamamoto and DeGroot 1974).

Following the administration of a single dose of TSH the follicle cell undergoes a series of changes. These ultrastructural changes in the mammalian follicle cell have been previously described in detail (Seljelid, Reith and Nakken 1970; Seljelid, Helminen and Thies 1971; Rapoport and Jones 1978).

Functionally there are two pools of vesicular elements in the apical region of the thyroid follicle cell: endocytotic and exocytotic. It has been shown that only one type of vesicle remains following the stimulation of the follicle cell (Bjorkman et al. 1974). Since it is assumed that the endocytotic response of the follicle cell to TSH is
more rapid than that of the exocytotic response, these vesicles have been classified as endocytotic (Bjorkman et al. 1974). After the removal of TSH stimulation, there is a change in the type of apical vesicles predominating and it is assumed that these vesicles are exocytotic (Bjorkman et al. 1974).

Exocytotic vesicles have been shown to arise from the golgi apparatus where thyroglobulin, which was synthesized in the rough endoplasmic reticulum, is packaged into microvesicles. These thyroglobulin-filled vesicles then migrate to the apical end of the cell, fuse with the plasma membrane and discharge their contents into the follicular lumen (Nadler et al. 1964; Fujita 1975).

The formation of endocytotic vesicles is more difficult to explain. Several authors maintain that the endocytotic response is due to the mass engulfment of colloid through the formation of pseudopodia on the apical surface of the follicle cell (Seljelid 1967b, 1967c; Ericson and Engstrom 1978). This engulfment of colloid by the follicle cell results in the formation of large colloid droplets within the apical region of the follicle cell. Colloid droplets may be observed within a few minutes following an injection of TSH to an animal and some workers maintain that this is one of the first morphological changes of the follicle cell induced by TSH (Ketelbant-Balasse et al. 1976; Ericson and Engstrom 1978).

Other populations of endocytotic vesicles occur in the apical region of the follicle cell and are apparently formed by the invagination of the apical cell membrane (Seljelid 1967a, 1967b; Ketelbant-Balasse et al. 1976). Chronic stimulation of the thyroid
follicle cell by TSH rarely results in the formation of pseudopodia and colloid droplets (Seljelid and Nakken 1968; Strum and Karnovsky 1971; Ketelbant-Balasse et al. 1976). It is possible that under prolonged stimulation such as during the development of a thyroid goiter, the micropinocytosis of colloid is more important in the endocytotic process than is the rapid uptake of colloid through the process of engulfment by pseudopodia (Seljelid, Reith and Nakken 1970; Ishimura, Okamoto and Fujita 1976; Ketelbant-Balasse et al. 1976).

Following the prolonged administration of an antithyroid substance several morphological changes occur which are associated with increased stimulation by TSH. Microvilli on the apical cell membrane increase in number and become longer (Olen 1969; Seibel and Knigge 1972). The function of these microvilli is unknown, although several authors have suggested that they facilitate the uptake of thyroglobulin into the follicle cell (Heimann 1966; Gaal, Kovacs and Sellers 1976). The number and size of mitochondria are greatly increased which may indicate increased production of thyroidal proteins (Olen 1969). In addition, numerous dense staining lysosomes are seen following prolonged stimulation of the thyroid (Seljelid 1967d). These lysosomes are thought to be responsible for the degradation of thyroglobulin to thyroxine (Seljelid 1967d; Itakawa and Kawada 1974). There is generally an increase in the vascularity of the gland (Thomas 1945) and microhemorrhage into the follicle lumen occurs (Zelligs and Wollman 1976). Tight junctions are also shown to become wide and elaborate following thiouracil administration (Tice, Wollman and Carter 1975), but the reason for this is unknown.
The activity of the thioureylene compounds have been most widely studied in the rat and little is known of the effects of the thioureylene compounds in the chick. It has generally been assumed that the bird's response to a goitrogenic substance is the same as a mammal's. Several studies have shown that avian goiters following antithyroid administration are similar to those of mammals (Mixner, Reineke and Turner 1944; Tanabe et al. 1965; Leung and March 1976), but Marine and Lenhart (1910) noted that the most common type of goiter found naturally in the fowl was a colloidal goiter. Colloidal goiter, although common in man, is very uncommon in laboratory animals and can generally be produced experimentally only by the withdrawal of the goiter-producing substance following the development of a hyperplastic goiter (MacKenzie 1958; Follis 1959; Olen 1969). MacKenzie and MacKenzie (1943) have also shown that the chicken does not respond to sulfaguanidinidine as a goiter-producing agent, whereas the rat, mouse and dog all produce large hyperplastic goiters following sulfaguanidine treatment. It is possible that, in spite of previous assumptions, the fowl may respond to a goitrogenic substance in a different manner than do common laboratory mammals.

There are very few accounts of the ultrastructure of the avian thyroid (Bestetti 1977; French and Hodges 1977). The normal avian thyroid ultrastructure has been described for a few species including the chicken (Fujita and Nagata 1962; Fujita 1963; Hilfer 1964; Bestetti 1977; French and Hodges 1977), pigeon (Sakai 1960; Muramoto 1964), and duck (Sakai 1960), yet the ultrastructural changes occurring
in the chick thyroid during hyperplastic and/or hypertrophic growth have not been previously reported.

The present study will report the histological and ultrastructural changes occurring in the hyperplastic and hypertrophic chick thyroid during the first ten weeks of growth and compare and contrast the effects of three different but commonly used goitrogenic compounds on the thyroid gland of the chick.
MATERIALS AND METHODS

Two hundred newly hatched White Leghorn chicks were divided into four groups of 50 birds each. One group, which served as a control group, was fed a standard University of Arizona chick starter mash diet. The remaining three groups were fed a diet of chick starter mash mixed with one of the following three goitrogenic compounds. All compounds were obtained from Sigma Chemical Co.

Group I : 0.1% Thiourea
Group II : 0.1% Propylthiouracil
Group III : 0.1% Methimazole

Birds were housed in two banks of electric brooders at the University of Arizona Poultry Center. The hover temperature of the electric brooders was maintained at approximately 95°F for the first 50 days. After 50 days, those birds not yet sacrificed in the control group were moved to outside floor pens. The other three experimental groups remained in the brooder cages. Temperatures in the floor pens ranged from daytime highs of approximately 80°F to nighttime lows of 45°F.

One day after hatch, six chicks were sacrificed by cervical dislocation, the body weights recorded to the nearest 0.1 gr, and the thyroid gland of each was excised and weighed on an Ainsworth analytical balance and recorded to the nearest 0.1 mg. Every five days thereafter, three birds from each of the four groups were sacrificed and the thyroid
glands removed. Immediately upon removal of the thyroid gland from the
bird one lobe was dissected in half in preparation for the following
tests.

1. One half of the dissected lobe was used for histological
sectioning and staining for examination under a light microscope.
2. The remaining half of the dissected lobe was used for thin
sectioning and staining for examination by transmission electron
microscope.
3. The remaining whole lobe of tissue was frozen and used for
assays of total DNA and total soluble protein content.

**Light Microscopy**

Tissue for histological examination was fixed in a 10% buffered
formalin solution prior to being embedded in paraffin. The paraffin-
embedded tissue was then sectioned to 5 μm sections. These sections were
dehydrated in alcohol and stained with hematoxylin and eosin.

**Electron Microscopy**

Tissue for ultrastructural analysis was cut to 1 mm squares and
fixed overnight in glutaraldehyde before being transferred to Millonigs
phosphate buffer for storage (Dawes 1971). Tissue was postfixed in 1%
osmium tetroxide for 2 hours, dehydrated in alcohol, cleared in propylene
oxide and embedded in Epon-Araldite (Dawes 1971). Sections were cut with
a glass knife on an LKB ultramicrotome at a thickness setting of 500 Å.
The sections were picked up on uncoated Pelco GC 300 copper grids
previously cleaned in acetone and acetic acid (Dawes 1971). Tissues
were stained with uranyl acetate and counter stained with lead citrate before examination by Phillips EM 200 transmission electron microscope.

DNA Assay

Thyroid tissue for protein and DNA assays was immediately frozen on dry ice and stored at -10°C until ready for use. The tissue was assayed for DNA according to the technique described by Setaro and Morley (1976, 1977) with minor modifications.

Tissue was completely homogenized in 0.9% physiological saline, centrifuged and washed twice each in saline, 0.6 N trichloracetic acid, and potassium acetate/ethanol (10g/L). The resulting pellet was then rinsed with absolute alcohol and dried. 1 N perchloric acid was added to extract deoxyribose and the resulting supernatant containing deoxyribose was allowed to react with 1.32 M diaminobenzoic acid (Aldrich Chemical Co.) for maximum fluorescence. The resulting yellow fluorescence of the solution was then read with an Aminco Bowman spectrofluorometer at an excitation wavelength of 420 nm and an emission wavelength of 520 nm. The results were compared against a standard curve to obtain absolute DNA amounts.

Protein Assay

The supernatant acquired through homogenation of tissue in 0.9% saline during the DNA assay was utilized in a protein assay described by Bradford (1976) to measure the amount of soluble protein within the gland. This assay involves the binding of Coomassie brilliant blue G-250 (Sigma Chemical Co.) to protein and monitoring the change in color.
from red to blue by use of a Beckman DU-2 spectrophotometer set to read the absorption at 595 nm. The results were compared against a standard curve to obtain absolute protein amounts.

**Statistics**

A two-way analysis of variance was run on the results of the DNA and protein assays. Regression lines were drawn from the means and standard deviations of the means for all data and for the first 30 days only. The homogeneity of the regression lines was tested by comparing the computed F values and the least significant difference (LSD) was calculated to determine the differences between regression lines (Steel and Torrie 1976).
RESULTS

Gland and Body Parameters

Body Size

Following the administration of a goitrogenic compound to one-day-old chicks there is a period of 5-10 days in which there is no statistical depression in the growth of birds if compared to controls. After this initial period, body weight was severely depressed by all three goitrogens but methimazole had the greatest depressant effect (fig. 1). Thiourea and PTU have a similar effect in retarding body growth. All three treatments were significant in depressing body weight when tested at the 95% confidence level.

Thyroid Size

Following the administration of PTU and methimazole there is a significant increase in thyroid size by 10 days while the increase in thyroid size caused by thiourea does not become significant until after 10 days of treatment (fig. 2). Thereafter, thiourea caused increases are very similar to those increases seen after methimazole treatment. The largest goiters were found in those birds fed methimazole and PTU. After 70 days of treatment, the thyroid weights of methimazole and PTU fed birds were over 200% greater than those of controls. Methimazole produced a slightly larger goiter than PTU at most sample dates (fig. 3).
Figure 1. Body weight changes following goitrogen administration.

The regression lines are statistically plotted and show changes in body weight following the administration of one of several goitrogenic compounds to one-day-old chicks for up to 70 days. LSD(.05) = 0.748; C = 2.83; TU = 6.02; PTU = 6.59; MMI = 12.95. All treatments differ from controls.
Figure 2. Thyroid weight changes for first 30 days of study.

The regression lines and means show increases in thyroid weight for the first 30 days of treatment with one of three goitrogenic substances. Each point represents approximately three animals. PTU△; MMI O; Tu ×; C ●. LSD(.05) = 0.792; C = .652; Tu = 1.845; MMI = 1.945; PTU = 2.810. All treatments are statistically different from controls.
Figure 3. Thyroid weight changes following goitrogen administration.

Regression lines are statistically plotted showing increases in thyroid weight for chicks placed on one of three goitrogens for up to 70 days. LSD(.05) = 0.46; PTU = 1.84; MMI = 2.42; TU = 1.10; C = 0.71.
For the first 30 days of treatment the increase in thyroid weight caused by thiourea increases at a rate similar to that produced by PTU and methimazole (fig. 2). After 30 days the variability in thyroid weights of thiourea fed animals is so great that the mean difference is not statistically significant.

DNA

The increase in absolute thyroid weights are coupled with increases in the total amount of DNA in the thyroid of all birds. Generally, DNA increases, denoting an increase in the total number of cells, follow any increases in thyroid size except in those birds fed thiourea (fig. 4). Following thiourea administration, the rate of DNA increase is similar to that seen in control birds. For the entire study, total DNA levels did not significantly rise above control levels in the thyroids of thiourea treated birds (fig. 5).

Total DNA in the thyroid gland of methimazole and PTU fed birds increases after 10 days of treatment (figs. 4, 5) and rapidly rises above control levels reaching maximal increases after 35 days of treatment. This increase is somewhat obscure since the variability between animals receiving the same goitrogen becomes progressively greater after 35 days of goitrogen treatment, and there is no significance to the differences between means at any treatment time level. On the other hand, the slope of the regression line showing DNA increases in PTU fed animals is significantly different from controls when tested at a 95% confidence level. The slope of regression lines for DNA increases in
Figure 4. Comparisons of thyroid weights and total DNA following treatment with three dietary goitrogens.

Each bar represents an average of three birds.
Thyroid weight - solid bar, DNA content - open bar.
Figure 5. Thyroidal DNA changes in response to goitrogen treatment.

Statistically plotted regression lines are showing increases in total DNA per thyroid gland over a period of 65 days following the administration of one of three different goitrogenic compounds. LSD(.05) = 2.25; C = 1.74; TU = 1.55; MMI = 3.35; PTU = 7.74.
methimazole fed birds is not significantly different from controls but the difference in the Y intercepts is significant.

Protein

Increases in total thyroidal protein were seen in all four groups studied (fig. 6). These increases in protein can be attributed to increases in total size of the gland and/or increases in colloid amounts. Regression lines for protein/mg thyroid weight is shown in fig. 7. Note that the increase in protein values is greatest in control animals. Methimazole treatment also causes an increase in protein/mg thyroid weight although not as rapid as seen in the controls.

Light Microscopy

In young control birds up to 15 days of age, the follicles of the thyroid gland are small and round or oval with a cuboidal epithelium (fig. 11). This type of follicle gradually becomes larger and distended, the cells becoming more flattened and squamosal as colloid accumulates in the follicle. By the 35th day the follicle cells are very flat and from this time until the end of the study the only changes in the control glands that may be seen is the gradual distention of the follicle as the follicle accumulates more and more colloid (fig. 27).

The administration of a goitrogenic substance has a drastic effect on the histology of the chick thyroid gland. After 5 days of treatment, there is essentially no difference in the follicle structure

1. For ease in comparison and handling, figures 8-41 are collected in Appendix A.
Figure 6. Total soluble protein changes following goitrogen administration.

Regression lines show increases in total soluble protein of thyroid glands after administration of a goitrogenic compound for up to 70 days. LSD(.05) = 23.94; C = 66.64; MMI = 113.73; PTU = 49.21; TU = 41.05.
Figure 7. Changes in thyroidal protein per mg wet weight after goitrogen administration.

Regression lines show changes in soluble protein per mg thyroid weight in response to treatment with one of three goitrogenic compounds for up to 70 days. 

LSD(.05) = 0.305; C = 1.042; MMI = 0.503; PTU = 0.15; TU = -0.018. Dotted lines are significantly different from solid.
of those birds fed methimazole or thiourea, while in those animals fed PTU the follicle has become larger, due mainly to the hypertrophy of the follicle cells. The cells are more cuboidal or columnar and there appears to be less colloid per follicle.

Following ten days of treatment there is a visible change in all of the glands removed from birds fed a goitrogen. These changes are similar to those seen in the PTU fed birds after 5 days of treatment. In all three groups, there is a gradual increase in the height of the follicle cell and a decrease in the amount of colloid in the follicle. This results in a collapsed appearance of the follicle. The greatest amount of hypertrophy and the least amount of colloid reabsorption is seen in birds fed thiourea (fig. 14). During this time, as follicles undergo a collapse due to colloid reabsorption, the follicle of the methimazole fed birds take on a very irregular folded shape (fig. 21). Folding of the follicle wall in those birds fed PTU is also evident but not to the extent seen in methimazole fed birds. The follicle wall usually remained round in whose birds fed thiourea, even after 35 days of treatment (fig. 22).

The maximum effect of the goitrogens methimazole and PTU occurs at or near 35 days of treatment. In the glands of PTU fed birds, almost no colloid is present within the follicles, most follicles being completely or almost completely collapsed onto themselves. This gives the gland the appearance of being a solid mass of cells (figs. 16, 18). The glands of methimazole fed birds are similar to those of the PTU fed birds after 35 days. Thiourea fed birds are apparently the least affected by the goitrogen. Colloid is still present following 35 days
of thiourea treatment although in reduced amounts when compared to controls (fig. 22).

From the 35th day of treatment to the end of the study, the follicles within the thyroids of birds fed PTU remained collapsed, cell height is very great and there is little colloid remaining in most follicles (figs. 17, 24). The vascularity of the gland which began increasing after approximately 20 days of treatment is maintained and the appearance of blood cells within the follicle lumen is not an uncommon sight.

Near the end of the study in thiourea fed birds there is a slight increase in the amount of colloid present in the follicles (fig. 26). This accumulation of colloid generally takes place in the outer follicles which in some cases become very distended and appear similar to those found in control animals. The vascularity of the gland increases but not to the extent seen in PTU fed animals. After 30 days of treatment the presence of blood cells in the follicle lumen is frequently noted, especially in those large follicles near the periphery of the gland.

Following the maximal collapse of the follicles after 35 to 40 days of methimazole treatment, there appears to be a gradual reappearance of colloid in the follicular lumen. The follicle cells remain columnar or cuboidal as the collapsed follicles slowly fill with colloid. Generally, the increase in colloid occurs in those follicles near the periphery of the gland although there is evidence that the follicles in the inner areas of the gland are also accumulating colloid to a smaller extent (fig. 23). The follicles at this time are usually
smaller than those seen when colloid is being depleted and are generally of uniform size and arranged in rows (fig. 19). The rows of follicles are difficult to see in those glands undergoing follicular collapse. The vascularity of the gland is similar to that found in PTU fed animals and occasional blood cells occur in the follicle lumen. It is interesting that the largest gland removed during this study was that of a methimazole fed bird with an abnormal amount of colloid. This gland was histologically more similar to the control glands than to those seen in the other goitrogen fed animals.

**Electron Microscopy**

The ultrastructure of the normal chick thyroid is similar to that described by French and Hodges (1977). The shape of the cell in the very young bird varies from squamosal to cuboidal. In the more mature birds the cells are almost always squamosal. The apical cell membrane is sometimes supplied with a scant number of microvilli. Normally, the microvilli are scattered over the apical surface and are extremely short and stubby. The lateral cell surfaces generally run parallel to each other as do the apical and basal cell membranes.

The cytoplasm appears to be granular, of varying density, usually, but not always, of a lighter nature than that of the follicular colloid.

The nucleus is generally the most prominent organelle of the cell, typically taking on a rounded appearance, although in some birds the outline is more irregular.
Mitochondria are generally found scattered throughout the cell, often almost completely surrounded by the ribosome studded membrane of the rough endoplasmic reticulum. Generally, mitochondria are oval shaped and as the bird matures there is an increase in the number of elongated and curved mitochondria as well as an increase in the overall size of the organelle.

Golgi bodies are normally found near the nucleus on the lateral and apical sides. Normally, they are very difficult to see being very flattened and small. The rough endoplasmic reticulum dominates the follicle cell cytoplasm. The general appearance of the rough ER is that of swollen cisternae filled with a fine granular light staining material and scattered randomly throughout the cell. Usually, the largest cisternae are in the lateral and basal parts of the cell.

Several other membrane-bound vesicles are also found in the cell, usually in the apical areas. Colloid droplets are sometimes seen, especially in the younger birds, but also occasionally in the more mature bird. The most conspicuous apical vesicles are very dense staining bodies which have been classified as primary and secondary lysosomes (Fujita 1975). Secondary lysosomes, which are formed by the fusion of a colloid droplet and a primary lysosome containing hydrolytic enzymes, are the most prominent types of vesicles seen in all ages of control animals. Pseudopodia rarely occur in control birds and are never seen on the same cell as a colloid droplet.

Following the administration of a goitrogenic substance several morphological changes occur. The most conspicuous changes include 1) the increase in size of the follicle cell accompanied by a further
distention of the endoplasmic reticulum beyond that seen in control birds, 2) an increase in the number and size of mitochondria, 3) an increase in the amount of dense staining vesicles in the apical region of the cell and 4) a change in the shape, size and number of apical membrane microvilli.

In PTU fed birds all the above changes were evident to some extent in those animals sampled five days after the administration of the goitrogen. The methimazole and thiourea fed birds showed only a slight response to the goitrogens after 5 days. Many cells sampled were no different from the control animals.

Changes found in the thyroids of birds sampled after ten and fifteen days of thiourea and methimazole treatment were quantitatively similar to those changes observed in PTU fed animals at the same time periods, but the response of the PTU fed birds was greater. The thyroids of PTU fed birds contained many collapsed follicles which were sometimes completely filled with long microvilli (figs. 30, 39). Golgi apparatuses are often prominent after 15 days of PTU treatment. The rough endoplasmic reticulum is concentrated in the basal end of the cell and there is a pronounced increase in the number of dense staining primary and secondary lysosomes. Many small light staining vesicles (possibly secretory and micropinocytotic) occur in the apical portions of the cell (fig. 31).

The number of dense staining bodies is not as great in those birds fed methimazole and thiourea after 15 days. Although large colloid droplets are occasionally seen in all three groups, there is
rarely evidence of pseudopod activity. The tallest cells observed were found in those animals fed thiourea (fig. 32).

By the middle of the study (35 days) and until 70 days all three experimental groups had considerable increases in the amount of dense staining bodies in the apical areas of the cell (fig. 33). What appears to be the fusion of small light staining vesicles with dense staining primary and secondary lysosomes may be seen in the apical region (fig. 34).

The endoplasmic reticulum was prominent in all three groups. In all birds the rough endoplasmic reticulum is usually concentrated in the lateral and basal portions of the cell. Occasionally in some cells large amounts of RER may occur in the apical areas of the cell as well.

Golgi bodies often occur in the apical and lateral areas of all three groups studied. Occasionally the golgi is dilated to some extent, especially after 30 or more days of methimazole feeding. Eventually some golgi bodies form round balls of golgi saccules in animals sampled after 35-40 days of thiourea treatment (fig. 35).

Mitochondria are often round and very enlarged at this time although there are also many elongated mitochondria still present. In thiourea fed animals, mitochondria which are very large and round often show signs of rupture, the cristae are broken and discontinuous giving the organelle an empty look (figs. 32, 35). This is most evident after 35 days of treatment. Nuclei in these cells are usually round while many in methimazole and PTU fed birds have an irregular shape and large invaginations which almost split the nucleus in half (fig. 36).
"Colloid" cells, in which the entire cytoplasm is dominated by large cisterns of endoplasmic reticulum are often numerous in the glands of all three experimental groups. Except for a few mitochondria and an occasional golgi apparatus, there are very few other organelles in these cells. In all three experimental groups, the colloid cells were interspersed among populations of normal hypertrophic cells (figs. 37, 41).

Birds that had been subjected to goitrogens for 35 days or more often had several types of cells within the same follicle. Colloid cells were interspersed among "normal" cells with round nuclei and small mitochondria, and elongated stacked RER or cells with irregular nuclei, larger mitochondria and more dilated endoplasmic reticulum.
DISCUSSION

The development of goiter in the bird and mammal is generally thought to be due to an increase in plasma levels of TSH (MacKenzie 1958). Since the thyroid glands of both species respond to TSH stimulation in a like manner it may be assumed that the development of goiter in the fowl is essentially similar to that of the mammal (Fujita 1975; Leung and March 1976). However, some differences in the development of a goiter in the chick are evident in this study which have not been previously reported for the mammal.

Increases in thyroid size following the administration of a goitrogenic substance is primarily due to cellular hyperplasia. Sinha, Anderson and Turner (1967) found that 30 days of methimazole treatment in the chick, increased thyroid size 68% by cellular hyperplasia. The remaining 32% of the thyroid weight increase was assumed (Sinha, Anderson and Turner 1967) to be a result of cellular hypertrophy. These workers did not believe that thyroid gland size increased by colloid accumulation of colloid within the follicular lumen is known to be a primary mechanism whereby young animals increase thyroid weight and follicle size (Hilfer 1964; French and Hodges 1977). Colloid accumulation in young control birds also occurred in the present study.

Although not all cells contain the same amount DNA, it may be assumed that they are all similar since the DNA contained in polyploid
cells and those cells undergoing replication make only a small contribution to the total gland DNA (Wollman and Brietman 1970). Therefore, increases in gland size should be reflected by increases in total numbers of cells per gland and, consequently, increases in the total DNA content of the gland.

Methimazole and PTU fed to chicks from hatch increases thyroid weight approximately three times that seen in control animals. This 300% increase in thyroid weight occurred as early as 15 days after the administration of methimazole or PTU in the food. Increases in DNA roughly parallel increases in thyroid size in PTU and methimazole fed birds. These results are similar to those found by Wollman and Brietman (1970) in the rat and mouse fed thiouracil for a period of 21 or 55 days and by Yamamoto and DeGroot (1974) in rats fed PTU for 31 days. These parallel increases suggest that the increase in gland size is due to cellular hyperplasia.

On the other hand, in thiourea fed birds, DNA values do not closely follow increases in thyroid weight gains. For the first 30 days of this study, thyroid weight in thiourea treated birds increased at a rate similar to that seen in control birds. The major increases in thyroid size seen during the first 30 days in thiourea fed birds do not appear to be caused by cellular hyperplasia as suggested for PTU and methimazole treatments. The histology of the thyroid gland of thiourea fed birds supports this suggestion. Although the gland is very active, cellular hypertrophy appears to take place to a greater extent than that seen in other experimental groups and the colloid diminishes and occupies a smaller percentage of the total area of the sectioned gland.
The toxicity of thiourea and other goitrogens found in many tissues and in a variety of animals may be responsible for DNA inhibition. Hydroxyurea, a compound similar in structure to thiourea inhibits cell replication in mammalian cells grown in culture (Grollman and Grollman 1970) and thiouracil inhibits cell replication by inhibiting the biosynthesis of pyrimidine nucleotides (Lindsay and Yu 1974). It cannot be determined whether or not PTU has the same effect as thiouracil since birds were not administered TSH alone but it appears unlikely that it does. It is apparent that thiourea inhibits cell division to some extent in the chick. Cytotoxic effects of thiourea on cell replication may be specific for thyroid tissue since the uptake and metabolism of thiourea is greatest in thyroid tissue. Thiourea may be metabolized by the chick thyroid to a compound similar to hydroxyurea or the drug may have a direct cytotoxic effect in the bird similar to the effect of hydroxyurea in the mammal.

Thiourea also appears to be toxic to the fowl in general. The highest death rate observed in any treatment occurs in those birds fed thiourea. This mortality rate was at least five times that seen in control and methimazole fed birds.

The histological appearance of the thyroids of PTU and methimazole fed birds indicate that these cells are not prevented from replicating as are those of the thiourea fed birds. Evidence of both follicle cell hypertrophy and hyperplasia are found in most follicles. Maximum cellular hyperplasia appears after 30 to 35 days of PTU and methimazole treatment. At this time the glands of PTU treated birds are larger, resulting in a greater amount of DNA in the glands sampled from
PTU fed birds. After 30 days the thyroids of PTU treated birds continue to increase in size by cellular hyperplasia. The cells of the gland remain hypertrophic and surround collapsed follicles containing little colloid.

The glands of methimazole fed birds are generally larger than the glands of PTU treated birds after 30 or more days of treatment but they contain less DNA than do glands from PTU birds. If growth of glands in PTU birds is due mainly to cellular hyperplasia, then it would be logical to assume that in methimazole fed birds sampled during the latter half of this study, growth is due mainly to factors other than just cellular hyperplasia. Histological examination indicates the growth of thyroids in the later stages of methimazole treatment may be due to colloid accumulation as well as cellular hypertrophy and hyperplasia.

Follicular colloid is composed primarily of thyroglobulin and other proteins (Pavlovic-Hournac and Delbauffe 1975, 1976) and measurements of total soluble protein content of the thyroid gland is a good indication of colloid content. Although total soluble protein may change with cellular hypertrophy or hyperplasia, increases in protein per mg thyroid weight are highest in control animals and lowest in PTU and thiourea treated animals. There is also a gradual increase in colloid and follicular distention in control animals and rapid cell proliferation in PTU treated animals which suggest that protein changes due to cell replication and growth are negligible when compared to that due to colloid accumulation.

The observed increases in thyroid weight due to colloid accumulation in methimazole fed birds after 35 days of treatment is compatible
with the rapid increases in protein/mg thyroid weight seen in the methimazole treated animals. This protein level is significantly greater in the methimazole treated group than in the PTU treated animals. These findings agree with Sinha, Anderson and Turner (1967) who reported a decrease from 68% at 30 days to 42% by 90 days in the amount of thyroid growth due to cell hyperplasia in chicks fed methimazole. These authors suspected cell hypertrophy as the main factor contributing to the growth not accounted for by cell hyperplasia. The present study indicates that gland growth may be due to colloid accumulation in methimazole fed chicks after 35 days of treatment.

Colloid accumulation has also been reported in rats fed various goitrogenic compounds but colloid does not accumulate until the animal has been subjected to prolonged periods of goitrogen treatment, sometimes as long as several years (MacKenzie 1958). In many of these cases the thyroid has also been shown to be cancerous (Griesbach, Kennedy and Purves 1945; Purves and Griesbach 1946; Glock 1949; Dalton et al. 1951; Sellers 1953).

Ultrastructural analysis of the follicle cells in birds treated with goitrogens support both the histological observations and the DNA and protein assays. In all groups of birds there were increases in the number of dense staining bodies near the apical region of the cell. Since, according to Seljelid, Helminen and Thies (1971) and French and Hodges (1977), the dense staining vesicles are either primary or secondary lysosomes it may be assumed that the reabsorption of colloid is rapidly taking place. This reabsorption is most rapid in PTU fed birds.
which have the greatest number of dense staining apical vesicles in their follicular cells.

The dynamics of colloid reabsorption are not well understood. Microvilli are considered by some authors to be possible indicators of colloid reabsorption (Olen 1969; Gaal, Kovacs and Sellers 1976) as are pseudopodia and colloid droplets (Fujita 1975). Microvilli are longest and most numerous in PTU fed animals (fig. 39) especially after 35 days of treatment while colloid droplets are rarely seen and pseudopodia are not common in any of the groups.

In the chicken, reabsorption of colloid during goiter development appears to be due mainly to micropinocytosis rather than phagocytosis of large colloid droplets by pseudopodia. These observations support the suggestion of Seljelid, Reith and Nakken (1970), Itakawa and Kawada (1974), Ketelbant-Balasse et al. (1976) and Kawada and Naito (1978) that colloid reabsorption occurs primarily by micropinocytotic processes and pseudopodia formation usually occurs only after acute TSH stimulation.

The presence of large colloid droplets within the cytoplasm of some follicle cells may arise by the fusion of many small endocytotic microvesicles (Seljelid, Reith and Nakken 1970) rather than from the mass engulfment of colloid by pseudopodia. The fusion of these light staining endocytotic microvesicles with dense staining primary and secondary lysosomes may be seen in fig. 34. It is possible that there are two parallel mechanisms of colloid reabsorption, micropinocytosis and phagocytosis. In the chicken micropinocytosis seems to be more important mechanism, at least after long term stimulation.
Exocytosis of colloid into the follicular lumen involves the budding of small exocytotic microvesicles from the golgi apparatus (Fujita 1975). These light staining exocytotic microvesicles are relatively indistinguishable from the endocytotic microvesicles and together they make up the large population of microvesicles in the apical region of the follicle cell. In methimazole fed birds there are often a greater number of microvesicles in the apical region than there are in the other groups. This could be due to an increase in the number of exocytotic microvesicles since the golgi are more dilated in methimazole fed animals than in other experimental groups.

The presence of the circular golgi apparatus in thiourea fed animals is difficult to explain. These golgi are similar to the "multimembranous" bodies seen by French and Hodges (1977) in control birds at 6 weeks of age except that the multimembranous bodies described by French and Hodges were wrapped around a small bit of rough endoplasmic reticulum. The presence of these circular golgi might be due to the toxicity of thiourea. Ortega (1969) reported similar structures in the liver cells of animals treated with 1000 ppm of DDT for 4 months.

The christae of the mitochondria in thyroid cells of thiourea fed birds are irregular and broken. This degeneration of mitochondria may be due to the toxic effect of thiourea. Pantic (1974) has also noted irregular looking mitochondria in animals fed goitrogens.

In PTU fed animals, thyroid enlargement is mainly due to cellular hyperplasia. In methimazole fed animals the initial development of the thyroid is due to cell replication but in the later stages enlargement is due to colloid accumulation. In thiourea fed birds, thyroid
enlargement is initially due to cell hypertrophy although some replication does occur. Later stages of enlargement have a small amount of colloid accumulation and cell atrophy resulting in no increase in protein per mg thyroid weight.

At 35 days of treatment, mean values of DNA in PTU fed birds are greater than those in methimazole fed birds although these differences are highly variable and therefore not significant. Increases in thyroid weights for the first 30 days of treatment are also greater in PTU fed animals. In addition, the effects of PTU administration are the earliest to appear suggesting that PTU is the more potent and rapid acting of the three drugs tested. The early onset of the effects of PTU on the thyroid are predictable according to the findings of Griessen and LeMarchand-Beraud (1973). Since PTU also affects the extrathyroidal deiodination of thyroxine (Hershman and Van Middlesworth 1962), there is a more rapid stimulation of TSH release by the pituitary occurring before the production of thyroxine by the thyroid is impaired.

Thioureylene antithyroid drugs are actively accumulated by the thyroid gland (Marchant et al. 1972). In mammals the thyroidal level of antithyroid compound rather than plasma levels appears to determine the degree of potency of the drug (Marchant et al. 1971). PTU has been found to accumulate to a greater extent in the rat thyroid than do other thioureylene drugs (Marchant et al. 1972) and this could account for the very high potency of PTU in the rat.

Methimazole and thiourea are also actively accumulated by the thyroid gland (Pittman, Beschi and Smitherman 1971; Marchant et al. 1972),
but in man, methimazole has a clinical therapeutic potency of approximately ten times that of PTU. The potency differences between methimazole and PTU in rats and man are probably due to differences in the metabolism of the drug within the thyroid.

In the chicken, it appears that PTU is the more potent drug although methimazole produces a larger gland and retards the growth of the bird to a greater extent. Methimazole does not promote the prolonged extensive cellular hyperplasia seen in the PTU treated animals. The greater weight of the thyroid in methimazole fed birds after 70 days is due to colloid accumulation and the retardation of body growth may be due to the direct inhibition of other pituitary hormones by methimazole (Muranyi-Kovacs, Rudai and Arnaud 1979). It has also been suggested by Pogorilor, Van Mannen and Sellers (1971) that methimazole may block cell receptors, thus preventing the action of any available thyroxine in the tissues of the animal.

Thiourea is by far the least potent of the three drugs tested. The thyroid glands of thiourea treated birds rarely have totally collapsed follicles. The toxic effect of thiourea on the thyroid is the most noticeable, although thiourea is the most rapidly metabolized drug of the three studied (Maloof and Soodak 1957).

Colloid reabsorption and the release of proteins to the follicular lumen are both stimulated by TSH. It is also possible that other factors may have important effects in the development of a goiter. The increased resistance of an animal to the effects of a goitrogenic agent with age (Sheline 1969) may be a possible explanation for the increase
in colloid seen in methimazole fed animals, however, this does not explain the responses of the bird to PTU and thiourea treatments.

Other factors, such as the extrathyroidal metabolism of the drug, toxic and other peripheral effects may influence the final response of the thyroid to a goitrogenic agent. These factors may have a much greater influence on the final development of a goiter in the chicken than they do in the rat even though the response of the rat to a goitrogen is more rapid.
Figure 8. Chick thyroid gland following the administration of propylthiouracil for 5 days.
Hematoxylin and eosin stain. (X 225)

Figure 9. Chick thyroid gland following the administration of methimazole for 5 days.
Hematoxylin and eosin stain. (X 225)
Figure 10. Chick thyroid gland following the administration of thiourea for 5 days. 

Hematoxylin and eosin stain. (X 225)

Figure 11. Thyroid gland of untreated 5-day-old chick. 

Hematoxylin and eosin stain. (X 225)
Figure 12. Chick thyroid gland following 15 days of PTU treatment.

There is a hypertrophy of follicle cells and increase in vascularity. Hematoxylin and eosin stain. (X 290)

Figure 13. Chick thyroid gland following 15 days of treatment with methimazole.

Follicles are smaller, cells are not as columnar as are those treated with other goitrogens. Hematoxylin and eosin stain. (X 225)
Figure 14. Chick thyroid gland following treatment with thiourea for 15 days.

Follicles are large and round, cells are very columnar. Hematoxylin and eosin stain. (X 290)

Figure 15. Thyroid gland from 25-day-old control bird.

Note the uniform increase in colloid (C) throughout the gland. Hematoxylin and eosin stain. (X 115)
Figure 16. Chick thyroid gland following 35 days of PTU treatment.

An increase in the vascularity and the presence of collapsed follicles can be seen. Hematoxylin and eosin stain. (X 225)

Figure 17. Chick thyroid gland following 55 days of PTU treatment.

Note that there is almost no colloid present throughout the gland. Hematoxylin and eosin stain. (X 115)
Figure 18. Chick thyroid following 35 days of MMI treatment. Note the vascularity and the abundance of cells. Hematoxylin and eosin stain. (X 115)

Figure 19. Chick thyroid gland following 55 days of treatment with MMI. An increase in the amount of colloid (C) and a uniformity to the size and arrangement of the follicles can be seen. Hematoxylin and eosin stain. (X 225)
Figure 20. Chick thyroid gland following 20 days of treatment with thiourea.

Note the great increase in the height of the follicle cells. Hematoxylin and eosin. (X 290)

Figure 21. Thyroid gland of chick treated with MMI for 20 days.

Note the great amount of folding (→) of the follicle walls. Hematoxylin and eosin. (X 115)
Figure 22. Chick thyroid gland after 35 days of thiourea treatment.

Note that colloid (C) is still abundant, vascularity has increased and there is little folding of the follicle walls. Hematoxylin and eosin stain. (X 225)

Figure 23. Edge of chick thyroid gland following 55 days of MMI treatment.

Increases in colloid (C) are greater at the edge of the gland (A) than in the middle of the gland (B). Hematoxylin and eosin stain. (X 50)
Figure 24. Chick thyroid gland following 70 days of PTU treatment.

The maintenance of hypertrophy and hyperplasia of the gland as well as an abundance of collapsed follicles is evident. Hematoxylin and eosin stain. (X 225)

Figure 25. Chick thyroid gland following 70 days of MMI treatment.

Note that the hypertrophy of the cells remain even though colloid has become more abundant. Hematoxylin and eosin stain. (X 225)
Figure 26. Chick thyroid gland following 70 days of thiourea treatment.

Note that the cells have become less hypertrophic. The follicles are still round. Hematoxylin and eosin stain. (X 225)

Figure 27. Thyroid gland of 70-day-old control bird.

The presence of large amounts of colloid and very squamosal follicle cells is evident. Hematoxylin and eosin stain. (X 225)
Figure 28. Thyroid follicle cells of normal 5-day-old chick.

Round nuclei (N), colloid droplets (CD), and a well developed endoplasmic reticulum (ER) are present. F - follicular lumen; B - basal membrane. (X 6,700)

Figure 29. Follicle cells of normal 25-day-old chick.

→ - lysosomes; F - follicular lumen; N - nucleus; CD - colloid droplet; ER - endoplasmic reticulum. (X 8,515)
Figure 28. Thyroid follicle cells of normal 5-day-old chick.

Figure 29. Follicle cells of normal 25-day-old chick.
Figure 30. Apical end of chick thyroid follicle cells after 15 days of PTU treatment.

Note the abundance of dense staining primary and secondary lysosomes (→) and the follicular lumen (F) filled with microvilli. N - nucleus; G - Golgi; ER - endoplasmic reticulum. (X 10,630)

Figure 31. Chick thyroid follicle cells following 20 days of PTU treatment.

Note the variety of vesicle types in the apical cytoplasm. CD - colloid droplet; F - follicular lumen; N - nucleus; mv - microvilli; L - 1° lysosome; M - mitochondria; 2 - 2° lysosomes. (X 29,220)
Figure 30. Apical end of chick thyroid follicle cells after 15 days of PTU treatment.

Figure 31. Chick thyroid follicle cells following 20 days of PTU treatment.
Figure 32. Chick thyroid follicle cells following 35 days of thiourea treatment.

Note the large abnormal mitochondria (M) and an abundance of golgi (G) and primary and secondary lysosomes (1, 2). N - nucleus; F - follicular lumen. (X 10,630)

Figure 33. Apical end of chick follicle cells following 66 days of methimazole treatment.

Note the abundance of dense staining secondary lysosomes and the colloid droplet (CD). F - follicular lumen; mv - microvilli. (X 24,645)
Figure 32. Chick thyroid follicle cells following 35 days of thiourea treatment.

Figure 33. Apical end of chick follicle cells following 66 days of methimazole treatment.
Figure 34. Apical region of chick thyroid follicle cell following 70 days of MMI treatment.

Dense staining secondary lysosomes fuse with light staining endocytotic microvesicles (→). M - mitochondria; N - nucleus; F - follicular lumen; ER - endoplasmic reticulum. (X13,520)

Figure 35. High magnification of thyroid follicle cell following the administration of thiourea for 35 days.

Note the circular golgi (G) and abnormal looking mitochondria (M). ER - endoplasmic reticulum; → - secretory vesicles. (X 17,840)
Figure 34. Apical region of chick thyroid follicle cell following 70 days of MMI treatment.

Figure 35. High magnification of thyroid follicle cell following the administration of thiourea for 35 days.
Figure 36. Chick thyroid follicle cells after 30 days of MMI treatment.

These cells have very irregular nuclei (N) and many dense staining lysosomes near the apical end of the cell. ER - endoplasmic reticulum; F - follicular lumen; End - endothelial cells. (X 6,700)

Figure 37. Two chick follicular cells after 45 days of thiourea treatment.

Differences between colloid cells (follicle A) and normal hypertrophic cells (follicle B) can be seen. N - nucleus; ER - endoplasmic reticulum; M - mitochondria; G - golgi. (X 6,700)
Figure 36. Chick thyroid follicle cells after 30 days of MMI treatment.

Figure 37. Two chick follicular cells after 45 days of thiourea treatment.
Figure 38. High magnification of chick thyroid follicle cell following 40 days of methimazole treatment.

Note the greatly enlarged and dilated golgi (G) and the abundance of secretory vesicles (sv) budding off. N - nucleus; L - lysosomes; M - mitochondria. (X 24,645)

Figure 39. High magnification of follicular lumen (F) following 66 days of PTU treatment.

Note the enlarged and elongated microvilli (mv). (X 17,840)
Figure 38. High magnification of chick thyroid follicle cell following 40 days of methimazole treatment.

Figure 39. High magnification of follicular lumen (F) following 66 days of PTU treatment.
Figure 40. Apical end of chick thyroid cells after 66 days of thiourea treatment.

N - nucleus; M - mitochondria; Mv - microvilli.
(X13,520)

Figure 41. High magnification of colloid cell.

G - golgi; N - nucleus; ER - endoplasmic reticulum; M - mitochondria. Thiourea treatment 20 days.
(X 10,630)
Figure 40. Apical end of chick thyroid cells after 56 days of thiourea treatment.

Figure 41. High magnification of colloid cell.
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