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The Retention of Goitrogens in the Blood and Tissues of Several Domestic Animals

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The Retention of Goitrogens in the Blood and Tissues of Several Domestic Animals

G. W. PIPES and C. W. TURNER

INTRODUCTION

During the past several years the investigation of simple organic compounds which inhibit the normal function of the thyroid gland has opened up new and fascinating opportunities for study in the field of endocrine chemistry. Through the use of these compounds, which produce what has been termed a "chemical thyroidectomy", our knowledge of the physiology and biochemistry of the thyroid has been greatly increased. The experimental use of these drugs has permitted an insight into the biosynthesis of thyroxine by the thyroid gland.

One of these goitrogens, thiouracil, has made possible quantitative studies of thyroid secretion rate and has elucidated the normal relationship of the thyroid gland to the processes of growth, reproduction and lactation.

Recent research has shown that inhibition of thyroid function in young, rapidly growing animals results in retarded proteinaceous growth and the accelerated deposition of fat. Goitrogenic drugs may bring us one step further in directing the endocrine functions of domestic animals into more efficient and profitable channels.

Since thiouracil is a valuable research tool and shows great promise as a fattening agent for livestock, it would be of interest to better understand its rate of absorption from the digestive tract and its retention in the tissues. If goitrogens are to be of practical importance in the fattening of livestock, it is essential to know if these compounds are present in the tissues in significant amounts to make the flesh unsafe for human consumption.

The objects of the present study were:

1. To investigate the rapidity of absorption of several goitrogens from the digestive tract.

2. To investigate the diurnal concentration of these compounds in the blood stream during oral administration.

3. To determine the goitrogenic activity of tissues from animals fed thiouracil and other goitrogens.

THE RELATIONSHIP OF CHEMICAL STRUCTURE TO GOITROGENIC ACTIVITY

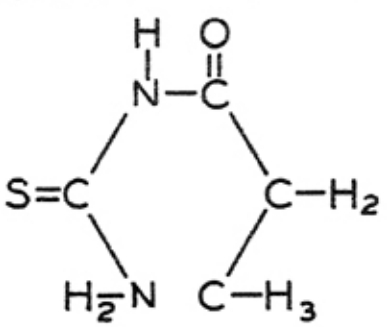
It has been demonstrated that many derivatives of aniline and of thiourea inhibit the synthesis of the thyroid hormone by the thyroid gland (Mackenzie, Mackenzie, and McCollum, 1941; Richter and Clisby, 1941; Kennedy, 1942; and Mackenzie and Mackenzie, 1943).

Astwood (1943a), from a study of 106 compounds of related structure, selected thiouracil for further investigation, on the basis of its high goitrogenic activity and low toxicity. Since this time thiouracil has been investigated extensively, (Astwood, 1943b; Paschkis, et. al., 1944; and Rawson, et. al., 1944a) and has been found to possess marked advantages over thiourea and certain other goitrogenic agents employed in the past.

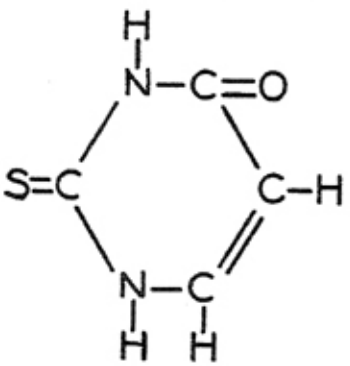
Since earlier studies indicated that the more active goitrogens could be considered to be derivatives either of aniline or thiourea, much investigational work has been concentrated on compounds possessing these groupings or closely related structures. The studies of McGinty and Bywater (1945a), Bywater, McGinty and Jenesel (1945), McGinty and Bywater (1945b), and Astwood et. al. (1945) has lead to a better understanding of the relationship of chemical structure to goitrogenic activity and has resulted in the discovery of goitrogens of increased potency. These investigators, through the use of quantitative dose-thyroid response methods have been able to make comparisons of the activity of many goitrogenic compounds (See Table 1). In these studies thiouracil was given a value of 1.0 by Astwood and 100 by McGinty. The rat was used as a test animal by these investigators.

Thioureas. -- Twenty-two derivatives of thiourea studied by Astwood, et. al. (1945) were found to be less potent and more toxic than thiouracil. The replacement of all four hydrogens of thiourea by methyl groups as in tetramethyl thiourea enhanced activity. The diethyl, isopropyl and diisopropyl derivatives were found to be the most potent of the open chain compounds, but the substitution of large constituents on the thiourea molecule as illustrated by diheptylthiourea resulted in decreased activity. The presence of polar groups such as amino or carbonyl attached to either of the nitrogen atoms of thiourea or substitution on sulphur atom reduced the activity. (See thiourea derivatives, Table 1).

McGinty and Bywater (1945a) have suggested that ring closure may produce greatly increased activity as is shown by comparison of propionylthiourea and thiouracil.



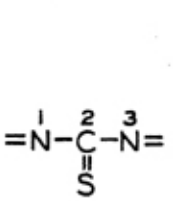
Propionylthiourea (0.14)



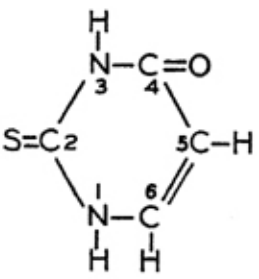
Thiouracil (1.0)

Astwood et. al., (1945) report that dithiobiurea, a compound containing two complete thioureylene groups, was completely inactive. An equally striking observation was the inactivity of sulfanilylthiourea, a

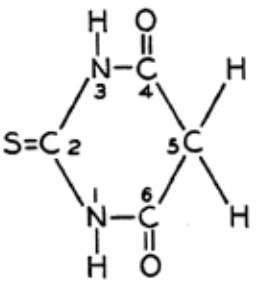
TABLE 1. -- COMPARATIVE ACTIVITY OF GOITROGENIC COMPOUNDS



Thiourea



Thiouracil

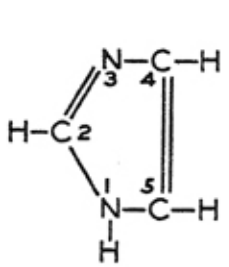


Thiobarbituric acid

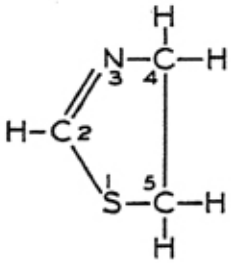
Compound	Formula	Estimated Activity Thiouracil = 1.0	Investigator
	Thioureas		
Thiourea	NH_2CSNH_2	0.12 0.09	1* 2
1, 1, 3,3-tetramethyl thiourea	$(CH_3)_2NCSN(CH_3)_2$	0.3	1
1,3-diethylthiourea	$C_2HSNHCSNHC_2HS$	0.4, 0.47	1, 2
1,-isopropylthiourea	$NH_2CSNHCH(CH_3)_2$	0.4	1
1,3-diheptylthiourea	$C_7H_{15}SNHCSNHC_7H_{15}S$	0.01	1
1,3-diacetylthiourea	$CH_3CONHCSNHCOCH_3$	0.01	1
1,3-diaminothiourea	$NH_2NHCSNHNH_2$	0.03	1
2-ethylisothioureasulfate	$(NH_2C(SC_2HS)NH_2\ H_2SO_4$	0.01	1
1-sulfanilythiourea	$NH_2CSNHSO_2$	0.003	1
1-propionylthiourea	$C_2HSCONHCSNH_2$	0.14	2
2,5-dithiobiurea	$NH_2CSNHNHCSNH_2$	0.01	1
Thiouracil		1.0	1&2*
Dihydrothiouracil	(5)-CH_2 (6) H_2	0.08	1
5-methylthiouracil	(5)-CH_3	0.7	1
5-ethylthiouracil	(5)-C_2H_5	3.5	1
5-isopropylthiouracil	(5)-$CH(CH_3)_2$	2.5	1
6-n-propylthiouracil	(6)-$CH_2\ CH_2\ CH_3$	11.0	1
6-isopropylthiouracil	(6)-$CH_2(CH_3)_2$	9.0	1
6-ethylthiouracil	(6)-$CH_2\ CH_3$	8.0	1
6-n-butylthiouracil	(6)-$CH_2\ CH_2\ CH_2\ CH_3$	3.0	1
6-n-amylthiouracil	(6)-$CH_2\ CH_2\ CH_2\ CH_2\ CH_3$	1.3	1
6-n-hexylthiouracil	(6)-$CH_2\ CH_2\ CH_2\ CH_2\ CH_2\ CH_2$	0.18	1
5,-methyl-6-ethylthiouracil	(5)-CH_3 (6)-$CH_2\ CH_3$	0.9	1
5,6-trimethylenethiouracil	(5,6)-$CH_2\ CH_2\ CH_2$-	0.3	1
5,-methyl-2-ethylthiouracil	(2)-SC_2H_5 (5)-CH_3	0.03	1
Thiobarbituric acid		0.0	2
		0.003	1
5,ethylthiobarbituric acid	(5)-$CH_2\ CH_3$	0.003	1
5,5,diethylthiobarbituric acid	(5)-$CH_2\ CH_3$,-$CH_2\ CH_3$	1.7,1.23	1, 2
5,5,diallythiobarbituric acid	(5)-CH_2 CH CH_2 -CH_2 CH CH_2	1.0	1

(1)*Astwood et al. (1945)
(2) McGinty et al. (1945)

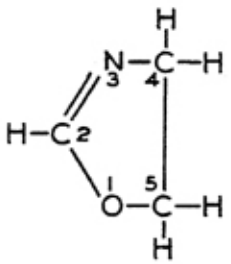
TABLE 1 (CONT.) COMPARATIVE ACTIVITY OF GOITROGENIC COMPOUNDS



Imidazoles

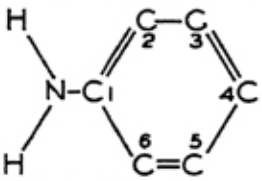


Thiazolines



Oxazolines

Compound	Formula	Estimated Activity Thiouracil = 1.0	Investigator
	Imidazoles		
2-mercaptoimidazole	N-HC(SH)NCHCH	1.5	1
2-mercaptobenzimidazole	$NHCSHNC_6H_4$	0.5	1
"	"	1.16	2
	Thiazolines		
2-mercaptothiazoline	$SC(SH)NCH_2CH_2$	1.3, 1.31	1,2
5,5,-dimethyl-2 mercapto-thiazoline	$SC(SH)NCH_2C(CH_3)_2$	0.3	1
	Oxazolines		
2-mercaptooxazoline	$OC(SH)NCH_2CH_2$	0.2	1
5,5-dimethyl-2 mercapto-oxazoline	$OC(SH)NCH_2C(CH_3)_2$	0.4	1



Amino Benzene

	Amino Benzene derivatives		
4,aminobenzoic acid	$H_2NC_6H_4COOH$	0.003, 0.003	2, 1
4,4,diaminodiphenylmethane	$H_2NC_6H_4CH_2C_6H_4NH_2$	0.25	1
Bis-(4 dimethylaminophenyl) methane	$(CH_3)_2NC_6H_4CH_2C_6H_4N(CH_3)_2$	0.25	1
2-amino-5-sulfanilythiazole (promizole)	$H_2NC_6H_4SO_2$ CCHNCNH$_2$S	0.18, 0.25	2, 1
Sulfadiazine	$H_2NC_6H_4SO_2NH$ CNCHCHCHN	0.1	1

(1)*Astwood et al. (1945)
(2) McGinty et al. (1945)

compound that contained within its molecule both the thiocarbamyl and the aminophenyl groupings, the only structures known to possess goitrogenic activity.

Imidazoles, Thiazoles and Oxazolines.--These workers have also demonstrated that five membered ring structures containing a thioureylene group may possess greater potency than thiourea (see Imidazoles, Table 1). Of the ten compounds studied in this series, 2-mercaptoimidazole was the most active.

The substitution of a nitrogen atom of the thioureylene group in a five membered ring by a sulfur or oxygen atom did not destroy the activity as was shown by 2-mercaptooxalzoline (see thiazoles and oxazolines, Table 1). The goitrogenic activity of these compounds indicates, as has been suggested by Astwood, that the essential structure for goitrogenic activity is not necessarily the entire thioureylene radical, but a $N - \overset{S}{\overset{\|}{C}} - X$ grouping where X is an O, S or N atom.

Thiouracils.-- The thiouracil derivatives studied by these investigators revealed some interesting relationships of structure to activity. Dehydrothiouracil which was found to possess approximately one-tenth the activity of thiouracil demonstrates the importance of the double bond between carbon atoms 5 and 6. Substitution on the carbon at position 5 by an ethyl group produced increased activity, but the substitution of a methyl or isopropyl side chain at this position resulted in decreased activity. The replacement of hydrogen atoms at carbon 6 by hydrocarbon substituents produced striking results. At this position the substitution of a propyl group produced a derivative which was claimed to be 11 times as effective as thiouracil. Longer hydrocarbon chains at position 6 resulted in progressively decreasing activity. The substitution of hydrogen at both the 5 and 6 positions by hydrocarbon side chains produced compounds possessing less activity than those substituted at 6 alone (see thiouracils, Table 1). As in the thioureas, the replacement of a hydrogen on the nitrogen atoms by a polar group or substitution on the sulfur atom resulted in decreased activity. The more effective compounds in this group exhibited greater toxicity than thiouracil, but toxicity increased at a slower rate than antithyroid activity. At physiologically equivalent dosages many of these compounds were found to possess far less toxicity than thiouracil.

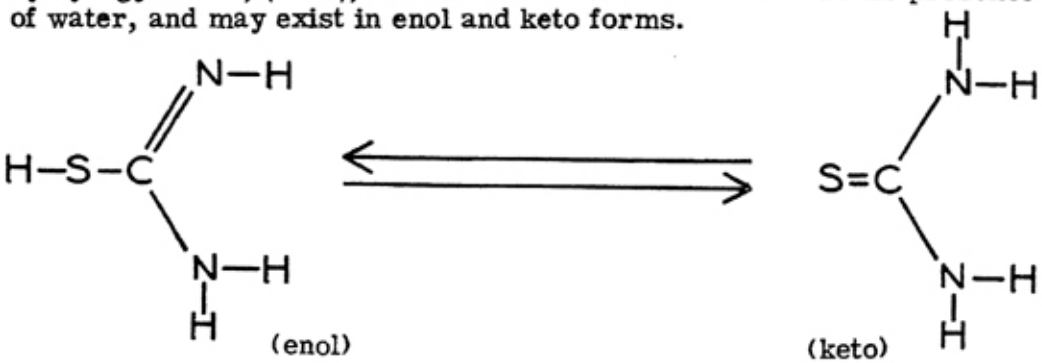
Thiobarbituric acids.-- The next modification of the thiouracil structure investigated by these workers was the effect of a carbonyl group at position 6 (see thiobarbituric acids, Table 1). The resulting thiobarbituric acid was found to be inactive and since this compound can be considered to be the keto form of 6-hydroxy thiouracil which had already been shown to lack goitrogenic properties, this observation was not surprising. However, when both hydrogens at position 5 are substituted by ethyl groups, the resulting compound is found to be more potent than thiouracil. As was suggested by Astwood et al. (1945) this activity may be due to the fact that enolization can no longer take place.

Aminobenzene derivatives.-- All of the compounds discussed so far can be considered to be derivatives of thiourea. The second active structure, the aminobenzine series, has also been investigated by Astwood et al. (1945). All active compounds in this series possessed an amino group attached to an aromatic ring. Sixteen benzene derivatives, lacking an aminophenyl group, were found to be inactive. This strongly suggests that the goitrogenic properties of these compounds are produced

by the aromatic amino group. The most effective compounds of this type were 4,4 diamino diphenylmethane and its derivatives. These drugs were found to be about twice as effective as thiourea and one-fourth as effective as thiouracil.

Sulfadiazine was found to be the most active of the sulfonamides as had previously been demonstrated (Astwood, 1943a).

The relationship of acidity or ease of oxidation to goitrogenic activity has not been studied in the derivatives of thiourea, but since many of the more active goitrogens are weak acids and also may act as antioxidants, these possibilities should be investigated. As has been shown by Gyorgy et. al., (1943), thiourea acts as an antioxidant in the presence of water, and may exist in enol and keto forms.



It is interesting to note in this respect that the studies of Astwood et al. (1945) have demonstrated that substitution on the sulfur atom of thiourea or thiouracil destroys goitrogenic activity. However, tetramethyl thiourea is among the most potent of the thioureas, and yet no hydrogen is available for enolization.

Taurog, Chaikoff and Franklin (1945) attempted to find the molecular structure essential for the inhibition of the in vitro conversion of inorganic iodine to thyroxine by thyroid tissue. These workers investigated forty compounds related to the sulfonamides and found a definite correlation between ease of oxidation and inhibitory activity. A free amino or free aromatic hydroxyl group was found to increase inhibitory activity. The acetylation of an amino group was found to reduce the activity.

While the comparative activity of goitrogens has not been extensively investigated in animals other than the rat, Vanderlaan and Bissell (1946) claim that these drugs possess approximately the same relative activity in the chick. As related to thiouracil, the comparative potency of the compounds studied by these investigators were found to be 6-propyl thiouracil 10; the three 6-butyl derivatives of thiouracil, 10; 6 ethyl thiouracil, 5; thiobarbital, 1; thiourea, 0.3. Jensen and Kjerulf-Jensen (1945) investigated the goitrogenic effect of 96 compounds in the rat. Compounds investigated included sulphonic acids, sulphones, organic thiocyantes and sulphides, thiols, thiamides, pyrimidine derivatives, many heterocyclic compounds and a number of compounds which form stable complexes with copper. These workers concluded that while all strongly active compounds studied were thioureas or thiouracils the effect was very specific since the displacement of a methyl group was sufficient to prevent it. Williams and Kay (1947b) confirmed the conclusions of Astwood et al. (1945) concerning the relationship of chemical structure to goitrogenicity. These investigators studied the action of over forty compounds including thiouracils, thioureas, anilides and iodinated benzoic

acid derivatives in the rat. The iodinated compounds demonstrated little or no effect and none of the compounds studied were more effective than the substituted thiouracils.

As thiourea was replaced by thiouracil, thiouracil has been replaced by 6 -propyl thiouracil in the treatment of thyrotoxicosis. (Astwood and Vanderlaan, 1945; Williams 1947 and others). It is not improbable that further research will provide compounds of greater potency and lessened toxicity.

At the present time, while extensive studies have been made of the goitrogenic activity of many chemical compounds in man and rat, little evidence is available to demonstrate their relative effectiveness in domestic animals. The marked variation in effectiveness in the rat as compared with the human indicates that large variations may exist between species. A study of the effectiveness of various goitrogenic substances as fattening agents for livestock, should yield valuable and essential information.

THE MODE OF ACTION OF GOITROGENIC COMPOUNDS

Since the earlier literature on the mode of action of thyroid inhibiting drugs has been reviewed by Schultze and Turner (1945), an extensive survey will not be included here. In view of the newer knowledge concerning the effect of goitrogenic compounds upon the chemistry of the thyroid, an attempt will be made to show how these drugs may prevent the biosynthesis of the thyroid hormone.

Since Mackenzie, Mackenzie, and McCollum (1941) first observed thyroid hyperplasia and hypertrophy in rats fed sulfaguanidine, it has been well established that thiouracil and other goitrogens block the formation of the thyroid hormone. Astwood et al., (1943) and Mackenzie and Mackenzie, (1943) reported that thyroid hypertrophy did not take place in hypophysectomized animals and the calorigenic effect of injected thyroxine was not prevented by thiouracil administration. These workers concluded that the deficiency of thyroid hormone brought about by goitrogenic drugs causes an excess of thyrotrophic hormone to be produced and released by the pituitary. Under the stimulation of thyrotrophin, the thyroid undergoes hypertrophy and the stored thyroid hormone is released. In spite of increased size and apparent activity, the thyroid gland in the presence of goitrogens is unable to synthesize the thyroid hormone, and is still subjected to increased stimulation by the pituitary. Additional evidence that thyroid enlargement is due to the thyrotrophic hormone has been given by Larson et al. (1945a) who compared the effects of thyrotrophin injection with thiouracil administration and observed similar histological and anatomical changes in the chick thyroid. Albert et al. (1947a) reported that thyrotrophic hormone lost activity during exposure to Lugol's solutions but could be reactivated by goitrogens and other reducing agents. Albert et al. (1947b) observed that exposure of thyrotrophic hormone to goitrogens resulted in augmented activity even when the goitrogen was presumably removed prior to bio-assay. While insufficient evidence is available at present to be conclusive, the two papers cited above indicate that the goitrogens may directly augment the thyrotrophic hormone in addition to their stimulation of thyrotrophic hormone secretion through suppression of thyroxine synthesis. Direct evidence that goitrogenic agents prevent the synthesis of thyroxine has been obtained through the use of radioactive iodine as an indicator of iodine metabolism.

Rawson, Tannheimer and Peacock (1944), Franklin et al. (1944), and Larson et al. (1945, 1945b) found reduced collection of radioactive iodine by the thyroid during thiouracil administration. While Larson suggested that thiouracil's ability to inhibit the collection of iodine may be its essential and primary effect. McGinty and Sharpe (1946) and Vanderlaan and Bissell (1946) later reported that thyroids enlarged by treatment with thiouracil possessed an enhanced ability to collect injected iodine. However, the effect was transitory and since the thyroid was unable to bind iodine in an organic form the iodine concentration rapidly fell to preinjection levels.

Franklin and Chaikoff (1944) and Schachner, Franklin and Chaikoff (1944) observed that sulfonamides prevented the formation of organic iodine but did not decrease the iodine concentrating capacity of the thyroid. Kestone et al., (1944) found reduced amounts of organic iodine in the thyroid after thiourea administration. Franklin, Lerner, and Chaikoff (1944) demonstrated that thiouracil prevented the formation of diiodotyrosine and thyroxine. These investigators found that the diiodotyrosine and thyroxine fractions of the thyroid contained reduced amounts of iodine in animals fed thiouracil.

In the biosynthesis of thyroxine it appears necessary for at least three chemical reactions to take place within the thyroid gland. The first of these reactions involves the liberation of iodine from iodides; second, the iodination of tyrosine to 3,5, diiodotyrosine; and third, the oxidative coupling of two molecules of diiodotyrosine to form thyroxine.

These reactions are believed to take place with thyrosine groups within the protein molecule and not with free tyrosine itself. Direct evidence that this series of reactions takes place within the thyroid gland has been given by Perlman et al. (1941), Mann, Leblond, and Warren (1942), and Leblond, et al. (1943) who have employed radioactive iodine in their investigations of this process. Reineke and Turner (1942) in their preparation of highly active iodinated proteins seem to have duplicated to a large extent the reactions that may produce thyroxine within the thyroid gland.

The investigations of Reineke and Turner (1942, 1946) as well as those of Harington and Rivers (1945) and others indicate that oxidative conditions are necessary for the conversion of diiodotyrosine to thyroxine. Further evidence suggesting the importance of the oxidative function of the thyroid has been reported by DeRobertis and Goncalves (1945). These workers, using microdissection techniques and supervital methods, found the normal oxidation reduction potential of thyroid cells to be +0.050 volts and that of the colloid to be -0.200 volts. Activation by cold or thyrotrophic hormone raised the oxidation-reduction potential of the colloid to that of the cells, +0.05 volts, while thiourea lowered the potential of activated cells and colloid alike to -0.200 volts. The oxidation-reduction potential was not depressed by sulfanilamide. An impressive array of evidence has accumulated to indicate that the goitrogenic activity of thiouracil and related compounds may be due to the inactivation of an oxidative enzyme system necessary for synthesis of the thyroid hormone. The presence of peroxidase in the thyroid and its inactivation by thiouracil or thiourea has been demonstrated by Dempsey (1944) and confirmed by DeRobertis and Grasso (1946). However Glock (1946) failed to isolate peroxidase from thyroid tissue. DeRobertis and Grasso have also found that thyroid extracts containing peroxidase will liberate iodine from iodides. Since the oxidative coupling of two molecules of paracresol into a quinol ether linkage is brought about by the presence of peroxidase

(Westerfeld and Lowe, 1942), it is not unlikely that this oxidative enzyme may also enter into a chemically similar reaction -- the condensation of two molecules of diiodotyrosine to form thyroxine.

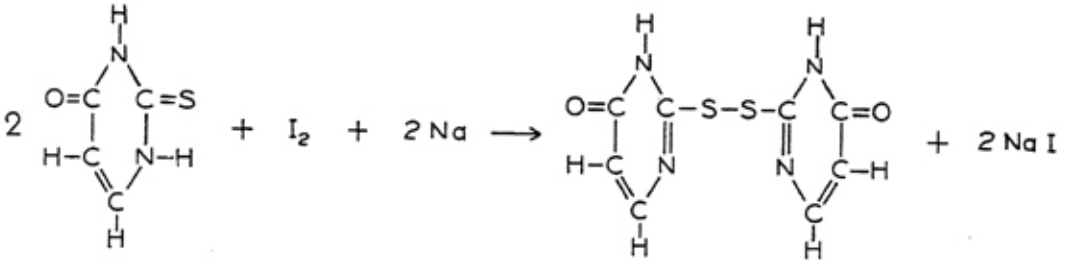
Dempsey (1944) observed that cytochrome oxidase is present in the thyroid but is not inhibited by thiouracil. Contrary to this observation Paschkis et al. (1945) found thiouracil inactivates cytochrome oxidase.

Other enzymes present in the thyroid which are not inhibited by various goitrogens include succinoxidase (McShan et al. 1946) and the proteolytic enzyme (De Robertis and Grasso 1946).

While xanthine oxidase (Keston 1944) and tyrosinase (Paschkis et al. 1944) are inhibited by thiourea, the importance of these enzymes in thyroxine synthesis has not been demonstrated.

In view of the discovery of acid and alkaline glycerophosphatase in the rat thyroid (Dempsey and Singer 1946), the effect of goitrogens upon this enzyme system should be investigated.

Miller et al. (1945) demonstrated that equivalent quantities of thiouracil and iodine react rapidly at the pH of body tissues to yield the disulfide of thiouracil.



The disulfide of thiouracil was found to react with several equivalents of iodine. These workers also observed that iodination of casein by the method of Reineke and Turner (1942) was prevented by thiouracil and that the iodination of tyrosine was unsuccessful in the presence of thiouracil. Goitrogenic aniline derivatives such as paraminobenzoic acid were found to react more slowly and with smaller quantities of iodine than did thiouracil.

While Astwood et al. (1943) reported that nontoxic levels of iodide failed to inhibit a hyperplastic response to thiourea or sulfaguanidine, the later studies of Astwood (1945) and McGinty and Sharpe (1946) revealed that iodide decreased the thyroid enlargement produced by thiouracil. Mackenzie (1947) reported that while small amounts of iodide produced a 50 to 100 per cent inhibition of the goitrogenic action of thiouracil, iodide augmented the goitrogenic effect of sulfaguanidine. This investigator concluded that thiouracil and the thioureas exert their effect by the reduction of iodine as fast as it is formed by the enzymatic oxidation of iodide, while sulfaguanidine inhibits an enzyme essential in the formation of thyroxine.

Dvoskin (1947) found that subcutaneous injection of elemental iodine completely inhibited the goitrogenic effects of thiouracil and sulfaguanindine. While this worker stated that on the basis of the dosages employed the action of elemental iodine is outside the range of a simple chemical neutralization of the goitrogens, this possibility still exists if the iodide concentrating ability of the thyroid and the rapid metabolism of thiouracil are considered. Jensen and Kjerulf-Jensen (1945) found no possibility of explaining the effect of goitrogens on the basis of inhibition of copper containing enzymes.

From the evidence available at the present time it seems highly probable that the mode of action of goitrogens may depend upon their chemical structure. Thiouracil and compounds of related structure may have a three fold action. First, these compounds through inactivation of peroxidase, prevent the liberation of iodine; second, through competitive action, prevent the iodination of tyrosine; and third, through the inactivation of peroxidase and lowering of the oxidation-reduction potential, prevent the oxidative coupling of diiodotyrosine to form thyroxine.

The less active sulfonamides and other aniline derivatives do not prevent the collection of iodine, inhibit peroxidase action or depress the oxidation-reduction potential of the thyroid. However, these compounds have been shown to react with free iodine (DeRobertis and Grasso, 1946) (Miller et al., 1945). The only known mechanism whereby these drugs may act is by competitive action for free iodine and the resultant iodination of these compounds in preference to tyrosine.

THE QUANTITATIVE DETERMINATION OF COMPOUNDS CONTAINING THE THIOUREYLENE GROUP

It has been demonstrated by Astwood (1943a), Astwood et al., (1945), Bywaters et al., (1945), McGinty et al., (1945a, 1945b), Williams and Kay (1947b) and others that many of the most active goitrogens contain the thioureylene grouping. Many goitrogenic compounds may be determined by methods based upon the reagent developed by Grote (1931) for detection of compounds containing the E=S grouping where E is any single non-metallic element. Grote's reagent was prepared through the reduction of sodium nitroferricyanide in sodium bicarbonate solution to aquoferrocyanide by hydroxylamine. Aquoferrocyanide on treatment with bromine reacts to produce sodium aquoferricyanide which on standing in sodium bicarbonate solution is changed to a compound of unknown structure. This compound produces characteristic color reactions with many compounds containing the E=S linkage. Grote (1931) reported that at a pH of 8 to 9 interference, due to ketones, aldehydes, creatine, thioethers and alkaloids, was practically eliminated.

Williams, Jandorf and Kay (1944) proposed a method based upon the use of a modified Grote's reagent for the determination of thiouracil in blood and tissues. This method required a twelve hour enzyme digestion and a time consuming pH adjustment. Danowski (1944), Campbell et al., (1944) and Fishberg and Vorgimer (1945) have also developed methods for the determination of thiourea and thiouracil. Chesley (1944) developed a more satisfactory method for determination of small amounts of thiourea in blood and urine. This investigator studied the effect of pH upon the reaction and pointed out the importance of time intervals in preparing a reproduceable reagent. Paschkis et al., (1945) adapted the method of Chesley (1944a) to the determination of thiouracil.

Christensen (1945, 1946) developed methods for the determination of thiouracil, thiobarbital and several substituted thiouracils. This investigator reported that, contrary to the observations of Williams, Jandorf & Kay (1944), acid deproteinizing agents were most useful for preparing protein free filtrates and that thiouracil was entirely ultrafiltrable below pH 3. Williams and Kay (1947a) substituted cadmium chloride for the sodium tungstate-copper sulfate protein precipitating agent used in their previous investigations (Williams, Jandorf and Kay 1944) and proposed methods for determination of several of the substituted thiouracils.

Olson, Ely and Reineke (1947) developed a process of aging the stock color reagent to produce a stable and reproducable reagent and eliminated the necessity reported by other investigators for establishing a standard curve with each set of determinations. These investigators also found that maximum color intensities could be developed in 5 to 15 minutes at a temperature of 50° C.

The investigations described in the present paper were concluded before the publication of the rapid method proposed by Olsen, Ely and Reineke. In our studies the lengthy method of Chesley (1944a) as modified by Paschkis et al. (1945) was used for the determination of thiouracil except in the studies of comparative blood levels produced by thiouracil, thiobarbital, and 6 n-propyl thiouracil when the methods of Christensen (1945, 1946) were employed.

Attempts to use the methods of Williams, Jandorf and Kay (1944) and other investigators for the determination of thiouracil in tissue extracts met with consistant failure due to the production of a red color and other side reactions which apparently destroyed the reagent.

EXPERIMENTAL METHODS AND MATERIALS

Our initial studies of the metabolism of several of the more effective goitrogens were made on fowls of the White Plymouth Rock breed. This animal was chosen due to its ready availability and its convenience as a laboratory animal. The birds employed in this investigation were reared and housed in an artificially-lighted and well ventilated animal room. The standard ration for birds of all ages consisted of the following ingredients:

Yellow corn meal	45 parts by weight.
Shorts	15
Soybean oil meal	15
Meat scraps, 50% protein	7
Alfalfa meal	10
Bran	5
Bone meal	0.5
Commercial salt	1.0
Cod liver oil (400 AOAC units vitamin D/gram)	0.25

Thiouracil and the other goitrogens employed were mixed with the feed in the following manner: A weighed amount was added to approximately a kilogram of feed in a small container. The container was shaken vigorously until the drug was evenly dispersed throughout. This mixture was then added to the main bulk of the feed and agitated in a rotary feed mixer.

Each group of birds was equally divided insofar as possible into subgroups of equal size and weight.

The three-week-old chicks were killed by ether and a four milliliter sample was drawn from the jugular vein into a dry centrifuge tube. Blood samples were taken from the older birds after the esophagus had been secured and the bird decapitated.

The blood samples were allowed to stand 15 minutes, stirred with a clean glass rod to break up the coagulum and were centrifuged. The serum was separated and stored at +5° C. until analyzed.

Thiouracil determinations were made by the method of Chesley (1944a) as modified by Paschkis et al. (1945) except for the studies of comparative blood levels of thiouracil, thiobarbital and 6 n-propyl thiouracil. The latter comparisons were made employing the method of Christensen (1945-46) for determination of these goitrogens.

The Effect of Increasing Dosage on the Thiouracil Content of the Blood of White Rock Chicks. -- Six groups of ten, three-week-old chicks were fed concentrations of 0.1 to 0.2 per cent thiouracil in the standard chick ration for a period of one week. Three hours after the morning feeding blood samples were taken from each chick and thiouracil was determined as previously described. The thiouracil content of the blood was found to increase with increasing dosages until the 0.6 per cent level was reached. (Fig. 1)

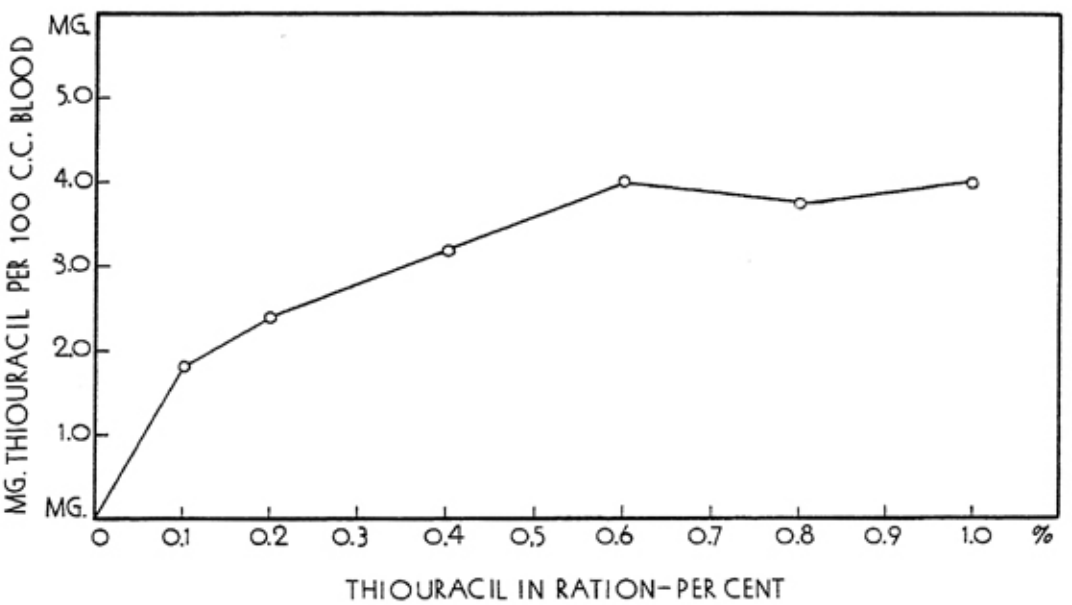


Fig. 1. Relationship of thiouracil concentrations in the ration to thiouracil concentrations in the blood of White Rock Chicks.

The 0.8 and 1.0 per cent levels did not produce further increases in the thiouracil content of the blood.

Similar results have been reported by Williams et al. (1944) and Williams (1944). Christensen (1945) suggested that higher dosages may result in increased uptake of thiouracil by tissue proteins and increased renal excretion.

Diurnal Concentrations of Thiouracil in the Blood of Chicks. -- Three groups of 72 three-week-old chicks were fasted for 12 hours and then allowed access for two hours to rations oontaining 0.1, 0.2, and 0.6 per cent thiouracil, respectively. Three birds from each group were sacrificed at two-hour intervals over a 24-hour period. Blood samples were taken and thiouracil was determined. Thiouracil concentrations (Fig. 2) were found to reach a peak two hours after the chicks had received feed containing 0.1 or 0.2 per cent thiouracil. After four hours the maximum blood level was reached in the group receiving 0.6 per cent until none could be detected after 14, 18, and 20 hours, respectively.

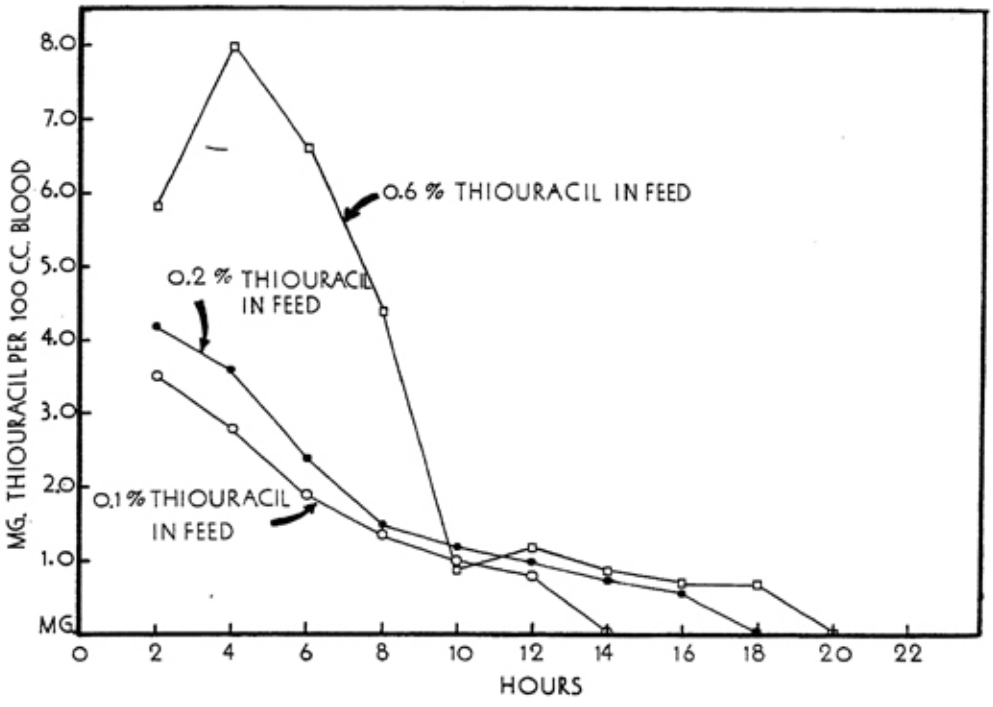


Fig. 2. Thiouracil levels produced and maintained in the blood of chicks by varying percentages of thiouracil in the ration.

Two-year-old Hens. -- Eighteen two-year-old White Leghorn hens were fasted twelve hours and fed a ration containing 0.6 per cent thiouracil for a two-hour period. Two birds were killed at intervals during the following 24 hours and blood samples were taken. The maximum thiouracil concentrations were found four hours after feeding. After 24 hours no trace could be detected in the blood. (Fig. 3).

The Effects of Feeding Interval on the Thiouracil Content of the Blood. -- Seventy-two three-week-old chicks were fed at four and one-half hour intervals during a 12-hour daylight period. The birds were allowed access for one hour at these intervals to feed containing 0.1 per cent thiouracil. Three chicks were killed each hour during a 24-hour period following the first feeding. The thiouracil content of the blood remained relatively uniform until ten hours after the last feeding (Fig. 4). Fourteen hours after feeding, only a trace could be detected in the blood.

Thiouracil Concentrations in the Blood Under Normal Feeding Conditions. -- Sixty-nine three-week-old chicks were fed a ration containing 0.1 per cent thiouracil during a normal feeding period. Three chicks were killed each hour for 16 hours and blood samples were taken. Samples were also taken after 24 hours. The maximum thiouracil content of the blood was found four hours after the chicks first received it in their feed. The thiouracil content of the blood declined rapidly until none could be detected after 14 hours (See Fig. 5).

Indirect evidence that thiouracil is not maintained in high concentrations in the blood of fowls has been given by Larson et al. (1945b). These investigators found strong inhibition of the collection of iodine by the chick thyroid one hour after injection of the thiouracil. Twenty-four hours after injection, the inhibiting effect had decreased. After 48 hours, the collection of iodine closely approached the normal level.

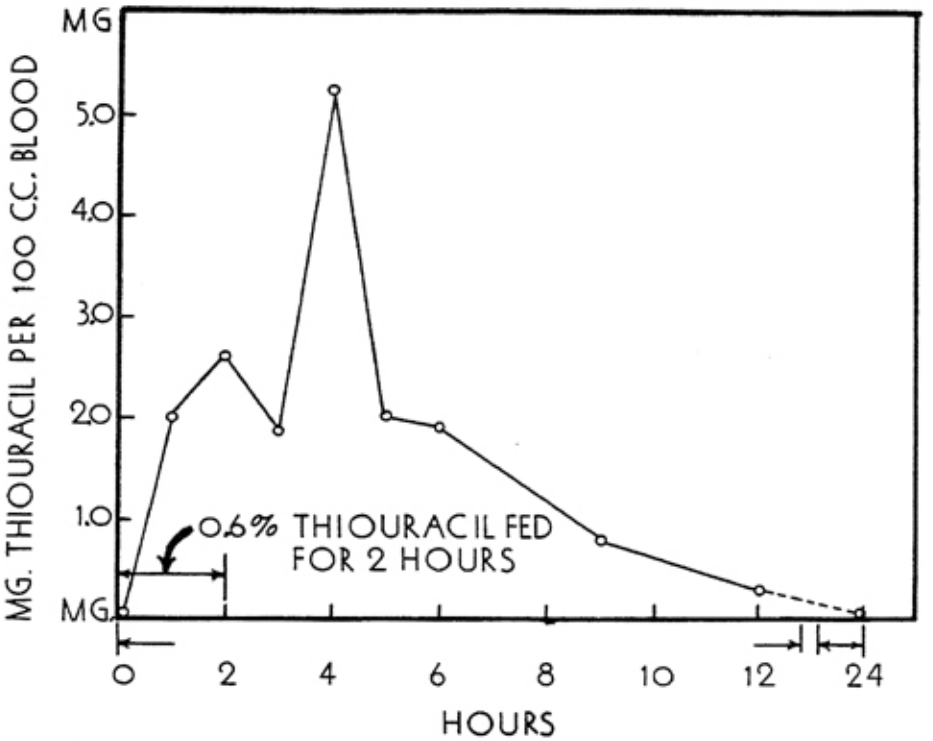


Fig. 3. Thiouracil concentrations produced in the blood of White Rock hen after a single feeding.

Andrews and Schnetzler (1946) observed an increased thyroid enlargement in fattening broiler by giving an additional feeding of thiouracil at 1:00 A.M. under artificial light. The increased thyroid size produced by additional feedings at a time when the thiouracil content of the blood is low, (Fig. 2 to 5) suggests the possibility of renewed thyroid secretion during this period.

The Non-Permeability of the Fowl Crop to Thiouracil. -- Since feed has been shown to remain in the crop for several hours (Heuser, 1945) and the absorption of thiouracil is rapid (Fig. 2 to 5), the possibility of absorption from the crop into the blood stream was investigated.

Single ligatures of silk thread were placed approximately one centimeter below the crops of six White Rock cockerels. Six non-ligatured cockerels and the six ligatured birds, were fed the standard mash plus 0.6 per cent thiouracil for a period of two hours. One bird from each group was killed and blood samples taken at hourly intervals for a six-hour period. The crops of all 12 birds were found to contain a considerable quantity of feed.

The blood of the non-ligatured birds was found to contain thiouracil in every case (Table 2) while only one of the ligatured fowls (Table 2, No. 3262) was found to contain thiouracil. On post mortem examination, faint traces of feed were found in the proventriculus of this bird indicating that the ligature had permitted water and finely ground feed to pass.

This technique for studying the absorption of thiouracil from the crop was not entirely satisfactory since the single ligature sometimes failed to prevent the passage of food from the crop and since it was impossible to measure the exact amount of thiouracil administered.

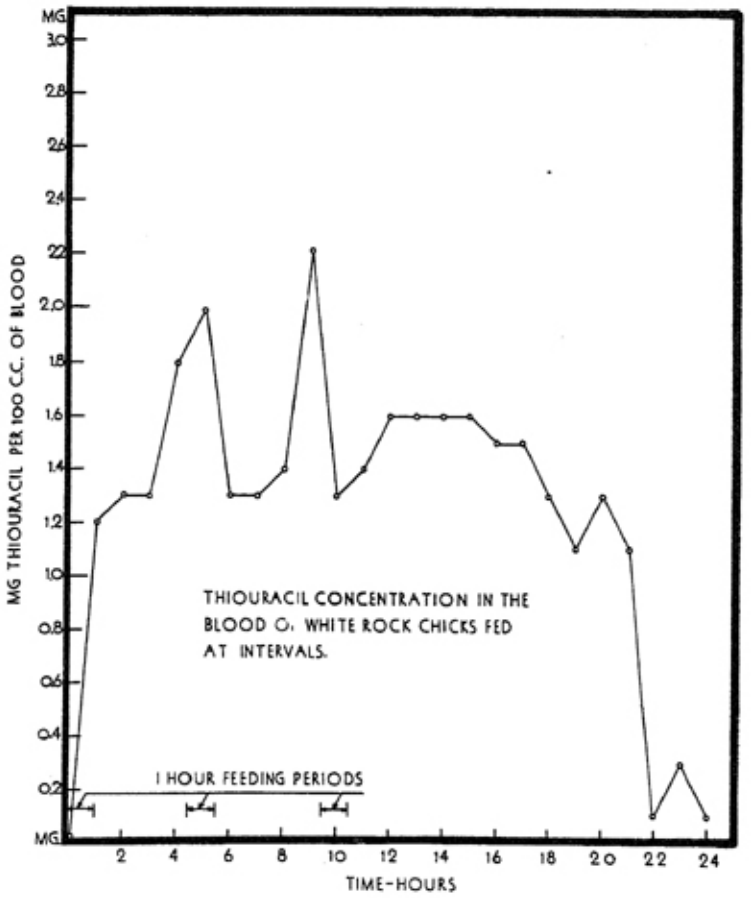


Fig. 4. The effect of interval feeding on the thiouracil content of the blood of chicks.

Table 2. ABSORPTION OF THIOURACIL FROM THE FOWL CROP

Time after Feeding Hours	Non-Ligatured		Ligatured	
	No. of Bird	Thiouracil per 100 ml. of Blood	No. of Bird	Thiouracil per 100 ml. of Blood
		mg.		mg.
1	3264	1.7	3251	0.0
2	3267	1.8	3253	0.0
3	3258	6.1	3254	0.0
4	3275	6.0	3255	0.0
5	3263	4.6	3262	2.4
6	3274	1.2	3261	0.0

Therefore a double ligature of silk thread was placed on the esophagus below the crop as before. Special care was taken to obtain a tight ligature on each fowl. Eleven six-week-old birds were ligatured in this

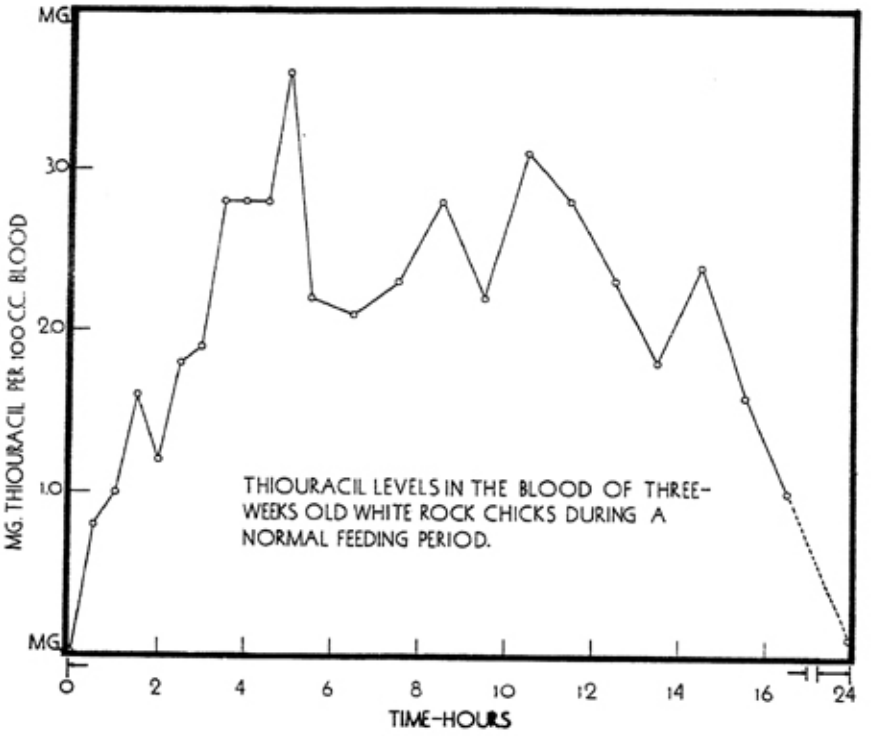


Fig. 5. Thiouracil concentrations in the blood of chicks under normal feeding conditions.

manner and 30 milliliters of a solution containing 75 milligrams of the sodium salt of thiouracil was placed in the crop of each, by the use of a syringe and a small rubber tube. Twelve non-ligatured, normal birds received equal amounts of thiouracil in a like manner. Two birds from each group, ligatured and non-ligatured were killed each hour for six hours and blood samples were taken.

As was to be expected from the previous studies (Fig. 2 to 6), the thiouracil content of the non-ligatured fowls was at a maximum (7.7 milligrams per 100 milliliters) one hour after administration. After six hours, the thiouracil concentration had declined to 1.2 milligrams per hundred milliliters of blood (Table 2).

Thiouracil was found in the blood of only one of the eleven ligatured fowls (Table 2, 3262).

Since the only evidence of thiouracil passage from the crop into the blood stream was at the four-hour period (Table 2), ten birds were double ligatured and 75 milligrams was administered as before. Blood samples were taken from all ten birds after four hours. The blood of one bird in this group (Table 3, No. 2611) was found to contain thiouracil but no trace of thiouracil could be detected in the blood of the other nine birds.

The presence of thiouracil in the blood of two of the esophagus-ligatured fowls can be explained only by the extreme difficulty of securing a perfectly tight ligature. As demonstrated in previous experiments,

single ligatures failed repeatedly to prevent the passage of feed from the crop into the proventriculus. There seems to be no question on the basis of this work, but that the crop is non-permeable to thiouracil.

Comparative Levels Produced in the Blood by Thiouracil, Thiobarbital and Propyl Thiouracil. -- Eighteen two-year-old White Leghorn hens were divided into three groups of equal size and body weight. One group of six was given 30 milliliters of an aqueous solution containing 150 milligrams of thiouracil. The other two groups were given an equal amount of thiobarbital and 6 n-propyl thiouracil respectively. The drugs were put into solution by the addition of 1 N sodium hydroxide and subsequent neutralization to pH 8.0.

Since at least three milliliters of blood were needed for each determination, it was necessary to use the birds in relays of two. The first five blood samples were taken from two birds in each group receiving the various goitrogens. Another pair of birds from each group was used for the second five samples and still another pair for the last five blood samples. In this manner blood samples were taken over a 24-hour period following the administration of the drugs. Thiouracil was found to reach a maximum concentration after three hours while the maximum concentration of thiobarbital and 6 n-propyl thiouracil was found after four hours (see Fig. 6). Thiobarbital reached a higher level than the other goitrogens studied but after 15 hours the thiobarbital level had dropped to the level of the other goitrogen and after this point was reached, there was no significant variation in the blood levels produced by the various goitrogens.

Comparative Levels Produced in the Blood of Chicks by Thiouracil, Thiobarbital and 6 n-Propyl Thiouracil. -- Since extremely large dosages were employed in the previous experiment and since these drugs were given in an aqueous solution, it was thought advisable to repeat this investigation under more practical conditions. Eighty-one four-week-old chicks were divided into three equal groups. The various groups were fed a standard ration containing 0.3 per cent thiouracil, thiobarbital and 6 n-propyl thiouracil respectively. After a two-hour feeding period blood samples were taken from three chicks in each group. Blood samples were taken at intervals for a 24-hour period after feeding.

The average goitrogen content of the blood of three chicks at each time interval was estimated as previously described.

Table 3. THIOURACIL IN THE BLOOD AFTER ADMINISTRATION TO ESOPHAGUS-LIGATURED FOWLS

No. of Bird	Thiouracil per 100 ml. of Blood
	mg.
3611	1.8
3521	0.0
3542	0.0
3548	0.0
3520	0.0
3575	0.0
3515	0.0
3588	0.0
3562	0.0
3513	0.0

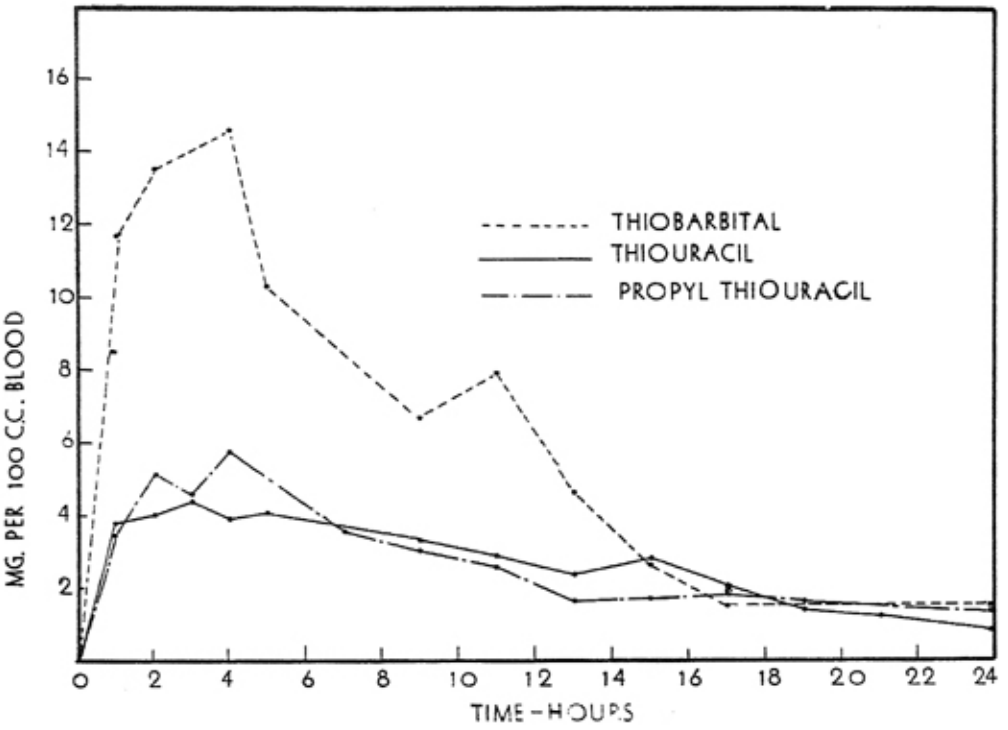


Fig. 6. Comparative levels produced and maintained in the blood of hens by equal amounts of thiouracil, thiobarbital and 6 n-propyl thiouracil.

Thiobarbital was found to reach a higher level than the other goitrogens as was demonstrated in the previous experiment (Fig. 7). A sharp drop in the thiobarbital concentration was noted after four hours but the level remained high in comparison to the other goitrogens throughout the 20-hour period. There was no significant difference observed in the blood levels of the other goitrogen. Both thiouracil and 6 n-propyl thiouracil followed the typical pattern that had been demonstrated for thiouracil in previous experiments.

Comparative Levels Produced in the Blood of Goats by Thiouracil and Thiobarbital.-- Three non-lactating female goats weighing about 95 pounds each were given three grams of thiouracil in their morning ration. These animals accepted the thiouracil and feed mixture readily and consumed the entire portion without objection. Blood samples were taken at intervals for a 24-hour period by inserting a No. 16 syringe needle into the juglar vein and allowing the blood to drip into a centrifuge tube.

This procedure was repeated at weekly intervals using the same goats and the following amounts of goitrogens: Thiouracil 5 grams, thiouracil 10 grams, thiobarbital 3 grams, thiobarbital 5 grams. As had been previously observed in the fowl, thiobarbital produced and maintained consistantly higher blood levels than thiouracil (Fig. 8).

Thiouracil Concentrations in the Blood of Sheep. -- A Shropshire wether weighing approximately 140 pounds was given 5 grams of thiouracil by capsule after this animal had refused feed containing the drug.

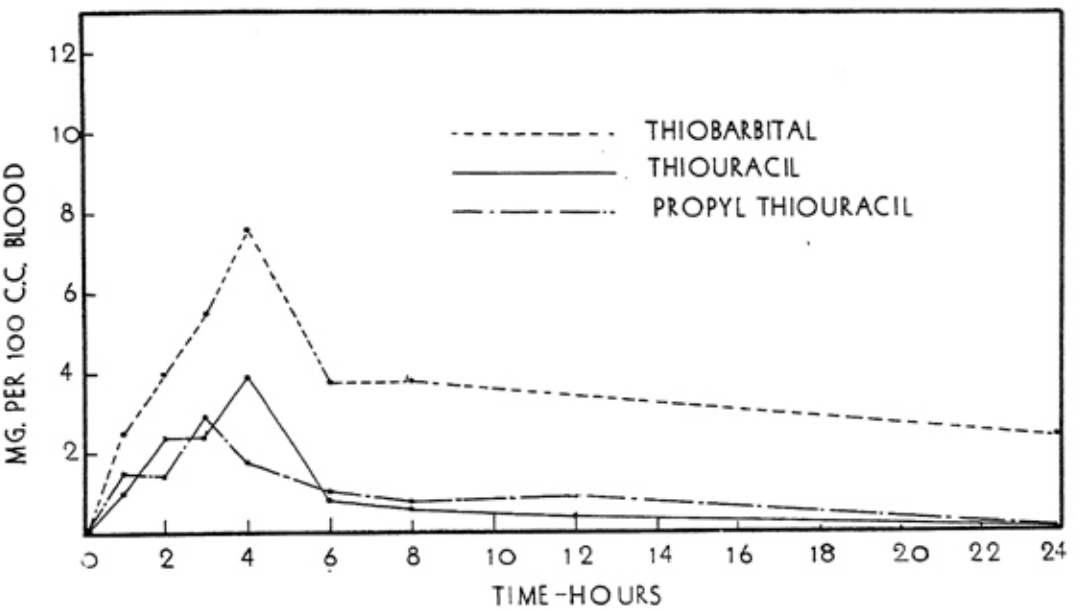


Fig. 7. Comparative levels produced in the blood of chicks by three of the more active goitrogens.

Blood samples were taken employing the same technique as was employed for goats. Samples were taken at four, six and twenty-four hour intervals after administration. The maximum thiouracil concentration was observed after six hours. After 24 hours only a relatively small amount of thiouracil remained in the blood stream (Fig. 9).

THE DISTRIBUTION OF GOITROGENIC DRUGS IN BODY TISSUES AND FLUIDS

The use of goitrogens in the treatment of thyrotoxicosis made necessary the investigation of the pharmacological properties of these compounds. Several workers have made intensive studies of the absorption, distribution, metabolism and excretion of the more active goitrogens.

Thiourea. -- Chesley (1944b) demonstrated that thiourea was quickly metabolized and excreted. Williams and Kay (1945) found that thiourea was rapidly absorbed and distributed throughout body tissues and fluids. These workers found the maximum thiourea concentrations in the blood of patients thirty minutes after administration.

However, 48 hours after the oral administration of 0.2 grams, thiourea could not be detected in the blood or urine of a normal subject. These investigators reported that 30 per cent of ingested thiourea could be recovered in the urine but none was present in the feces.

Tetraethylthiourea. -- Williams (1945) found concentrations of 5.8 to 7.0 milligrams of tetraethylthiourea as compared to 3.2 to 4.0 milligrams of thiouracil per 100 ml. in the blood of rats fed equal amounts of these drugs. The thyroid tissue of these animals contained 19.8 to 20.4 milligrams of tetramethylthiourea and 10.6 to 14.6 mgs of thiouracil per 100 grams dry weight. In the human tetraethylthiourea was found to produce and maintain consistently higher levels in the blood than thiouracil. Small quantities of tetraethylthiourea were found in the blood ten

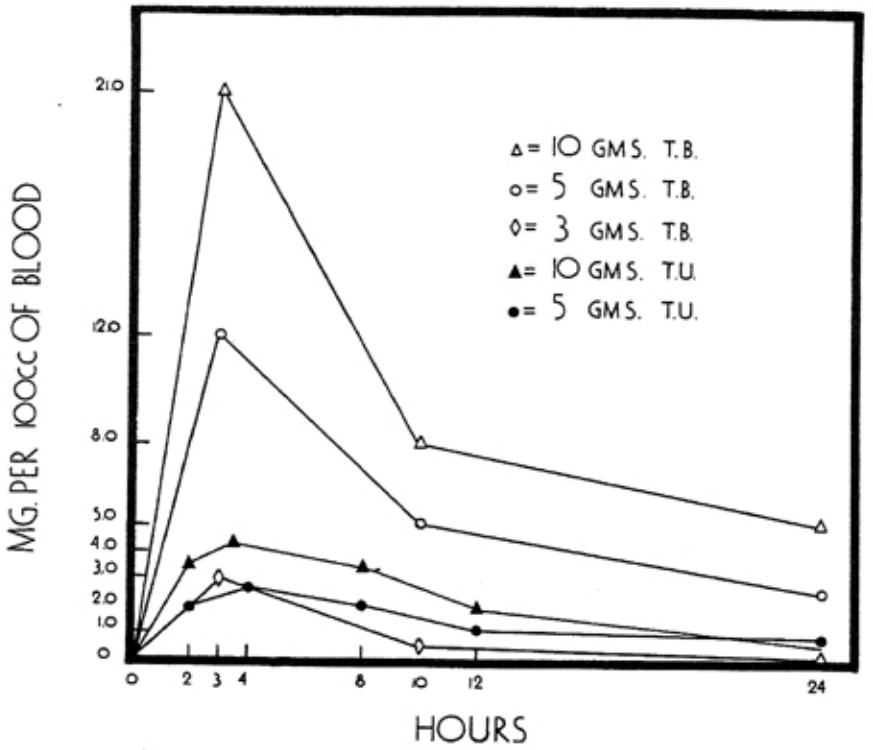


Fig. 8. Thiouracil and thiobarbital levels in the blood of goats after various dosages.

hours after administration of 0.2 grams. At the same dosage level and after an equal period thiouracil could not be detected in the blood. Smaller percentages of tetraethylthiourea were excreted in the urine than was thiouracil at equal dosages. No correlation was found between distribution levels and goitrogenic effect, since thiouracil proved to be more goitrogenic than tetramethylthiourea.

Diethylthiourea. -- Williams (1945) reported that while diethylthiourea produced slightly higher blood levels than thiouracil, there was less goitrogenic effect.

Thiouracil. (a) Absorption from Gastrointestinal Tract. -- Williams et al. (1944b) reported that 50 per cent of orally administered thiouracil was absorbed in one hour and less than 25 per cent was destroyed in the gastro-intestinal tract of the rat. The secretions of the stomach, duodenum and jejunum but not the ileum were found to destroy thiouracil. Thiouracil was not present in the stools of either the human or rat, indicating that 100 per cent was destroyed or absorbed. Williams and Kay (1944) reported that comparison of intervenous with oral administration indicated that approximately 15 per cent of the thiouracil ingested is destroyed in the gastro-intestinal tract.

(b) In Blood. -- Williams, Kay and Jandorf (1944) found that thiouracil was rapidly absorbed into the blood stream. It was observed in the human that a dose of 0.2 gram produced the highest blood level (2.3 milligrams per 100 ml.) 15 minutes after administration. After this time thiouracil concentrations in the blood declined until after eight hours only 0.3 milligrams per 100 ml. was present. In one patient traces of thiouracil persisted in the blood for three days but none could be detected

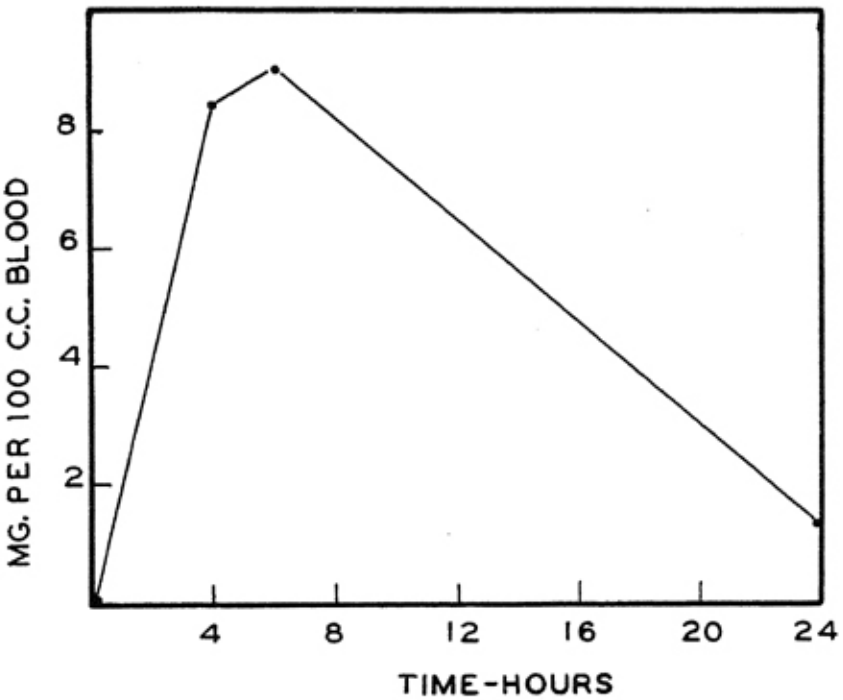


Fig. 9. Thiouracil concentrations in the blood of a sheep after a single dosage.

after this period. In most subjects, however, thiouracil concentrations in the blood declined rapidly after administration ceased and only traces could be detected after 24 to 48 hours. Divided doses produced a more uniform level than single large dosages. Twenty-four to forty-eight hours after administration began a relatively uniform level was observed in the blood regardless of the level of dosage. Williams (1944) reported that the administration of 0.1 grams produced a blood level of 2.0 milligrams two hours after administration. A rapid decline was observed after this period and thiouracil could not be detected in the blood after 48 hours. When thiouracil was not administered during the sleeping hours, concentrations tended to be low the following morning. On dosages up to 0.6 grams thiouracil disappeared from the blood stream at intervals varying from a few hours to three days after therapy ceased. With larger dosages thiouracil was found in the blood for five days, although in all cases the quantity became small one day after cessation of treatment. Paschkis et al. (1945) found a maximum thiouracil concentration in the blood two hours after a patient received a 1.0 gram dosage. In all cases thiouracil disappeared rapidly from the blood and, unless large doses were given in the evening, the concentration fell to almost imperceptible levels during the night.

Pipes and Turner (1946) reported that thiouracil could not be detected in the blood of the fowl 16 to 20 hours after oral administration. Pipes and Turner (1947) found a similar pattern of rapid elimination of thiouracil from the blood stream of the rabbit, the goat and the sheep. Ely, Olson, and Reineke (1947) found that thiouracil was practically eliminated from the blood plasma of goats, 12 hours after oral administration of 0.015 grams per kilogram body weight and 24 hours after administration of 0.12 grams per kilogram body weight.

(c) Excretion in Urine. -- Williams, Kay and Jandorf (1944), Williams (1944), Williams and Kay (1947a) reported that approximately one third of the drug ingested is excreted unchanged in the urine. Paschkis et al. (1945) observed that 24 to 57 per cent of 1.0 gram dosage was excreted in the urine during the first 24 hours and 10 to 15 per cent during the next 24-hour period. Thiouracil has been detected in urine in small concentration as long as five days after therapy ceased but in all cases the quantity was small after 48 hours.

(d) Concentrations in Milk and Eggs. -- Williams, Kay and Jandorf (1944) reported concentrations of 9 to 12 milligrams of thiouracil per 100 ml. in milk from patients which had received 1.0 gram of thiouracil. Monroe and Turner (1946) demonstrated that extreme thyroid enlargement occurred in infant rats nursing mothers receiving thiouracil. Andrews and Schnetzler (1945), found 7.0 to 15.0 and 8.0 to 12.0 milligrams of thiouracil per 100 grams in the yolks and whites of eggs from hens fed 0.2 per cent thiouracil in the ration. A definite thyroid enlargement was observed in chicks hatched from such eggs.

(e) Concentration in Tissue. -- Thiouracil has been found in varying amounts in practically all tissues of the body (Williams and Kay, 1944; Williams, 1947). Highest concentrations were found in the bone marrow, thyroid, adrenals, ovaries and pituitary. Relatively small concentrations were found in the testes and liver. Muscle was found to contain from 8.2 to 0.0 milligrams per 100 grams (dry weight). In patients which received thiouracil over five days, the thiouracil concentration was many times higher in the tissues than in the blood. Pipes and Turner (1947) found thiouracil concentrations of less than 5 milligrams per 100 grams in the tissues of several species. Franklin et al. (1947) reported that thiouracil concentrations in the muscle of swine fed 0.2 per cent thiouracil for 34 days were 6 ± 3 milligrams per 100 grams. One day after removal of thiouracil from the ration the level dropped to 2 ± 1 milligrams per 100 grams and none could be detected after three days.

Franklin et al. (1948) demonstrated that the thiouracil content of the tissue of fowls after a three week feeding period was 3 ± 2 milligram per cent. After administration ceased the thiouracil content fell to 0.1 milligram per cent after 24 hours and less than 0.05 milligram per cent after 48 hours.

(f) Destruction by Tissues. -- In a series of in vitro experiments Williams (1944) demonstrated that essentially all tissues possess the ability to break down thiouracil. In some cases more than 75 per cent was broken down in two hours. Pituitary, thyroid and adrenal tissue were most active and muscle and pancreas tissue least active.

These workers also reported that 94 per cent of injected thiouracil could be recovered if the entire carcass of the rat was analyzed one minute after injection. However the carcass and the urine contained only 65 per cent after 1 hour, 30 per cent after 3 hours and 22 per cent after 10 hours. At the end of 24 hours only a trace of thiouracil could be detected in the carcass and only 12 per cent had been excreted in the urine. While the breakdown products of thiouracil are not known, William, Kay and Jandorf (1944b) found increased excretion of neutral sulfur in the urine.

Substituted Thiouracils. -- Williams and Kay (1947a) found that the destruction of thiouracil was almost twice as rapid as that of any of its derivatives with substituents in the 6-position -methyl, ethyl, propyl, butyl or amyl. Thiouracil was found in the bodies of rats in concentrations of 7 to 16 milligrams per 100 grams. Derivatives with an odd number of carbon atoms were found to accumulate in the body in highest con-

centrations. However the ethyl and butyl derivatives accumulated in the largest concentrations in the thyroid gland. No essential difference in blood levels produced by 6 n-propyl thiouracil and thiouracil were observed with patients receiving less than 100 milligrams daily. When the dosage was 100 milligrams or above, 6 n-propyl thiouracil was found in higher concentrations in the blood than thiouracil. No proportional relationship has been established between the concentrations produced in the blood or thyroid tissue by these drugs and their goitrogenic activity (Williams, 1945; Williams and Kay, 1947a).

Intensive investigation has demonstrated that thiouracil and many of the more active goitrogen are quickly absorbed from the gastrointestinal tract, differentially distributed throughout the body and rapidly broken down by the body tissues or excreted in the urine. These investigations have been confined for the most part to the rat and man. The question of species variation in metabolism of the goitrogens has not been extensively studied.

DETERMINATION OF THIOURACIL IN TISSUES

Preparation of a Standard Reference Curve. -- Existing methods for determination of thiouracil in tissue (Williams, Jandorf and Kay, 1944) have not proved satisfactory in this laboratory. Therefore, a method based on hypertrophy of the rat thyroid was developed.

The rats employed were female Wistar albinos of the Missouri strain weighing from 120 to 140 grams each at the beginning of the test. A standard reference curve was prepared in the following manner: Sufficient thiouracil was blended with fresh chicken tissue in a Waring blendor to give thiouracil concentrations of 0.005 to 20.5 per cent or 5 to 50 milligrams per 100 grams of tissue.

The tissue-thiouracil mixtures were combined with the standard ration (see page 13) to give 95 per cent meat diet on a wet basis. After packaging in 300-gram lots this mixture was stored at -18°C.

Six groups of ten rats each were fed the ration containing 0.0, 5.0, 10.0, 17.0, 25.0, and 50.0 milligrams of thiouracil per 100 grams of tissue. The daily feed for each group consisted of a freshly thawed 300-gram lot of the mixture described above. After two weeks on this diet, the rats were killed with ether and body weight was recorded. The thyroids were weighed immediately to the nearest 0.1 milligram on a Roller-Smith torsion balance. The average thyroid weight per 100 grams body weight was found to increase with increasing thiouracil dosage until the 25 milligram level was reached. At the 50 milligram level no further increase in thyroid size was observed (Fig. 10) (Table 4).

Estimation of Thiouracil and Thiobarbital in Chicken Tissue. The fowls employed in these experiments were six-week-old White Plymouth Rock chickens which had received thiouracil or thiobarbital in the ration for the preceding week. The birds were slaughtered three hours after the morning feed since previous experiments had demonstrated a high level of thiouracil in the blood at this interval after feeding. The birds were skinned, dressed as for market and were ground in a Straub E 4 mill. In each analysis, every effort was made to duplicate the conditions under which the standard reference curve was prepared.

Estimates of the goitrogenicity of tissue were made on eight groups of birds receiving varying amounts of goitrogens. Four groups received 0.1, 0.2, 0.4, and 1.0 per cent thiouracil in their respective rations.

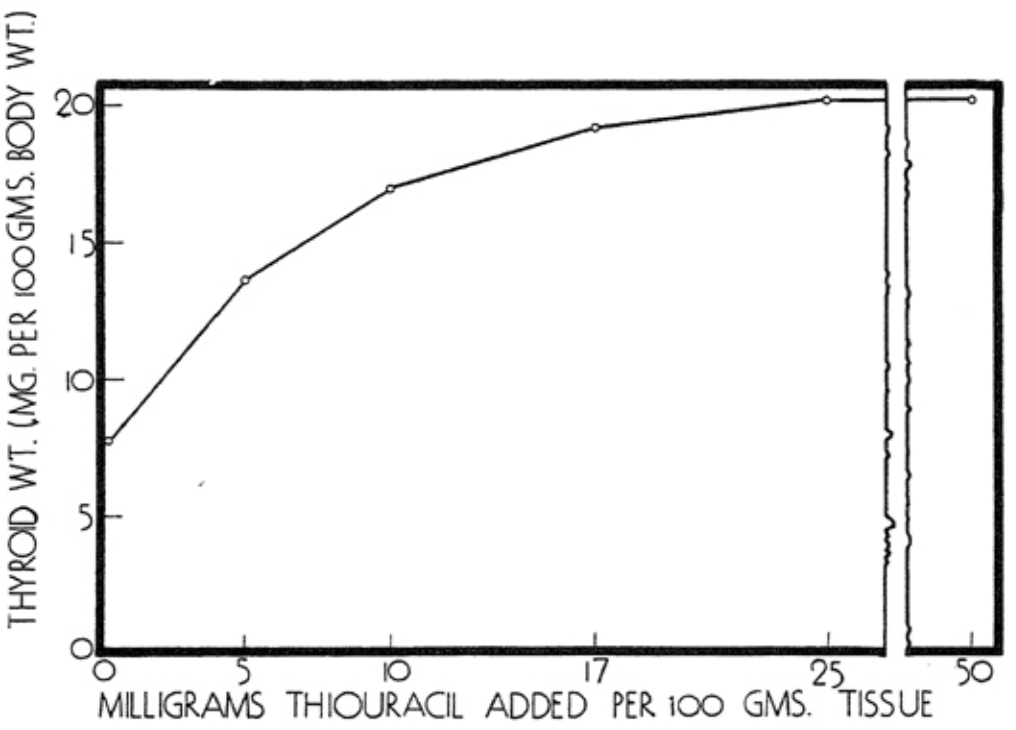


Fig. 10. Relationship of thiouracil concentration in meat to the thyroid weight of rats on the ration.

Two additional groups were fed 0.1 per cent thiouracil and were used to test the possibility of accelerated enzymatic action due to freezing and thawing of the tissue before feeding. The tissues from one of the latter groups was prepared as previously described, frozen, and a portion thawed daily before feeding. Birds from the other group were killed daily while receiving 0.1 per cent thiouracil and fed to rats in a freshly prepared ration.

The two remaining groups were fed 0.1 per cent thiobarbital.

The data obtained in these experiments were analyzed according to methods for analysis of variance (Snedecor 1940).

Results. -- Tissues from birds receiving 0.1, 0.2, and 0.4 per cent thiouracil were found to produce a slight but significant increase in size of the thyroid glands of the rats consuming the tissues (Table 5). Birds fed at 1.0 per cent thiouracil level produced tissues without significant goitrogenic effect.

Freezing and thawing of tissue did not tend to reduce goitrogenicity since a larger increase in thyroid size was observed in rats fed thawed tissue than in rats fed fresh tissue from birds receiving equal amounts of thiouracil.

The tissues from one group of birds receiving 0.1 per cent thiobarbital produced a definite goitrogenic effect but the tissues of the other group failed to increase thyroid size significantly while slight but significant increases in the thyroid size was observed in rats fed tissues from fowls receiving thiouracil or thiobarbital. It is evident that all tissues assayed contained less than the equivalent of 5 milligrams of thiouracil per 100 grams of meat.

TABLE 4. THE EFFECT OF FEEDING CHICKEN TISSUE CONTAINING THIOURACIL ON THE THYROID GLAND OF THE RAT

Thiouracil added per 100 grams of chicken tissue mg.	Average Body Weight gm.	Average Thyroid Weight mg.	Average Thyroid Weight per 100 grams body weight mg.
0.0	152.5	10.90	7.72
5.0	140.6	19.06	13.60
10.0	136.2	23.14	17.00
17.0	141.3	27.27	19.30
25.0	156.4	31.63	20.22
50.0	160.0	32.38	20.22

Thiouracil Retention in the Tissues of Swine. -- Since female rats were not available at the time of this investigation, male rats varying in weight from 140 to 240 grams were employed. However since Monroe and Turner (1946) have shown that thyroid secretion rate is a linear function of body weight in the male rat, it was not considered that a serious error was introduced by the rather large variation in body weight of these groups. The tissue was provided by cross bred pigs which had received 0.0, 0.05, and 0.1 per cent thiouracil respectively in the ration during a 28 day ad libitum feeding period. Composite samples of lean and fatty tissue were ground and combined with a feed mixture as described in the previous assays. Tissue from the control animals was divided into three portions and 0.0, 5.0 and 10.0 milligrams of thiouracil per 100 grams was added.

In all other respects the conditions of this experiment were identical with our previous studies on chicken tissue.

TABLE 5. RETENTION OF GOITROGENS IN CHICKEN TISSUE

Per cent of Goitrogen in Ration of Birds Providing Tissue	Average Body Weight of Rats gm.	Average Thyroid Weight mg.	Average Thyroid Weight per 100 grams body weight mg.
0.0	152.5	10.90	7.20
0.1 Thiouracil	166.9	17.10	10.20
0.1 "	155.2	16.14	10.40
0.1 " (Fresh tissue)	158.7	13.22	8.30*
0.2 Thiouracil	148.7	18.10	12.20
0.4 "	160.9	19.20	11.90
1.0 "	132.7	11.08	8.35*
0.1 Thiobarbital	142.5	17.10	10.20
0.1 "	142.6	10.45	7.50

*not significant

Results. -- The addition of 5.0 to 10.0 milligrams of thiouracil per 100 grams of pork tissue produced significant increases in the size of the thyroids of the rats consuming the tissue (Table 6). Pork from swine receiving 0.05 per cent thiouracil failed to produce a significant goitrogenic effect. The group receiving 0.1 per cent thiouracil produced tissues possessing a goitrogenic effect slightly higher than that produced by the addition of 5.0 milligram of thiouracil per 100 grams.

Retention of Thiouracil in the Tissues of Other Domestic Animals. -- Estimates of the goitrogenic content of the tissues of rabbits, goats, calves and sheep were made. Since previous experiments demonstrated that large amounts of thiouracil (5 to 25 milligrams per 100 grams) could be easily detected but that smaller quantities could not be accurately estimated, it was not considered necessary to set up standard reference curves.

The animals providing the tissue for these experiments were fed thiouracil or thiobarbital for at least seven days previous to slaughter. All animals were slaughtered within three to five hours after receiving the last dosage of the goitrogen. Composite samples of tissue were taken and were prepared in a ration as previously described. Female rats weighing between 100 and 120 grams were employed in these studies except as otherwise noted.

Rabbits. -- Tissues from three groups of white New Zealand rabbits fed 0.1, 0.2, and 0.4 per cent thiouracil in the ration and tissues from three groups fed 0.1, 0.2, and 0.4 per cent thiobarbital were fed to male albino rats ranging in body weight from 180 to 240 grams. No significant increase over the controls was observed in thyroid weight of any of the groups of rats consuming the tissue from these animals (Table 7).

Goats. -- Tissues from a young 70 pound female goat which was fed 4 grams of thiouracil per day failed to produce a significant goitrogenic effect when compared with control tissue (Table 7).

TABLE 6. THE EFFECT OF PORK CONTAINING THIOURACIL UPON THE THYROID GLAND OF THE RAT

Thiouracil added per 100 grams of Pork mg.	Average Body Weight of Rats gm.	Average Thyroid Weight mg.	Average Thyroid Weight per 100 Grams Body Weight mg.
0.0	185.0	11.84	6.40
5.0	176.2	13.30	7.55
10.0	174.0	17.49	10.05
Thiouracil in ration of swine providing tissue %			
0.05	205.9	14.58	7.12
0.1	164.1	12.59	7.67

TABLE 7. THE RETENTION OF GOITROGENS IN THE TISSUES OF DOMESTIC ANIMALS AS SHOWN BY INCREASED THYROID SIZE OF RATS CONSUMING THE TISSUE

Animal Providing Tissue	Amount of Goitrogen in Ration	Sex of Rats	Body Weight gms.	Average Thyroid Weight mgs.	Thyroid Weight per 100 Grams Body Weight mgs.
Rabbit	0.0	Male	200.8	12.45	6.20
Rabbit	0.1% Thiouracil	Male	210.0	16.55	7.90
Rabbit	0.2% "	Male	249.4	13.50	5.41
Rabbit	0.4% "	Male	239.5	17.87	7.46
Rabbit	0.1% Thiobarbital	Male	226.4	16.16	7.15
Rabbit	0.2% "	Male	260.6	14.78	5.67
Rabbit	0.4% "	Male	243.9	16.30	6.68
Goat	0.0	Female	123.1	13.17	10.70
Goat	4.0 grams daily	Female	129.4	11.34	8.76
Sheep	0.0	Female	153.1	12.74	8.32
Sheep TU-	4.0 grams daily	Female	112.0	9.22	8.23
Calf #55 TU-	4.0 grams daily	Female	125.6	14.54	11.57
Calf #77 TU-	2.0 "	Female	121.2	11.73	9.67

Sheep. -- Rats fed tissue from a 170-pound Shropshire wether which had received four grams of thiouracil per day failed to show a significant increase in thyroid weight when compared with the controls (Table 7).

Calves. -- Tissues were obtained from two male Holstein calves which were fed thiouracil during a fattening experiment. Calf No. 55 weighed 100 pounds when thiouracil administration began and received one gram of thiouracil three times per day for nine days and two grams twice per day for thirty-six days. When slaughtered this animal weighed 152 pounds. Calf No. 77 weighed 75 pounds and received one gram of thiouracil twice per day for 10 days, two grams twice per day for seven days and one gram twice per day for seven days. This calf weighed 106 pounds when slaughtered. The diet of these calves consisted of whole milk fed at 10 per cent of body weight. Failure to gain weight at a normal rate was probably due to housing the animals in an unheated barn and the interference of thiouracil with the normal thyroid function of maintaining body temperature.

Although control calf tissue was not available for comparison, it appears that tissues from calf No. 55 produced a slight but significant goitrogenic effect (Table 7). However tissues from calf No. 77 failed to produce a goitrogenic effect if compared with control goat or sheep tissue.

Discussion. -- While the metabolism of the goitrogens has been extensively studied in man and the rat, little data is available to demonstrate that the metabolism of these compounds is as rapid and complete in domestic animals. This problem has been ignored by the majority of

investigators who have supplied abundant evidence to prove that thiouracil is a rapid and efficient fattening agent for livestock and poultry. The present studies indicate that, as had been assumed previously, the conclusions of clinical research can be extended to the domestic animals. In all species investigated, thiouracil followed the typical pattern of rapid absorption, destruction and excretion as has been demonstrated in the rat and human by Williams, Kay and Jandorf (1944b). It is logical to assume from the present work that much of the knowledge of goitrogen metabolism in the human and rat can be directly applied to farm animals.

Williams (1944) and Paschkis et al. (1945) have observed in the human that unless large doses of thiouracil are given late in the evening, the concentrations in the blood drop to imperceptible levels by the following morning. The investigations reported herein indicate that similar conditions can be expected in domestic animals and stress the importance of feeding thiouracil throughout the day rather than in single feedings. From a theoretical view point at least small divided dosages should produce a greater goitrogenic effect than single large dosages.

Williams (1945) and Williams and Kay (1947a) failed to find a proportional relationship between blood levels produced by various goitrogens and their goitrogenic activity. In the present studies thiobarbital was found to produce and maintain blood levels over twice as high as thiouracil. Although Williams and Kay (1947a) have shown that the substituted thiouracils are more slowly metabolized than thiouracil, these compounds may be suitable for use as fattening agents if their activity is sufficient to permit the use of low dosages. It seems likely, however, that retention in the tissues may present a more serious problem than encountered with thiouracil.

Biological methods for determining the goitrogenicity of tissue, while lacking sensitivity at low concentration, are suitable to demonstrate that no hazard exists in the use of meat from thiouracil fed animals for human consumption.

The development of rapid and reliable chemical methods for determination of thiouracil in animal tissues would permit an investigation of the rate of disappearance of this compound from the tissues after administration is discontinued. In order to demonstrate definite goitrogenic activity in the present investigation it was necessary to use tissues from animals which had received thiouracil or thiobarbital on the day they were sacrificed. Variations in the goitrogenic acitivity of the tissues of thiouracil-fed animals of different species can be explained by the small amounts of thiouracil present and the lack of sensitivity of the method employed.

A practical consideration of the evidence now available indicates that no hazard is involved in human consumption of meat from thiouracil-fattened livestock.

The low toxicity of thiouracil has been demonstrated by its use in treatment of thyrotoxicosis. Initial dosages of 1.0 gram per day and maintenance dosages of 0.2 to 0.4 grams have been administered for long periods with few toxic reactions. While in a small percentage of cases leucopenia and agranulocytosis have developed, these toxic reactions have appeared only after continous administration at these high levels.

As shown in the present studies, tissues from animals receiving even 10 to 20 times the amount of thiouracil necessary for optimum fattening contain less than 25 milligrams of thiouracil per pound. At this concentration it would be practically impossible for a human to consume

sufficient meat to obtain even the lower medicinal dosages of thiouracil, since a daily consumption of eight pounds of meat would be required.

In the present investigations it was necessary to employ tissues from animals fed thiouracil on the day of slaughter to demonstrate definite goitrogenic activity. Since destruction and excretion are rapid in living tissues, the usual time lapse of 48 to 72 hours between the fattening pen and the slaughter house should reduce thiouracil concentrations to imperceptible levels.

It has been assumed that cooking process should practically eliminate any thiouracil present in tissues since Williams (1945) reported that 20 per cent of a thiouracil solution is destroyed by heating at 100°C for five minutes and 100 per cent after five minutes in the autoclave under fifteen pounds pressure at 105°C. However, Franklin et al. (1948) found that cooking meat for one hour at 130°C. had no effect on the thiouracil level, but that most of the compound disappeared from the tissue after storage for one week at 4°C.

SUMMARY

1. In the chick, increasing thiouracil levels above 0.6 per cent in the feed failed to produce corresponding increases in the thiouracil concentrations in the blood.
2. Thiouracil, when fed to the chick or the mature fowl in concentrations up to 0.6 per cent, is speedily absorbed from the intestinal tract and is rapidly eliminated from the blood stream. Twenty four hours after feeding no trace of thiouracil can be detected in the blood.
3. Feeding at intervals a ration containing thiouracil produces and maintains more uniform blood levels than large single feedings.
4. The ad libitum feeding of a ration containing thiouracil produced a relatively uniform level in the blood during the day. Thiouracil concentrations in the blood drop to almost imperceptible levels during the night and early morning hours.
5. In esophagus-ligatured fowls, thiouracil does not pass through the crop into the blood stream. Apparently the fowl crop gland is nonpermeable to thiouracil.
6. Thiobarbital produces and maintains higher blood levels in the chick, the mature fowl, and in the goat than does thiouracil. The 6 n-propyl thiouracil in spite of its greater goitrogenic effect does not produce blood levels significantly higher than thiouracil.
7. A similar pattern of speedy absorption from the intestinal tract and rapid disappearance from the blood is produced by thiouracil in the fowl, the sheep, and the goat.
8. Thiouracil, when added to chicken or pork tissue in amounts ranging from 5 to 25 milligrams per 100 milligrams, produces proportional increases in the thyroid gland of rats consuming the tissue.
9. Tissues from birds fed thiouracil in the ration at levels up to 1.0 per cent produced barely perceptible enlargement of the thyroid gland when fed as 95 per cent of the diet (wet basis). In all cases the goitrogenic effect was less than that produced by the addition of 5 milligrams of thiouracil per 100 grams of tissue.
10. Tissues from rabbits fed thiouracil or thiobarbital in the ration at levels as high as 0.4 per cent failed to produce a definite goitrogenic effect.

11. While tissues from swine receiving 0.05 per cent thiouracil in the ration did not exhibit a goitrogenic action, tissues from swine receiving 0.1 per cent produced a goitrogenic effect slightly in excess of pork tissue to which 5 milligrams of thiouracil per 100 grams had been added.
12. Tissues from sheep and goats fed 4 grams of thiouracil per day failed to produce significant increases in the thyroid size of rats.
13. Beef from veal calves fed up to 4 grams of thiouracil per day failed to produce increased thyroid size in rats when compared with normal sheep or goat tissue.
14. Biological methods for estimation of thiouracil in tissue while lacking sensitivity at low concentrations are suitable to demonstrate that thiouracil is not retained in muscular tissue in amounts in excess of approximately 5 milligrams per 100 grams. In many assays the presence of thiouracil in the tissues could not be demonstrated.

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