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THE METABOLISM OF THYROXINE

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THE METABOLISM OF THYROXINE

R. A. MONROE AND C. W. TURNER

INTRODUCTION

The thyroid gland is not a newcomer to medical literature. The ancients, although ignorant of its importance, were fascinated and baffled by this shield-shaped entity situated in the neck without apparent reason. Many centuries were to elapse before the vital role of the thyroid was suspected and finally established.

Meanwhile, several curious theories about the thyroid were proposed. Some writers maintained that the purpose of the gland was to moisten the larynx and protect the vocal chords. Others were touched by the aesthetic qualities of the thyroid, whose sole function, supposedly, was to add rotundity and beauty to the neck. Another theory which gained some popularity was that the thyroid functioned in some mysterious manner during intra-uterine life, after which time it became a useless vestige.

These theories, while valueless except from a historical viewpoint, serve to illustrate the natural tendency to focus attention on the gland itself. Even today, when it is known that the chief function of the gland is to produce a hormone which affects every tissue in the body, most of the research in thyroid physiology is directed toward a more complete understanding of the gland, its mechanisms and responses. It goes without saying that it is essential that these functions be elucidated. Equally important, however, are such unsolved, and virtually unstudied questions as the mode of action of the thyroid hormone in peripheral tissues and the fate, in general, of the hormone in the organism. For example, what factors at the cellular level affect the level of the thyroid hormone in the blood or in the tissues? Does the hormone level, on the other hand, affect the tissues only in a quantitative sense, or are different mechanisms brought into play by variations in the hormone level? And what happens to the hormone after it has exerted its action on the cells? How long does it remain in the body? Does exogenous thyroid hormone act in a manner similar to the endogenous hormone? If not, what factors determine the effectiveness of exogenous hormone?

Seemingly, a list of questions such as these could go on endlessly. However, these examples are sufficient to indicate the complexity of the problems faced by future research in thyroid physiology. It is evident, therefore, that any single study can only scratch the surface of the work which still is necessary for a complete understanding of these problems.

The present study represents an attempt to answer some of the questions presented by the problem of thyroxine metabolism. Among the aspects of this problem studied are the absorption and excretion of various thyroxine containing compounds, the length of time that thyroxine remains in the body of the chick, and several of the factors affecting this duration period.

Before these results can be interpreted properly, however, it is first necessary to have in mind a good understanding of the overall picture of the problem of thyroxine metabolism. It is attempted, in the review of literature, to present just such a rounded perspective. For convenience, the literature review is presented in sections, each section representing one of the major aspects of the problem.

Because of the fact that the preponderance of thyroid research in the past has been aimed at the gland itself, it is felt that the present study represents a step in a direction which, as yet, has not received the attention that its importance merits. It is hoped, also, that this study will shed some light on the possibilities of the metabolism of thyroxine as a subject for investigation and will, as a consequence, serve as a stimulus for research in that direction.

REVIEW OF LITERATURE

Absorption of Thyroidally Active Compounds in the Gastrointestinal Tract

In view of the obvious importance of the thyroid hormone in clinical therapy and its effect on the production processes of livestock, the problem of the absorption of thyroactive substances from the gastro-intestinal tract is of great importance. Unfortunately, relatively few investigations have been conducted for the specific purpose of evaluating the oral efficacy of these compounds. However, the literature does offer information which sheds some light on the extent of absorption of various thyroxine compounds.

Absorption from the Stomach. Cohn (1932) showed that iodine can be absorbed by the stomach. After isolating the stomach of a dog and ligating the duodenum near the pyloric sphincter, he introduced a potassium iodide solution. Two hours later only sixty-nine percent of the introduced iodine remained. Elemental iodine, on the other hand, was not absorbed *per se,* but only after its conversion to iodide. By a similar technique Schittenhelm and Eisler (1932) calculated that about forty percent of an alkaline thyroxine solution was absorbed from the stomach in fourteen hours.

Absorption from the Intestines. Wide variations exist in the reported observations concerning the oral effectiveness of thyroxine. Apparently the oral efficacy depends on the form in which thyroxine is fed. The extent of these differences is indicated by observations of various thyroxine compounds on urinary iodine excretion (Boe and Elmer, 1931; Elmer and Rychlik, 1934) and on basal metabolism (Thompson *et al.*, 1933, 1934).

Thyroxine seems to be less active when fed in pure crystalline form than

when fed in the form of its alkali salts. Schittenhelm and Eisler (1932) found that iodine in the form of thyroxine in alkaline solution introduced into an isolated loop of intestine in the dog was ninety percent absorbed. On the other hand, of the same amount of crystalline thyroxine administered orally to man, only fourteen percent was absorbed.

Apparently desiccated thyroid is more easily absorbed than pure crystalline thyroxine but about equally as well as its di-alkali salt (Asimoff and Estrin, 1931; Harington and Salter, 1930).

The only hypothesis set forth thus far to explain the differences in activity of the various thyroidally active compounds is one which considers the solubility in water of the compounds as the principal source of difference. According to this idea, the most soluble forms of thyroxine (desiccated thyroid, in which the thyroxine is in protein combination, and the di-alkali salt of thyroxine) are more readily absorbed and hence more active than pure crystalline thyroxine.

In this connection, another suggestion becomes apparent. Concomitant with the greater water solubility, it would seem reasonable to assume a more uniform distribution of the thyroactive substances which, in itself, might be conducive to an increased amount of absorption. The relative values of solubility and uniform distribution of a substance in determining its absorption is problematical. The subject is mentioned only because the solubility of the substance fed might be altered in its passage (e. g., due to pH changes) in which case the distribution of the material might be important.

The question arises, also, as to the extent of destruction of thyroactive compounds in the gastro-intestinal tract. It seems quite likely that such substances as desiccated thyroid and its potent protein, thyroglobulin, undergo the usual processes of protein digestion. Indeed, the work of Barnes *et al.* (1931, 1932) substantiates this belief. On the other hand, it should be mentioned that Barnes and Bueno (1933) observed an anaphylactic response in guinea pigs to the injection of thyroglobulin into the intestines after the animals had been sensitized previously by a subcutaneous injection of thyroglobulin, indicating that some thyroglobulin was absorbed unaltered. It is possible, however, that these results were due to experimental trauma. In fact, it seems incredible that a molecule the size of thyroglobulin (mol. vol. 675,000, Heidelberger and Pederson, 1935) could pass intact through the intestinal mucosa.

Despite the probable extensive digestion of thyroid protein in the gastro-intestinal tract, the actual destruction of thyroxine seems highly improbable in the light of the observations of Harington and Salter (1930) and Barkan and Kinigsepp (1932). After intensive enzymatic treatment *in vitro* they observed no appreciable destruction of diiodotyrosine.

It is obvious, at any rate, that some of the active part of the hormone reaches the blood stream unaltered after oral administration, since a physiologic response is obtained. Indeed, the blood iodine picture after ingestion of thyro-

active substances can serve as an indication of the rate of absorption of the hormone.

Behavior of Blood Iodine after Oral Administration of Thyroidally Active Compounds. Numerous observations have been reported on the behavior of blood iodine after the administration of various iodine containing compounds. Some of these investigations are pertinent to the problem of thyroxine absorption and yield interesting information on the rate at which orally administered thyroxine compounds reach the general circulation. Veil and Sturm (1925) found that a small dose of thyroid extract caused a marked rise in blood iodine, the peak of which was obtained in two hours. After this time the iodine level of the blood dropped rapidly. In 24 hours it had returned to normal. Schittenhelm and Eisler (1932), who followed the fate of thyroxine in alkaline solution introduced into the duodenum of the dog, noted a similar peak in the blood iodine curve after 2 hours. Once again, it should be noted that the rapidity of increase and decrease of iodine in the blood depends on the form of thyroxine administered.

Biosynthesis of Thyroxine

From the foregoing section, it will be suspected that inorganic iodide is very readily absorbed from the gastrointestinal tract and, indeed, this suspicion has been proved to be a fact by many investigators. Since it is well known that iodine is an essential component of the thyroid hormone—or, more specifically, of thyroxine, the active constituent of the hormone—and that the thyroid gland takes up iodide preferentially, it is obvious that the process of iodide entrapment by the thyroid is of primary importance among the mechanisms of thyroxine biosynthesis.

Fixation of Iodide by the Thyroid Gland. Many investigators have shown that iodide introduced into the organism is rapidly trapped by the thyroid gland (Leblond, 1942; Lein, 1943; Perlman *et al.*, 1941). When tracer doses of I^{131} are administered, the iodide is rapidly incorporated into diiodotyrosine and thyroxine molecules. Within 15 minutes after injection of tracer doses of I^{131} Taurog and Chaikoff (1947) observed that 95 percent of the radioactivity present in the thyroid could be accounted for by organically bound I^{131}—about 80 percent as diiodotyrosine and 10 to 15 percent as thyroxine. Thus, it would appear that the mechanism of iodine fixation by the thyroid might depend on its rapid conversion to organically bound forms. However, it has been shown that the thyroid retains its capacity to fix iodide even when the conversion of iodide to organic forms does not occur. Thus, when large doses of iodide are administered, much of the iodine taken up by the thyroid remains as inorganic iodide for some time (Leblond, 1942; Wolff and Chaikoff, 1948). Moreover, it has been shown that the thyroid glands of thiouracil-treated animals remain capable of concentrating iodine even though the ability to synthesize organic

iodine compounds is destroyed (Franklin *et al.*, 1944; Astwood, 1945; McGinty and Sharp, 1946; Vanderlaan and Bissell, 1946).

Many workers believe the iodine in the thyroids of goitrogen-treated animals to be free inorganic iodide (Baumann *et al.*, 1944; McGinty and Bywater, 1945; Taurog, Chaikoff and Feller, 1947). Indeed, Taurog *et al.* state that it is not precipitated with the proteins by trichloroacetic acid and that it passes readily through a dialysis membrane. Salter (1949), however, found the opposite to be true after prolonged treatment with thiouracil. He observed that the iodine could be precipitated by heat coagulation or treatment with acetone and is partially retained after dialysis. Consequently, Salter is dissatisfied with the viewpoint that the iodine in the thyroid glands of thiouracil-treated animals is merely an accumulation of dissolved iodide. Rather, he maintains that something more complex may be happening.

Salter proposes an interesting theory as a possible explanation of the mechanism of iodine entrapment by the thyroid. He suggests that the cellular proliferation in the thyroid caused by thiouracil administration is accompanied by an expanded intracellular enzyme system with a high affinity for iodine. These "iodases" would present an increased amount of colloidal carrier, with which part of the dissolved iodide could become associated. Salter points out that, to be effective, such a system would have to be capable of participating in the oxidation of iodide to elemental iodine, a step which is prerequisite to the iodination process in thyroxine synthesis (Harington, 1933). It is possible that two components are involved in such an iodine carrying system—an "iodase" and, say, a peroxidase, since peroxidases are known to liberate iodine from iodide (Sumner and Somers, 1943), and since peroxidase is a normal constituent of the thyroid gland (DeRobertis and Grasso, 1946). Such an enzyme system would be analogous to the now well-known, stepwise, enzymatic process in biological oxidations of making molecular oxygen available to cells. In view of the complete lack of supporting experimental evidence, Salter's theory must be considered only with considerable caution.

Biochemical Reactions in Thyroxine Synthesis. Whatever the mechanisms employed by the thyroid gland for the fixation of iodide and the subsequent liberation of elemental iodine, they must be, in the normal gland, extremely rapid. As noted above, 15 minutes after the injection of tracer doses of I^{131}, 80 percent of the radioactivity in the thyroid was in the form of diiodotyrosine and 10 to 15 percent was in the form of thyroxine (Taurog and Chaikoff, 1947). Over a period of 50 hours the diiodotyrosine iodine level decreased to about 70 percent of the total iodine present in the gland, while the thyroxine level increased to about 25 percent of the total. These shifting percentage levels are indicative of the validity of the theory, set forth by Harington (1933) and supported by the work of Johnson and Tewkesbury (1942), that diiodotyrosine is the biological precursor of thyroxine.

Harington (1944) has reviewed the evidence in support of his theory.

Among this evidence is the important work of Mann, Leblond and Warren (1942), which is probably the first really strong evidence that diiodotyrosine is actually the natural percursor of thyroxine. They studied the relative specific activities of thyroxine iodine and diiodotyrosine iodine at intervals after the injection of I^{131}. (Specific activity is defined as the number of radioactive units per unit weight of total iodine in a given fraction.) It was found that 48 hours after injection, the specific activity of the thyroxine fraction was greater than that of the iodide fraction and less than that of the diiodotyrosine fraction at 0.5, 8, and 48 hours. Therefore, the only possible source of I^{131} for thyroxine was from diiodotyrosine. Taurog and Chaikoff (1947) using a similar technique but with a larger number of animals showed beyond any doubt that diiodotyrosine is indeed the natural percursor of thyroxine.

It should be noted that recent evidence has been presented which indicates that another step should be recognized in the conversion of iodide to thyroxine via diiodotyrosine. Fink and Fink (1948), using filter paper partition chromatography, have detected what appears to be monoiodotyrosine in thyroid hydrolysates of I^{131}-injected rats. They believe that monoiodotyrosine is normally present in the thyroid, but admit the possibility that it might be an artifact of hydrolysis. Taurog *et al.* (1949), however, have studied the specific activity of monoiodotyrosine, from measurements of I^{131} on paper chromatograms, and conclude that monoiodotyrosine is a precursor of diiodotyrosine and not a hydrolytic product.

Enzymes Involved in the Biosynthesis of Thyroxine. Johnson and Tewkesbury (1942) presented good evidence to show that the coupling of two diiodotyrosine molecules to form one of thyroxine is an oxidative process. When Morton and Chaikoff (1943) demonstrated that surviving slices of thyroid tissue have the ability to concentrate I^{131} and synthesize diiodotyrosine and thyroxine *in vitro*, it became possible to study this problem further. That an intracellular enzyme system is involved in thyroxine synthesis is indicated by the fact that thyroid slices are capable of this function, whereas homogenates are not. That is to say, cellular organization cannot be destroyed without disrupting the mechanism of thyroxine synthesis. Studies conducted under anaerobic conditions or with inhibitors of cytochrome oxidase reduced materially the amount of thyroxine synthesized (Schachner *et al.* 1943, 1944). The cytochrome-cytochrome oxidase system, therefore, apparently plays an important part in the incorporation of iodine in diiodotyrosine and thyroxine. Various goitrogenic compounds have also been shown to inhibit this *in vitro* reaction (Franklin *et al.*, 1944; Taurog et al., 1945), confirming the work on intact animals of the MacKenzies (1943) and Astwood *et al.* (1943).

Dempsey (1944) was able to demonstrate the presence of a peroxidase in the thyroid gland and suggested that this enzyme might also be involved in thyroxine synthesis. Peroxidases are known to liberate iodine from iodides and may be concerned in biological iodinations (Keston, 1944). The observations

of Westerfeld and Lowe (1942) indicate that peroxidase may have a role in the coupling of two diiodotyrosine molecules to form thyroxine. An interesting series of experiments were performed by DeRobertis and Grasso (1946) which lend definite weight to this possibility. They found that thiourea, but not the sulfonamides, inhibits peroxidase activity. In addition, a concomitant drop in the redox potential of the thyroid follicle was noted, indicating a cessation of the secretory activity of the gland.

Other enzyme systems in the thyroid gland have been studied, including phosphatases (Dempsey and Singer, 1946) and proteolytic enzymes (DeRobertis *et al.*, 1941, 1946). At present, however, phosphatase concentration cannot be related definitely to thyroxine synthesis, although, in some cases, it seems to be affected by it.

Proteolytic activity in the thyroid gland will be considered in another section.

Possibility of an Exchange Reaction. In considering the studies on thyroxine synthesis conducted with I^{131}, the possibility should be mentioned that a reaction may occur whereby the non-radioactive iodine of diiodotyrosine may be replaced by I^{131} from inorganic iodide (exchange reaction). Miller *et al.* (1944) have demonstrated that such an exchange can occur. Chaikoff and Taurog (1949), however, point out that the following experimental observations indicate that the iodination of diiodotyrosine and thyroxine by I^{131} represents the formation of new molecules of these compounds, rather than the results of a simple exchange reaction.

(1) When surviving slices were incubated in a Ringer medium to which radioactive iodide had been added, a large part of the radioiodine was soon incorporated into thyroxine and diiodotyrosine. Organic binding of iodine was greatly reduced, however, when tissue organization was disrupted by homogenization; in this case, nearly all of the radioiodine was recovered in the inorganic iodine fraction.

(2) The conversion of radioactive inorganic iodide to thyroxine and diiodotyrosine by surviving thyroid slices was greatly inhibited under anaerobic conditions or in the presence of cytochrome oxidase inhibitors. These findings strongly imply the participation of an intracellular enzyme system in the formation of these iodinated compounds.

(3) It has been shown by the MacKenzies (1943) and Astwood *et al.* (1943) that goitrogenic compounds depress hormone formation by the thyroid gland. These same compounds were found to inhibit both the *in vitro* and the *in vivo* conversion of inorganic radioactive iodide to thyroxine and diiodotyrosine by thyroid tissue. A single injection of 10 mg. of thiouracil into a rat completely abolished, for some time, the thyroid's capacity of converting circulating radioactive iodine to diiodotyrosine and thyroxine.

Possible Regulatory Function of Inorganic Iodine in the Synthesis of Thyroxine. A discussion of thyroxine synthesis would not be complete without some mention of the long perplexing problem of why iodine, an essential element in the formation of thyroxine, can sometimes act in a manner inhibitory to thyroid function, as in the well-known alleviation of Graves' syndrome by iodine therapy. One wonders, too, whether the inorganic iodine level of the blood or in the thyroid itself may not play a regulatory role in thyroid function.

At any rate, it is clear that iodine administered in sufficient amounts can reduce the output of the thyroid gland. Various theories have been proposed in an attempt to explain this effect, prominent among which are those that suggest an effect on the mechanisms of hormone release. This is a reasonable assumption since, by the Law of Mass Action, a diminished release, with a consequent accumulation, of thyroid hormone would result in a markedly decreased rate of thyroxine synthesis. Salter (1940) has suggested that with therapeutic doses of iodine the secretion of thyroid hormone tends toward storage in the follicle instead of flowing directly into the bloodstream. De-Robertis and Nowinski (1946) believe that iodine may affect a proteolytic enzyme system, which they believe to be necessary for colloid release. A discussion of these theories, however, belongs in another section. At the moment, it is more pertinent to consider the possible effects of inorganic iodine on the process of thyroxine synthesis.

It is clear from the observations mentioned earlier that, although the rapid conversion of iodide to organically bound iodine is one of the mechanisms which allow the thyroid to trap iodine, it is by no means the only one. Even when diiodotyrosine and thyroxine syntheses are nearly completely stopped by the administration of iodide, the iodide concentration of the thyroid is several hundred times that of the plasma (Wolff and Chaikoff, 1948). However, the rise and fall in this thyroid iodide closely parallels the plasma iodide curve. Moreover, after the concentration of inorganic iodine in the thyroid drops below a certain level (20 to 35 gamma percent in the case of the rat), thyroxine synthesis once more occurs. It should be emphasized, therefore, that even in the *normal* thyroid, as opposed to the hyperthyroid state in Graves' disease, that inorganic iodine is capable of inhibiting thyroxine synthesis, and that the degree of inhibition is correlated with the plasma iodide level.

The exact mechanisms involved in this inhibition are as yet unknown. Perhaps the depression of colloid release or the reversal of the direction of secretion, as mentioned above, are the major factors. Again, the diminution of thyrotrophic hormone activity by iodine (Rawson *et al.*, 1945) seems a likely possibility. Since iodination of some enzymes is known to result in their inactivation (Herriott, 1937), Morton *et al.* (1944) have suggested a similar effect on the thyroid enzymes concerned in thyroxine synthesis. The latter workers also proposed the inhibition of the formation of an intermediate in the synthesis of thyroxine. They reason that if the oxidizing agent involved in the liberation of I_2 from KI (or I^-) were used up by the excessive amount of I^- present, there would result a decreased formation of hypoiodous acid (intermediate in thyroxine synthesis proposed by Johnson and Tewkesbury, 1942), since I_2 is necessary for the formation of HIO. In this connection it is interesting that the iodination of tyrosine depends on the presence of hypoiodous acid in proper concentration and that this reaction is inhibited by inorganic iodine (Li, 1942).

It appears, then, that the biosynthesis of thyroxine is a process which takes

place rapidly in the normal gland, and can be fairly well defined as a stepwise process involving the liberation of elemental iodine from iodide, the iodination of tyrosine and, finally, the oxidative coupling of two diiodotyrosine molecules. Of the specific enzymatic mechanisms involved, however, not much can be said at present. Normally, this process is probably governed largely by the thyrotrophic hormone of the anterior pituitary. It has been shown that in the presence of excessive amounts of inorganic iodine thyroxine synthesis practically ceases. Whether this effect is due to direct action of the iodide or to indirect effects, through the inhibition of hormone release, is not definitely known. At any rate, the regulatory properties of inorganic iodine are a fortunate happenstance, since they offer an effective system for the prevention of toxic amounts of hormone being formed.

Before leaving the subject of thyroxine synthesis to consider the problems involved in the release of the hormone from the gland, it should be mentioned that reports in the literature usually leave one elementary fact unsaid. Consequently, those unfamiliar with the subject are often left unnecessarily confused. In reading the literature one would be led to think of the steps in the synthesis of thyroxine as analogous with a series of test tube reactions. Actually, of course, the reactants are constituents of proteins. Thus, the formation of the hormone should be thought of as the alteration of a protein to form an active complex. The final product, of course, is known as thyroglobulin, which makes up the "colloid" of the thyroid gland.

Release of Hormone by the Thyroid Gland

Once the thyroid hormone has been synthesized in the gland its immediate course may take one of two directions. It may be stored in the colloid of the thyroid follicle or it may be released directly into the circulation. The exact mechanism by which the thyroid hormone is delivered from the gland into the bloodstream has yet to be determined. In truth, it would probably be more accurate to think of the process of hormone release as a rather complex interrelationship of several mechanisms. Indeed, the problems posed by the two aforementioned routes of conveyance of the hormone from the thyroid to the general circulation may differ to some extent. For example, the thyroglobulin stored in the acini, in order to be released into the blood, must first be broken down in preparation for its reabsorption by the follicular cells and then must cross the cell in its passage to the circulatory system. These are problems not encountered in the direct release of the hormone.

In any case, the thyroid gland is primarily a homeostatic organ and, as such, is under the influence of many factors. Much of the activity of the thyroid is mediated by the anterior pituitary. Most certainly, therefore, the thyrotrophic hormone must play a major part in the stimulation of thyroid hormone release.

Action of the Thyrotrophic Hormone. The action of the thyrotrophic hor-

mone in the release of the thyroid hormone may be demonstrated by the familiar technique of comparing the effects of ablation of the pituitary against the effects of thyrotrophic hormone administration.

Houssay *et al.* (1931) and Sturm (1934) studied the effects of hypophysectomy on the blood iodine level in dogs and noted that after an initial, transitory hyperiodemia, the iodine level fell below that of normal dogs. Recently, improvements in micro-techniques for the determination of iodine and thyroxine have made possible the extension of the scope and accuracy of this type of measurement. Taurog, Chaikoff and Bennett (1946) showed that hypophysectomy in the rat brought about a 50 percent drop in the level of protein-bound iodine in 3 days. However, the thyroxine content of the thyroid gland was not altered by hypophysectomy. Thus, a lowered thyroxine level in the blood (as indicated by protein-bound iodine determination) is not sufficient in itself to bring about a release of thyroxine from the thyroid gland. Clearly, also, even though the thyroid may contain a normal amount of thyroxine, the action of the thyrotrophic hormone is necessary to effect its release.

Further evidence indicating that the thyrotrophic hormone is a causative agent in the release of thyroxine from the thyroid can be seen in the fact, long known, that administration of thyrotrophic hormone results in a marked decrease of thyroid iodine (Loesser, 1931; Shockaert and Foster, 1932; Grab, 1932). This represents a decrease not only in total thyroid iodine, but particularly in the organically bound iodine fraction (Closs *et al.*, 1932; Morton *et al.*, 1941). Associated with the drop in thyroid iodine is a rise, as might be expected, in the iodine content of the blood (Closs *et al.*, 1932; Schittenhelm and Eisler, 1932). Again, this increase applies to an elevation of organically bound iodine and, particularly to be noted, to a heightened level of thyroid hormone (Zunz and La Barre, 1935; Morton *et al.*, 1941).

Thus, it is obvious that the rate of release of thyroxine from the thyroid is regulated by the thyrotrophic hormone of the anterior pituitary. Perhaps other factors can effect the release of thyroxine directly also, but no such factor has as yet been clearly demonstrated.

In conjunction with the foregoing observations, it has also been found that hypophysectomy lowers severely the rate of iodine fixation by the thyroid and, for all practical purposes, stops the formation of thyroxine from diiodotyrosine (Morton *et al.*, 1942). Curiously, however, the formation of diiodotyrosine from organic iodide was not interfered with by hypophysectomy. As might be expected, both processes are speeded up remarkably by administration of thyrotrophic hormone (Hamilton and Soley, 1940; Hertz *et al.*, 1940; Leblond and Sue, 1941; Morton *et al.*, 1941).

Perhaps these observations belong properly in the previous section on thyroxine synthesis. However, in the present case the two processes, thyroxine formation and thyroxine release, cannot be considered entirely independent of one another, at least not at the present time. All of the effects noted above on

the rate of formation of thyroxine could be explained by the Law of Mass Action. In the case of hypophysectomized animals, the cessation of thyroxine release would naturally result in an inhibition of the conversion of diiodotyrosine to thyroxine. By the same token, the rate of iodine fixation would likewise be diminished. Thus, the thyrotrophic hormone may exert a part of its action on the rate of thyroxine formation indirectly through its effect on the mechanism(s) concerned with the release of thyroxine from the thyroid.

On the other hand, the extremely rapid appearance of intracellular colloid droplets in thyroid epithelium after administration of thyrotrophic hormone presents some strong evidence in support of the idea of a direct stimulation of the thyroxine synthesis process. Dvoskin (1947), in agreement with numerous other investigators, noted a marked increase in the number of droplets as early as one hour after thyrotrophic hormone injection in the chick. It is evident, at any rate, that the thyrotrophic hormone is concerned with both the synthesis of the thyroid hormone and its release from the gland. And, in view of the evidence presented above, one is strongly inclined to believe that the action is direct in both cases.

Possible Enzymatic Mechanisms Involved in the Release of Thyroxine. A hint as to the means by which the hormone is released from the thyroid gland is offered by the experiments of DeRobertis (1941), who, following the suggestion of Gersh and Caspersson (1940), has demonstrated the presence of a proteolytic enzyme in the colloid of active thyroid follicles. Administration of thyrotrophic hormone resulted in increased proteolytic activity within the thyroid follicle. From these findings, DeRobertis proposed the theory, supported by Dziemian (1943), that the thyroid hormone is released from the stored colloid by its being reabsorbed by the cells, a process which will not take place until the colloid is broken down by enzymatic activity. In furtherance of this theory DeRobertis and Nowinski (1946) studied the proteolytic activity in pathological human thyroids. Severe toxic goiters, they found, showed enzymatic activity twice that of the normal gland, whereas in simple colloid goiters the activity was about 30 percent subnormal.

Phosphatase activity apparently is also related to the process of thyroid hormone release. At present, however, it is impossible to define this relationship. As an example, phosphatase concentration in the thyroid, as determined by histochemical methods, is increased after thiouracil treatment but decreased after exposure to cold (Dempsey, 1949). And yet, colloid is released in both cases. Likewise, an increase in phosphatase is noted on exposure to heat, while hypophysectomy depresses the enzyme. Both of these states are characterized by a storage of colloid. Apparently, therefore, phosphatase concentration is altered by the degree of thyroid activity, but no cause-effect relationship can be offered in explanation.

Doubtless, future investigations will clarify this problem and disclose other

mechanisms involved in the process of hormone release by the thyroid. It is only reasonable to assume that such a complex process must certainly demand the harmonious interaction of several such systems.

Rate of Hormone Secretion by the Thyroid Gland. Fortunately, more definite information has become available recently concerning the rate at which the thyroid gland secretes its hormone. The goitrogen method proposed by Dempsey and Astwood (1943) has stimulated investigations of the thyroid secretion rate of many species, including the domestic fowl, goats and calves (Schultze and Turner, 1945), rats (Griesbach and Purves, 1945; Monroe and Turner, 1945), mice (Hurst and Turner, 1948) and ducks (Billier and Turner, unpublished). All of these studies show thyroid secretion rates of the same order of magnitude (2 to 10 micrograms D,L-thyroxine per 100 grams body weight, depending on species and state of activity of the gland). The rates at which the thyroid glands of various species have been found to secrete are, in order: > rats > ducks > goats > chicks > calves > pullets and cockerels > two-year-old hens.

Recently Taurog and Chaikoff (1947) have studied the rate of turnover of I^{131} in the rat thyroid by the method of Zilversmit *et al.* (1943). From these data they calculated that the rat secreted 1.5 micrograms of thyroxine iodine daily per 100 grams body weight. Since the thyroxine present in the thyroid is levo-rotatory (Harington and Salter, 1930) and this form has been found to possess twice the activity of racemic thyroxine (Reineke and Turner, 1945), their figure agrees well with that obtained for rats at this laboratory by the goiter prevention method (Monroe and Turner, 1946).

Many workers have found that the level of protein-bound iodine in the blood is correlated with and, hence, indicative of the state of thyroid activity (Salter *et al.*, 1941; Lowenstein *et al.*, 1944; Talbot *et al.*, 1944; Winkler *et al.*, 1946). Recently, Chaikoff *et al.* (1947) have added to this observation. By injecting tracer doses of I^{131}, they observed the rate of formation of plasma protein-bound iodine in normal, thyroidectomized, and thyrotrophin-treated rats. Associated with the diminished protein-bound iodine level in thyroidectomized animals and the augmentation of the hormone level by thyrotrophic hormone, they observed a decrease and increase, respectively, in the rate of appearance of radioiodine in the protein-bound iodine fraction of plasma. This technique may prove more useful, due to increased accuracy of determination, than the usual protein-bound iodine determinations. Recently, H. G. Turner (unpublished data) has observed a striking correlation between the level of plasma protein-bound iodine and the thyroid secretion rate of rats (as reported by Dempsey and Astwood, 1943) kept at various environmental temperatures, thus indicating that plasma protein-bound iodine is indeed a valid measure of thyroid activity.

Possible Regulatory Function of Inorganic Iodine in Thyroid Hormone Release. It has already been seen that inorganic iodine may act as a regulating

factor in thyroxine synthesis. Some evidence has been presented which indicates that inorganic iodine may also have some effect on the release of thyroxine from the thyroid.

Both DeRobertis (1941) and Dziemian (1943) reported that the proteolytic activity of follicular colloid was inhibited by administration of iodide. Moreover, *in vitro* studies have shown that iodine can act directly on the enzyme, causing loss of activity (DeRobertis and Nowinski, 1946). These findings shed some light on the long-puzzling problem of how, at one time (simple colloid goiter), iodine administration may act as a stimulant to thyroid secretion and, at another (thyrotoxicosis), as an inhibitor. In the former case the iodine would be rapidly utilized in thyroxine synthesis, while in the latter case the iodine could act as an inhibitor to thyroxine release.

Another means by which inorganic iodine may inhibit thyroid hormone release is indicated by the finding that the activity of the thyrotrophic hormone can be abolished, *in vitro*, by treatment with inorganic iodine (Albert *et al.*, 1946). It is of interest to note that these workers found that thyrotrophic hormone was inactivated by reaction with elemental iodine but was not affected by iodide. That such a reaction may occur *in vivo* is indicated by the observation that injections of elemental iodine cause thyroid atrophy in normal animals and prevent goiter in thiouracil-treated animals (Dvoskin, 1947).

Obviously, the picture presented here is incomplete. Much further clarification is needed before it can be understood how the thyroid hormone is released from its parent gland into the general circulation, to be conveyed to the peripheral tissue where the hormone can exert its known effects.

The Metabolic Circuit of the Thyroid Hormone

Just as the mechanism(s) responsible for the release of the thyroid hormone into the general circulation has yet to be definitely demonstrated, so the form in which thyroxine is conveyed to the peripheral tissues is very much a controversial issue. It has been fairly well established that in the thyroid gland thyroxine is intimately associated with a protein, and exists in the form of thyroglobulin. Some workers have reported the presence of thyroglobulin in the blood and lymph of the thyroid (Hektoen *et al.*, 1927; Lerman, 1940). But Salter (1949) is of the opinion that these are results of abnormal cases and do not indicate a true picture of normal thyroid physiology.

Most workers have agreed that thyroglobulin is altered in some way previous to or during its release from the thyroid. Many proposals have been offered regarding the nature of the circulating thyroid hormone, none of which, until recently, was backed by really strong evidence.

The Nature of the Thyroid Hormone in the Blood. There seems to be no doubt that the circulating thyroid hormone can be precipitated quantitatively with the plasma proteins (Taurog and Chaikoff, 1948; Salter and Johnston, 1948). Moreover, the iodine thus precipitated is largely in the form of thy-

roxine. After the hydrolysis of plasma with strong alkali, Elmer *et al.* (1934), using Leland and Foster's butanol extraction method, could account for forty to sixty percent of the protein-bound iodine as thyroxine. Slightly lower percentages were obtained by Bassett *et al.* (1941) after enzymatic hydrolysis of plasma.

These results, in themselves, would seem to be good supporting evidence for the theory that circulating thyroxine is bound in a peptide linkage (Harington and Salter, 1930; Salter, Lerman and Means, 1933; Means, 1937). Indeed, Riggs *et al.* (1942) are of the opinion that thyroxine, being an amino acid, is incorporated in the molecules of plasma protein.

However, Trevorrow (1939) and Taurog and Chaikoff (1947, 1948) have demonstrated that thyroxine can be extracted from plasma with butanol without a preliminary hydrolysis. In fact, Taurog and Chaikoff (1948) extracted 73 to 93 percent of the plasma protein-bound iodine in this manner. Of this amount, only 10 to 15 percent was re-extractable with 4 N NaOH·5% Na_2CO_3, which washes out diiodotyrosine and inorganic iodide. Incidentally, these figures serve well to illustrate the preferential release of thyroxine by the thyroid, since, it will be remembered, only 25 percent of the iodine in the thyroid is present as thyroxine (Wolff and Chaikoff, 1947).

In addition to the above evidence, Taurog and Chaikoff (1948), in an excellent study, have shown:

1. That thyroxine added to plasma could be recovered to the extent of about 80 percent; therefore, at least 80 percent of the circulating hormone acts like thyroxine.
2. Thyroxine added to plasma precipitated quantitatively with the proteins and could not be dialyzed; again, showing the similarity between the behavior of added and "natural" thyroxine.
3. Protein-bound iodine (determined after administration of I^{131}) was distributed quantitatively between two immiscible solvents in exactly the same manner as a thyroxine carrier.

Thus it appears that the circulating thyroid hormone is thyroxine in weak combination with plasma proteins, perhaps by absorption (Trevorrow, 1939) or weak covalent bonds (Cohn, 1946; Cit. Salter, 1949).

Distribution of Thyroxine in Plasma Protein Fractions. Whatever the strength of the association between thyroxine and plasma protein, it appears that the thyroxine is unevenly distributed among the various protein fractions. Salter *et al.* (1941) and Bassett *et al.* (1941) found that protein-bound iodine resided chiefly with the albumin fraction. In later studies (Salter *et al.*, 1946) it was found that while the larger albumin fraction accounted for most of the iodine, a higher concentration peak was observed in the alpha-beta-globulin fraction. Taurog and Chaikoff (1948) confirmed the finding that thyroxine is concentrated more in these smaller protein fractions.

Thyroid Hormone in Body Fluids. Some information is available regarding the iodine concentration of various body fluids. Most of the data is indirect, due primarily to the fact that it is difficult to analyze tissue fluids directly. In practice, therefore, it is usual to determine simultaneously the contents of plasma and lymph (which, of course, tissue fluid ultimately becomes) and thus to estimate the probable intermediary figures. Salter (1949) summarizes the results of several such investigations (Hahn and Schurmeyer, 1932; McCullagh, 1935; Wallace and Brodie, 1937; Salter *et al.*, 1946) as follows:

(1) The "hormonal" iodine of the body fluids is lower than that of the plasma.
(2) The protein of body fluids is lower than that of the plasma.
(3) There is relatively more iodine in the clear fluids than their total protein content would indicate on a proportionate basis. In other words, the higher iodine-containing fractions of the plasma proteins apparently have leaked through the capillary wall preferentially.
(4) The iodine concentration of the body fluid is low and approximately that of the plasma.

The Nature of Thyroid Hormone in Tissues. The work of Wallace and Brodie (1937) shows that the inorganic iodine concentration in all tissues is the same as that of the plasma. In the past, several workers have reported values for organic iodine distinctly above that of plasma (Schittenhelm and Eisler, 1932; Lohr and Willmanns, 1937; McClendon and Foster, 1941). The techniques of analysis used in these investigations, however, usually yield high values due to impurities.

Recently, Salter and Johnston (1948) have obtained results on protein-bound iodine of tissues (muscle) similar to those of plasma, and, as in plasma, the iodine content varied among different protein fractions. Moreover, the tissue protein-bound iodine reflected changes in thyroid activity. The investigators concluded that the naturally occurring organic iodine was not free thyroxine and from this fact, suggested that the iodine might be incorporated into a peripheral enzyme system, for which they propose the name "thyrenzyme."

Distribution of Thyroxine in the Tissues. Recently, Gross and Leblond (1947), have conducted studies on the distribution of thyroxine in the various tissues of the rat, using large amounts of thyroxine labelled with I^{131}. As soon as 2 hours after the injection of radiothyroxine 30 percent of the radioactivity could be detected in the jejuno-ileal contents, 14 percent in the liver, and 11 percent in the muscles. After 24 hours, the detectable amounts had dropped to 0.2 percent, 8 percent, and 1.2 percent, respectively, indicating that the metabolic circuit of thyroxine is in a highly dynamic state. Similar distributions were obtained with physiological doses, although the percentage of the injected dose was lower in each case. Earlier investigations, although based on less sensitive methods of analysis, showed similar trends of thyroxine distribution (Zawadowsky and Asimoff, 1927; Krayer, 1928; Asimoff *et al.*, 1931; Muller and Fellenberg, 1932; Schittenhelm and Eisler, 1932, 1933).

Unlike Asimoff *et al.* (1931) and Muller and Fellenberg (1932), who noted some accumulation of iodine in the thyroid after hormone administra-

tion, Gross and Leblond could find no appreciable radioactivity in the thyroid after 24 hours. Apparently, therefore, thyroxine does not ordinarily enter the thyroid gland. It is possible, however, that after a longer period of time, some thyroxine may be destroyed with a consequent liberation of iodide, which would then be trapped by the thyroid.

Also, some earlier investigators (Schittenhelm and Eisler, 1932, 1933; Sturm and Schneeberg, 1933; Joliot *et al.*, 1944) claim that the pituitary is characterized by the selective ability to fix thyroxine iodine. Leblond (1949), using physiological doses, was unable to confirm this finding.

In any case, after the thyroid hormone has been conveyed to the peripheral tissues to exert its action on the cells, it may take either or both of two directions. It may be excreted in the urine or feces, or it may be destroyed in the tissues or in some organ system, such as the liver or kidneys. As will be seen, the latter route (destruction) is the more probable means of hormone disposal in most cases.

Excretion of Thyroxine

Conclusions regarding the elimination of thyroidally active substances introduced into the organism must be based largely on indirect observations on the effect of the administration of these compounds on iodine balance. While studies of this sort may leave something to be desired in accuracy and specificity, they nevertheless give a valuable insight into the general picture with regard to the routes by which and the relative rates at which thyroactive compounds are excreted.

The excretion of thyroxine and its related compounds is dependent on numerous variables. Prominent among these are the route of administration, dosage, and the form in which the compound is introduced.

Iodine Excretion after Peroral Administration of Thyroidally Active Compounds. It has already been seen that relative rates of urinary iodine elimination have been used as a criterion of the absorbability of crystalline thyroxine as compared with the same substance in alkaline solution (Elmer and Rychlik, 1934). In the latter case the percentage of urinary iodine elimination was increased approximately twice as much after oral administration as after ingestion of the pure crystalline compound. Likewise, Asimoff and Estrin (1931) observed only a slight increase in urinary iodine after feeding large amounts (sixty milligrams) of crystalline thyroxine to dogs. Both groups of workers feel that the slight increment in urinary iodine elimination following the ingestion of thyroxine is due to an incomplete absorption of the compound in the gastro-intestinal tract. This point is emphasized even more by the rapid and extensive urinary elimination of orally administered inorganic iodide (Abderhalden and Slavu, 1909; Elmer and Rychlik, 1934). That incomplete absorption of thyroxine is not the only cause of the less marked urinary excretion of iodine, however, is demonstrated by the observation that even after an intravenous injection of thyroxine the iodine excretion is both less and longer

delayed than after an injection of an equivalent amount of potassium iodide (Boe and Elmer, 1931).

Observations concerning the iodine elimination in the urine after feeding desiccated thyroid vary a good deal. Fellenberg (1926) found comparatively little iodine excreted in the urine of man after feeding thyroid (17 to 29 percent), whereas Asimoff and Estrin (1931) found that dogs excreted much more of the administered iodine (over 90 percent). Evaluation of these observations is made difficult because of the fact that, regardless of total iodine content, thyroid preparations contain large and variable amounts of diiodotyrosine, which is readily absorbed and excreted in the urine (Schittenhelm and Eisler, 1932; Foster and Gutman, 1930; Sainton *et al.*, 1934).

Iodine Excretion after Parenteral Administration of Thyroidally Active Compounds. The amount of iodine excreted in the urine after the injection of thyroxine apparently does not differ appreciably from that after preoral administration of thyroxine in alkaline solution. Following the intravenous injection of thyroxine Boe and Elmer (1931) observed urinary iodine values (13 percent of the injected dose) after 24 hours that agreed very closely with those reported by Elmer and Rychlik (1934) for iodine excretion (14 percent) after oral administration of thyroxine in alkaline solution. However, in the former case, iodine elimination was observed to be more rapid, 11 percent of the injected dose being excreted in 6 hours as compared with 7 percent after the ingestion of thyroxine. Recently, Gross and Leblond (1947) have reported that, after the subcutaneous injection of rats with thyroxine labelled with I^{131}, about 20 percent of the radioactivity can be detected in the urine after 24 hours.

Excretion of Thyroxine in the Urine. It is important to note at this point that various investigations have shown that the iodine present in the kidney after the administration of thyroxine or thyroid extracts is not in the form of the hormone but as simple iodide (Veil and Sturm, 1925; Zawadowsky and Asimoff, 1927; Gross and Leblond, 1947). Likewise, Elmer and Scheps (1934), using the chemical method of Leland and Foster (1932), demonstrated that thyroxine, *per se*, is not present in the urine of either normal or thyrotoxic patients. Apparently, therefore, the kidney does not play a primary role in the excretion of thyroxine.

Excretion of Thyroxine in the Feces. It should be expected that the oral administration of thyroactive materials would result in a larger excretion of iodine in the feces than in the urine. The researches of Schittenhelm and Eisler (1932) substantiate this supposition. They found that 53 percent of the iodine fed to man as crystalline thyroxine is excreted in 24 hours, and 86 percent within the next few days. When the thyroxine is fed in alkaline solution, however, only 9 percent of the iodine so administered could be found in the feces in 24 hours. Likewise, when Elmer and Luczynski (1933) fed thyroxine to rabbits in alkaline solution only 15 percent of the iodine remained in the gastro-intestinal tract after 12 hours.

Even after parenteral administration of thyroxine, the iodine level of the feces is increased. Krayer (1928) reports that as much as 86 percent of the iodine can be found in the feces of the rat after the intravenous injection of thyroxine. Gross and Leblond (1947) have reported similar figures after subcutaneous injection of radioactive thyroxine. They found, however, that only about one-half of the I^{131} in the feces was soluble in *n*-butanol—i. e., was present as thyroxine—and that this recovered thyroxine was somewhat less active biologically than commercial preparations of thyroxine or thyroxine prepared by the investigators. The work of Schittenhelm and Eisler (1932) indicates even less excretion of intravenously injected thyroxine.

Part of the thyroxine excreted in the feces after parenteral administration of thyroxine is apparently excreted through the wall of the intestines (Schittenhelm and Eisler, 1932; Gross and Leblond, 1947). Perhaps a more important excretion route, however, is through the liver and the bile, for many investigators have observed that the liver takes up iodine from the blood and releases it into the bile (Kendall, 1919; Krayer, 1928; Elmer and Luczynski, 1933; Barnes, 1933; Gross and Leblond, 1947; and others).

Destruction of Thyroxine

One thing is certain; thyroxine and thyroid extracts, after their introduction into the organism, must be either excreted or deactivated. Otherwise there would be an accumulation of the hormone in the body. This circumstance may occur in thyrotoxicosis but certainly not in the normal organism.

We have seen that some thyroxine is excreted *per se* in the feces. The remaining portion of the hormone must, therefore, be deactivated, presumably by some breakdown process. Probably the increased excretion of iodine in the urine after administration of thyroactive materials reflects this process to some extent. At any rate, it must be assumed that thyroxine must undergo fairly extensive inactivation, since only a fraction of it can be accounted for by excretion. Moreover, the inactivation process must be fairly rapid in view of the fact that the hormone has been shown to disappear from the blood and tissues in a short time (Gaebler and Strohmaier, 1942; Gross and Leblond, 1947; and others), although its physiological effects have been reported to last for considerable periods (Plummer, 1921, and many others).

Despite the fact that thyroxine resides only briefly in the bloodstream, it has been demonstrated that it retains full biological potency when incubated *in vitro* with blood for forty-eight hours (Muller and Fellenberg, 1932). Apparently, therefore, thyroxine is not decomposed in the bloodstream, but must await destruction elsewhere in the body.

Some decomposition of thyroxine may take place, of course, in the peripheral tissues during the process of stimulation of that tissue by the hormone. However, Abderhalden and Wertheimer (1928) perfused muscle tissue with thyroxine solution and found that substantial amounts of thyroxine were bound

by the tissue but without loss of activity, as shown by a positive reaction in tadpoles. It may be argued, however, that (1) the tadpole reaction is not a sensitive quantitative test and, therefore, that some thyroxine might have been destroyed without detection; and (2) that the tissue was probably not stimulated to increase its metabolism in view of the well known difficulty in eliciting such a response with pure thyroxine *in vitro* and, therefore, that the tissue may behave differently toward thyroxine *in vivo*. At any rate, however, it is logical to suppose that more decomposition of thyroxine may take place in some organ system more specialized for this sort of reaction. Moreover, it seems inconceivable that thyroxine should be destroyed during the process of exerting its action on the cells. Else how could such a profound response be elicited by the small amount of thyroxine necessary to exhibit its effect?

Role of the Kidneys in the Destruction of Thyroxine. As we have seen, the kidneys play an important part in the excretion of iodine but, apparently, not in the excretion of thyroxine *per se*. Presumably, therefore, this substance is altered in some way before its excretion through the kidneys into the urine.

Role of the Liver in the Destruction of Thyroxine. It has long been known that the liver has an important place in iodine metabolism and, moreover, is the site of many detoxication reactions. It would seem a likely place for the inactivation of thyroxine to occur. The available evidence seems to bear out this assumption. Despite the failure of many early workers to show the presence of thyroid hormone in the liver after administration of thyroactive substances (Romeis, 1922; Abelin and Scheinfinkel, 1925), it is now known that this organ does contain the hormone, even after the administration of physiologic doses of thyroxine (Leblond, 1949). The failure of the earlier workers was no doubt due to the insensitivity of their assay method. (Gudernatsch tadpole assay.)

In 1927, however, Zawadowsky and Asimoff were able to demonstrate the presence of thyroid hormone in the liver. Their observations were made possible by the development of an improved biological test (axolotl metamorphosis) in their laboratory (Zawadowsky *et al.*, 1925, 1927, 1928). In connection with their observations on liver tissue, these investigators noted a curious occurrence. When they administered large amounts of thyroid extract, there was a lapse of 3 to 5 hours before detectable amounts appeared in the blood. But from this time until 20 hours after administration, the hormone present in the blood gave a strong positive response. They concluded that the liver can act as a sort of reservoir for excess amounts of thyroid hormone and that after a certain threshold has been reached, the hormone is released gradually into the circulation in an unchanged form. They apparently did not appreciate the fact that, with their assay method, there might be some destruction of the hormone even though a considerable amount seemed to be released unchanged by the liver. The fact that these workers obtained a negative reaction from the bile of their experimental animals indicates that the hormone is destroyed in

its passage through the liver. Therefore, on the two points of retention and destruction by the liver, their observations are not in accord. Later, however, Zawadowsky (1933) became convinced that active thyroid hormone is excreted into the bile after administration of thyroidally active compounds. This view is upheld by Krayer (1928) and Asimoff and Estrin (1931), who also used the axolotl assay.

Mathieu and Barnes (1932) also assumed complete destruction of the thyroid hormone by the liver, but on rather infirm grounds. They base their conclusion on the fact that when bile from dogs treated with thyroglobulin was administered to a second group of dogs, the latter's oxygen consumption did not increase.

The fact that the thyroid hormone is excreted into the bile unchanged by the liver is shown irrevocably by the work of several investigators who have found, by chemical fractionation, that as much as one-half to two-thirds of the administered hormone may be recovered in the bile (Barnes, 1933; Barnes and Chang, 1933; Elmer and Luczynski, 1933).

Some interesting data on the regulatory action of the liver on thyroxine metabolism has been provided recently by Kellaway *et al.* (1945). They studied the effects of thyroxine on the heart rates of thyroidectomized, partially hepatectomized rats. Doses in excess of the maintenance requirement caused a greater heart rate increase in these animals than in the thyroidectomized controls. With amounts just large enough for the maintenance of a normal heart rate, however, there was no increase in heart rate in the partially hepatectomized animals. They interpret these data to indicate that normally there is a physiologic balance between the thyroid secretion rate and the rate of utilization of the hormone. Under these conditions, the liver is believed not to be called upon to exert a decomposing action on thyroid hormone. If, however, the hormone is present in excessive amounts, the liver is stimulated in some way to inactivate the excess thyroxine. These investigators believe that the mechanism is one of destruction rather than simple excretion, since, when they ligated the bile duct of thyroidectomized rats and injected large amounts of thyroxine they obtained no increase in heart rate.

Another interpretation of their data suggests itself. The liver may be able to inactivate most of the thyroxine injected in normal doses. With excessive doses, the liver, although possibly stimulated to greater inactivation of the hormone, may not be able to cope with the increased amount of thyroxine, resulting in an increased excretion into the bile. This thyroxine would then be released into the gastrointestinal tract to be partially reabsorbed in the general circulation and, hence, to exert its action on the heart before the liver had another chance at inactivating it. This possibility is given weight by the work of Elmer and Luczynski (1933), who could find no thyroxine in the bile of fasted rabbits as compared with an appreciable amount in the bile of animals injected with thyroxine. In this connection, it is interesting to note that the

livers of rats injected with a physiologic dose of radioactive thyroxine contained a smaller percentage of the injected dose than did the livers of rats injected with a larger dose (Leblond, 1949).

Rate of Destruction of Thyroxine. It is apparent, at least, that the rate of destruction of thyroxine is greatly influenced by the dosage administered. Various estimates have been presented concerning the duration of thyroid hormone in the organism, which, by inference, indicates the rate of destruction of the hormone. The most recent estimates have been based on the measurement of the turnover rate of I^{131} by the method proposed by Zilversmit *et al.* (1943). Utilizing this method, Taurog and Chaikoff (1947) estimated that the rat used up 1.5 micrograms of thyroxine iodine (equivalent to 2.3 micrograms thyroxine) daily per 100 grams body weight. This is equal to the thyroxine iodine content of the thyroid gland of the rat. Likewise, Taurog *et al.* (1947) calculated that 1.5 percent of the thyroxine in the dog thyroid was replaced every hour. Recently, Salter and Johnston (1948) have calculated that 1 to 2 micrograms of hormonal iodine were degraded daily in the muscles of the rat.

Although these observations vary slightly, they represent strikingly constant values compared to the wide variations in the reported observations of the earlier literature concerning the duration of action of thyroactive compounds. Classically, the thyroid hormone has been thought of as a slow-acting hormone, capable of acting over long periods. The above observations drive home the falsity of this reasoning.

As an example, Plummer (1921) makes the general statement that the effect of 14 milligrams of thyroxine injected intravenously into thyroidless patients may not have worn off completely in 8 weeks. Baumann and Hunt (1925) calculated that it took about 65 days following complete thyroidectomy in the rabbit for the thyroid hormone in the tissues to become exhausted. Other estimates range from a few days to several weeks (Kendall, 1917, 1923; Gaddum. 1929; Thompson *et al.*, 1929-1934; and others).

It should be emphasized that these workers studied the duration of the *effects* of thyroid hormone administration, while the recent investigations indicate the length of time that the hormone itself remains in the organism. It is possible (and probable) that the thyroid hormone, since it affects nearly every cell in the body, produces cellular changes (especially in the anterior pituitary) which manifest themselves long after the causative agent has disappeared.

Possible Biochemical Reactions in the Destruction of Thyroxine. The biochemical mechanisms by which thyroxine is destroyed may be only speculated upon at the present time. It has been generally assumed that the most likely method of thyroxine degradation is its deiodination (Salter, 1940). There is much evidence to indicate that this assumption is justified; e. g., the increased inorganic iodide content of many tissues after the administration of thyroid hormone (Veil and Sturm, 1925; Zawadowsky and Asimoff, 1927). Also, Foster and Gutman (1930) maintain that the closely related diiodotyrosine is rap-

idly broken down in the body with the liberation of iodide. Be this as it may, there are many other possible mechanisms worthy of consideration.

Perhaps, since thyroxine is an amino acid, its inactivation follows the usual path of amino acid metabolism—deamination and/or decarboxylation. If deamination took place, the end product would be an alpha-keto acid or, in the event of a secondary reduction, an alpha-hydroxy acid. Credence is lent this possibility by the observation of Foster and Gutman (1930) that after the administration of massive doses of diiodotryrosine, a compound identified as 3,5-diiodo-4-hydroxyphenyl lactic acid was found in the urine. If the naturally occurring L-thyroxine is deaminated to form its alpha-keto analogue, it is possible, also, that it may be reaminated in such a way as to become the physiologically inactive dextro-rotatory isomer (Du Vigneaud and Irish, 1937). Either of these possibilities is the more interesting in view of the fact that Gross and Leblond (1947) admit the possibility of an active, butanol-soluble, iodine compound in the feces.

Another possible method of thyroxine inactivation is the breaking of one or both of the rings. This process would seem highly improbable, since drastic chemical treatment would be necessary to accomplish the feat *in vitro*. However, tissues are sometimes capable of amazing accomplishments, as shown in the present instance by Bernheim and Bernheim (1944). They found that, *in vitro*, various tissues—liver, kidney, heart, and skeletal muscle—were capable of breaking the tyrosine ring.

Although none of the above reactions for thyroxine has been demonstrated in the living organism, all of them are possible, singly or in combination. Such speculations make for a tremendously complicated picture, and it is obvious that much investigation is needed. Perhaps, with radioactive isotopes becoming readily available, something can now be done in this direction.

EXPERIMENTAL

It appears obvious from a review of the literature on the subject that much more research is needed on all phases of the problem of thyroxine metabolism. Various questions come to mind and problems are posed which are in dire need of extension and clarification. To mention a few, what are the relative rates of absorption of orally administered thyroactive compounds and what factors are operative in governing this relative absorbability? How can the feeding of these compounds be made more effective? To what extent does the destruction of activity play a part in the economy of the thyroid hormone? Where in the body does this destruction take place and, again, what are the operative factors?

It was in an attempt to answer some of these and similar questions that the following investigations were undertaken.

Absorption of Thyroidally Active Substances

It has been shown in the first part of this paper that reports vary consid-

erably concerning the absorbability of various thyroactive substances. Apparently the chemical form in which the hormone is fed and, consequently, its solubility in water are among the more important factors in determining the extent of absorption from the alimentary tract.

Thus, pure crystalline thyroxine, which is virtually insoluble in water, is reported to have the least effect on the BMR of any thyroidally active compound when administered orally (Thompson *et al.*, 1933, 1934). Monosodium thyroxine reportedly is somewhat more active (and, likewise, is more soluble), while thyroxine in alkaline solution (di-alkali form) shows activity of the same order of magnitude as that evoked by the intravenous injection of the same substance (Thompson *et al.*, 1933).

Likewise, it will be remembered that the increment in urinary iodine excretion after ingestion of an alkaline thyroxine solution (14 percent in 24 hours) is more than double that after peroral crystalline thyroxine (6 percent in 24 hours) (Elmer and Rychlik, 1934). The inference is, of course, that the thyroxine in alkaline solution was over twice as readily absorbed.

That desiccated thyroid is easily absorbed is shown by the work of Asimoff and Estrin (1931). After the administration of desiccated thyroid, 93 to 95 per cent of the iodine contained therein was excreted in the urine, whereas after thyroxine only a small increment (4.5 to 8.5 percent) in urinary iodine could be detected. The investigators explain these differences as due to differences in absorption of the two substances. It must be noted, however, that desiccated thyroid contains, in addition to thyroxine, considerable amounts of diiodotyrosine, which is very readily absorbed and excreted in the urine (Foster and Gutman, 1930; Sainton *et al.*, 1934). Taurog and Chaikoff (1947) and Wolff and Chaikoff (1947) have found recently that about 70 percent of the gland's total iodine is present as diiodotyrosine iodine.

The investigations of Fellenberg (1926) on man indicate a much less marked absorption of desiccated thyroid, as shown by a smaller urinary iodine excretion (16.6 to 28.6 percent in 24 hours). The difference between the results of Fellenberg and those of Asimoff and Estrin may be due in part to species differences, and to different dosage levels, which are known to affect the percentage of iodine eliminated in the urine (Elmer, 1938).

At any rate, it is clear that the absorption of desiccated thyroid is superior to that of thyroxine (Harington and Salter, 1930). Quite possibly thyroxine occurs in desiccated thyroid in a readily water soluble form (peptide combination) and, consequently, is easily absorbed.

It should be mentioned in this connection that, even when injected, desiccated thyroid displays potency four to five times that expected from its thyroxine content, even assuming all the thyroxine to be present as L-thyroxine, and assuming L-thyroxine to have twice the potency of the racemic mixture (Frieden and Winzler, 1948). Thyroidal activity in these experiments was measured by the goiter prevention method in thiouracil-treated rats.

With respect to these findings, the possibility should be noted that the thyroid gland may contain substances unrelated to thyroxine which affect the thyroid-pituitary axis (Mansfeld, 1943).

Unlike the above observations on desiccated thyroid, there are no reports in the literature comparing the relative oral effectiveness of synthetic iodinated proteins and thyroxine. Schultze and Turner (1945), showed that 0.009 percent iodinated casein (thyroprotein) in the diet was sufficient to maintain the thyroid glands of thiouracil-treated chicks at a normal weight, as compared with approximately 2.0 micrograms of D,L-thyroxine injected subcutaneously. The thyroprotein used in this study contained 2.7 percent thyroxine as determined by the method of Reineke *et al.* (1945). On this basis a 100-gram chick would receive about 35 micrograms of thyroxine daily. In other words, thyroxine was only about 4.5 percent as effective when fed in the form of thyroprotein as it was when injected. If, as it is assumed, the thyroxine is present in thyroprotein in the levo form (Reineke and Turner, 1943), this figure should be even lower. In the light of more recent results, however, these figures should be revised, as will be seen shortly.

Frieden and Winzler (1948) in the study reported above found that thyroprotein was only one-half as active when injected as expected from its L-thyroxine content. The thyroxine determination was carried out by the method of Reineke *et al.* (1945).

Recently, however, Reineke (personal communication) has found, using an isotope dilution method, that only 18 to 29 percent of the "thyroxine" determined by his former method is true thyroxine. Apparently some iodine-containing compound having similar solubility characteristics in *n*-butanol can be hydrolyzed from thyroprotein. Correcting Schultze and Turner's data on this basis, their chicks would be receiving about 7.5 to 9.0 micrograms L-thyroxine in the feed daily. Assuming, for the moment, that L-thyroxine possesses twice the biological activity of the racemic mixture, thyroxine would then be about 15 percent as effective when fed in the form of thyroprotein as when injected as an alkaline thyroxine solution. If this figure is taken as an absorption percentage, it compares fairly well with the absorption value reported by Fellenberg (1926) for desiccated thyroid. And, since only 25 percent of the iodine in the thyroid is thyroxine iodine (Wolff and Chaikoff, 1947), the absorption of desiccated thyroid reported by Asimoff and Estrin (1931) should probably be lowered to about this percentage of their observed value, which would then indicate about 20 percent absorption.

In a similar manner, by correcting the data of Frieden and Winzler (1948) in regard to the amount of thyroxine in thyroprotein, the discrepancies observed between the relative activities of desiccated thyroid and thyroprotein become very much less apparent.

Likewise, some other seeming contradictions reported in the literature disappear. Reineke *et al.* (1945) determined quantitatively the amount of thyrox-

ine in iodinated casein (thyroprotein) and have found that it contains twenty to thirty times as much thyroxine as U.S.P. thyroid. On this basis, one would expect a concomitant physiologic response when equal amounts of desiccated thyroid and thyroprotein are compared, assuming, of course, that the absorbability of the respective products is equal. According to a previous report, however, thyroprotein, assayed by the metabolic response of guinea pigs, is only four times as effective as desiccated thyroid (Reineke and Turner, 1943). From these data, therefore, it would appear that thyroprotein is absorbed only 12.5 to 20 percent as easily as desiccated thyroid. When these results are corrected according to Reineke's recent observations, the results reported in both papers come into nearly perfect accord.

It is unfortunate that all observations reported above are, to a greater or lesser degree, indirect measurements of the actual amounts of thyroactive substance absorbed from the gastro-intestinal tract. It seemed worthwhile to pursue this study further, especially since a method is now available which, it is believed, would give a more accurate indication of the relative activity of the various thyroxine compounds. Also, it seemed that if solubility is the important factor, a more valid comparison would be obtained by administering all three forms of thyroxine in the same physical state, i. e., in solid form as contrasted, for example, with feeding the disodium salt already in solution.

Methods and Materials. The technique used in these experiments for determining the activity of the thyroxine compounds was essentially the same as that proposed by Dempsey and Astwood (1943) for measuring the thyroid secretion rate. In general, this technique embodies the hypothesis that the amount of thyroxine necessary to counteract the effects of thiouracil (compensatory hypertrophy) and maintain the thyroid gland at normal weight is equivalent to the amount of hormone normally produced by the thyroid. It was thought that, for our experiments, this method could be used to advantage. By including thyroxine in crystalline, monosodium, and disodium forms in the feed of thiouracil-treated chicks, the amount of each form required to maintain thyroid glands at normal weight could be ascertained. Thereby we should obtain a fairly accurate measure of the relative oral effectiveness of these thyroxine compounds.

The chicks used in these experiments were day-old White Plymouth Rocks purchased from a local hatchery. As they were obtained, the chicks were placed in various groups. One group served as a control; each other group received 0.1 percent thiouracil and one of the various doses of whichever form of thyroxine was under consideration. In the preliminary attempts fairly wide dosage intervals were used. The results of these preliminary attempts are not reported, since they were intended primarily for the orientation of the proper dosage ranges. When the proper range had been determined, the dosage interval was narrowed. In order that we might feel assured that the proper dosage levels had been attained, the latter runs were repeated. The results were re-

ported in Table 1 and Figures 1 and 2 represent a combination of the data obtained in these two runs, with the exception of the groups receiving 0.00003 percent by weight of monosodium and disodium thyroxine, where only one was conducted.

The basal ration fed to the chicks in these and in all subsequent experiments, except where otherwise noted, was compounded as follows:

	Parts by Weight
Yellow corn meal	45
Shorts	15
Soybean oil meal	15
Alfalfa meal	10
Meat scraps (50% protein)	7
Bran	5
Bone meal	0.5
Common salt	1
Cod liver oil (400 A.O.A.C. units vitamin D per gram)	0.25

TABLE 1.--EFFECT OF D.L-THYROXINE AND ITS SALTS IN THE FEED OF THIOURACIL TREATED CHICKS
(White Plymouth Rock 3-week-old chicks)

Form of Thyroxine	Dosage %	Corrected Dosage %	No. of Chicks	Body Weight gm.	Thyroid Weight mg.	Thyroid Weight per 100 gm. Body Weight mg.	Estimated Normal Requirement %
				MALES			
Control*	------	--------	48	160.1	8.9	5.6	
Crystalline	0.000050	0.000050	26	145.2	23.1	15.9	
"	0.000075	0.000075	21	173.1	8.9	5.1	0.000074
"	0.000100	0.000100	23	162.6	4.5	2.8	
Monosodium	0.000025	0.0000243	18	161.9	33.5	20.7	
"	0.000030	0.0000291	7	141.0	15.4	10.9	
"	0.000035	0.0000340	31	153.7	9.7	6.0	
"	0.000040	0.0000388	20	163.5	5.2	3.2	0.000035
Disodium	0.000025	0.0000236	24	161.7	34.5	21.3	
"	0.000030	0.0000283	7	133.7	21.7	20.3	
"	0.000035	0.0000330	33	150.5	13.3	8.8	
"	0.000040	0.0000378	16	169.3	9.6	5.6	0.000038
				FEMALES			
Control*	------	--------	47	148.2	9.2	6.2	
Crystalline	0.000050	0.000050	15	151.7	18.6	12.3	
"	0.000075	0.000075	18	161.6	10.9	6.7	
"	0.000100	0.000100	14	156.1	4.8	3.1	0.000078
Monosodium	0.000025	0.0000248	18	156.9	43.1	27.5	
"	0.000030	0.0000291	11	139.5	22.7	16.3	
"	0.000035	0.0000340	28	147.8	7.9	5.3	
"	0.000040	0.0000388	19	146.6	6.6	4.5	0.000036
Disodium	0.000025	0.0000236	15	140.8	42.6	30.3	
"	0.000030	0.0000283	12	121.3	25.3	20.9	
"	0.000035	0.0000330	28	143.3	16.6	11.6	
"	0.000040	0.0000378	22	151.7	10.9	7.2	0.000039

* All groups except controls received 0.1% thiouracil in their feed.

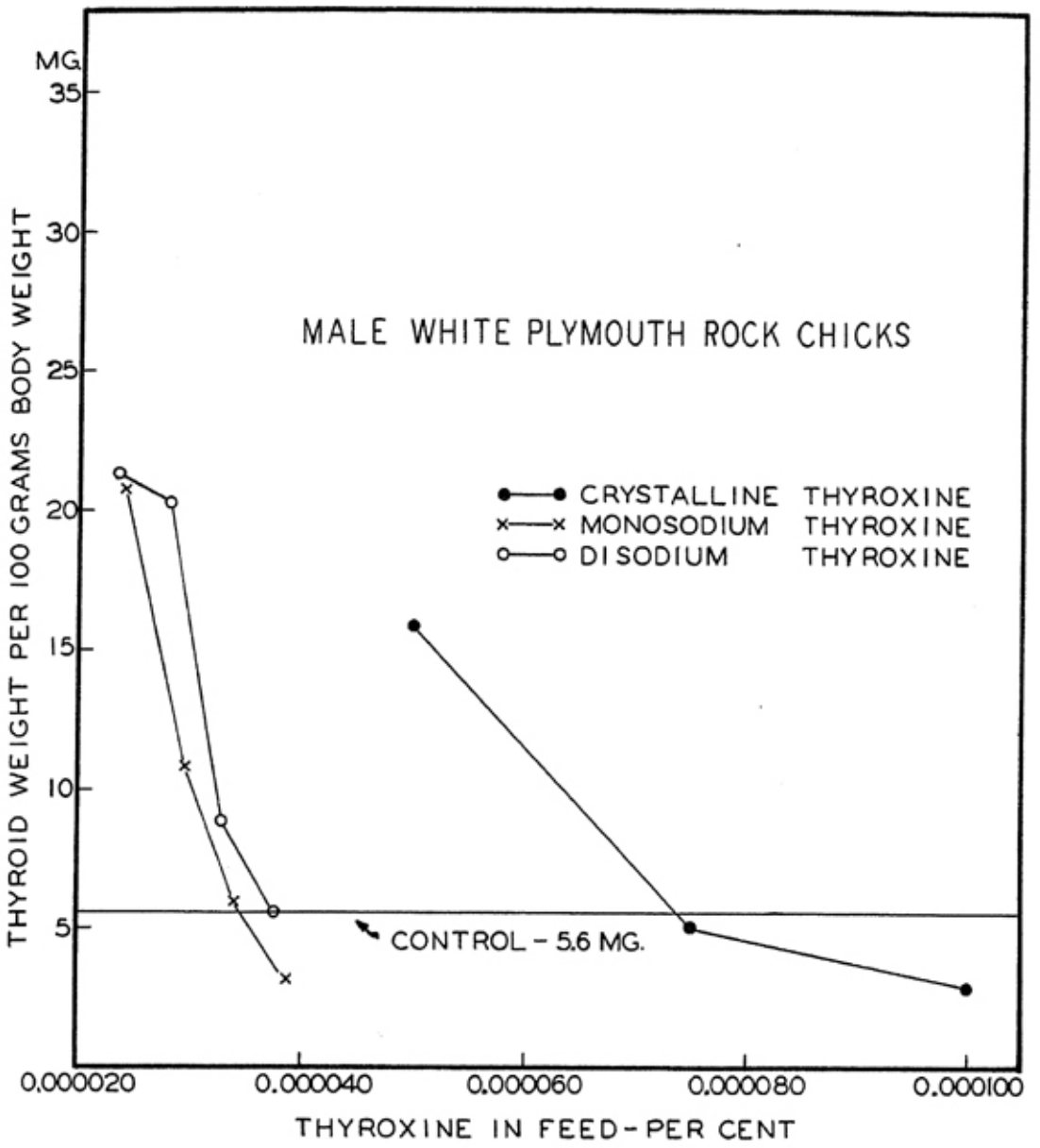

Fig. 1.—Plot of data showing the relative oral effectiveness in male chicks of thyroxine in crystalline, monosodium, and disodium forms.

Each experiment was of three weeks duration. At the end of this period the chicks were killed, sexed, and their body weights determined. The thyroid glands were removed and weighed immediately.

It should be mentioned that some difficulty was encountered in determining the dosage range and interval for the chicks receiving crystalline thyroxine. For some inexplicable reason, one or two of the earlier attempts indicated dosages misleadingly high. However, several successive attempts yielded reasonably repeatable results. It was found, however, that it was impossible to narrow the dosage interval to the same extent as with the salts of thyroxine, due

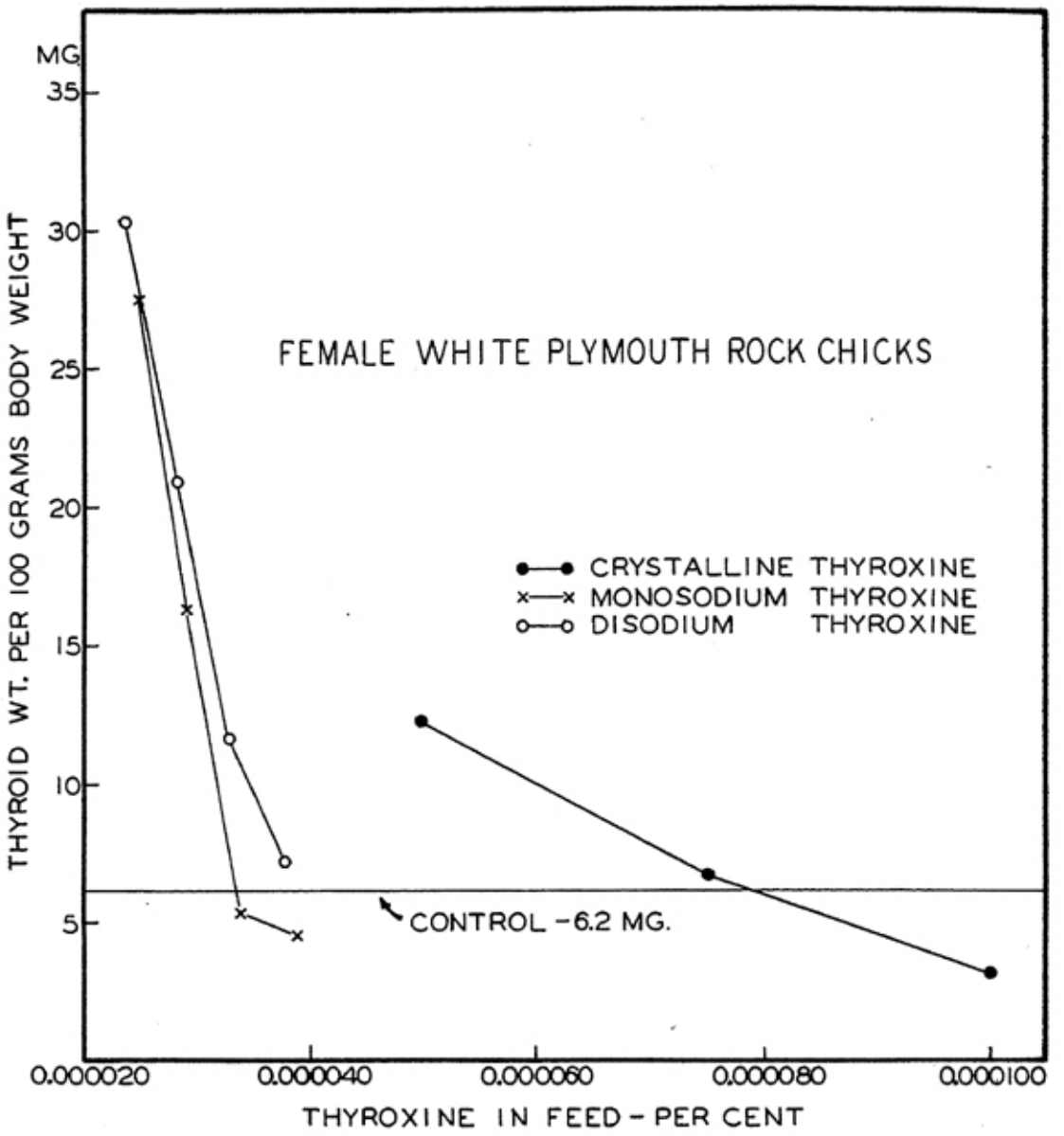

Fig. 2.—Plot of data showing the relative oral effectiveness in female chicks of thyroxine in crystalline, monosodium, and disodium forms.

to the fact that the variability of thyroid weights within dosage groups confused the interpretation of the data.

The crystalline D,L-thyroxine used was a commercial preparation purchased from the British Drug House, London. The monosodium and disodium salts were made from this stock by the method outlined by Harington (1933). These salts were checked for purity by iodine analyses, which showed that the monosodium salt contained 63.70 percent iodine and the disodium salt, 60.91 percent, as compared to theoretical percentages of 63.46 percent and 61.69 percent, respectively.

Both the thiouracil and thyroxine were mixed in the feed as the proper

percentage by weight. In this way, of course, a certain error was introduced. The addition of sodium atoms to the thyroxine molecule causes a diminution of the actual amount of thyroxine received by the groups treated with thyroxine salts. This error is taken into account in the presentation of the data (Table 1; Figures 1 and 2).

In a similar manner a commercial preparation of desiccated thyroid (Viobin Corporation) was assayed for thyroidal potency. The results of this assay are shown in Figure 3.

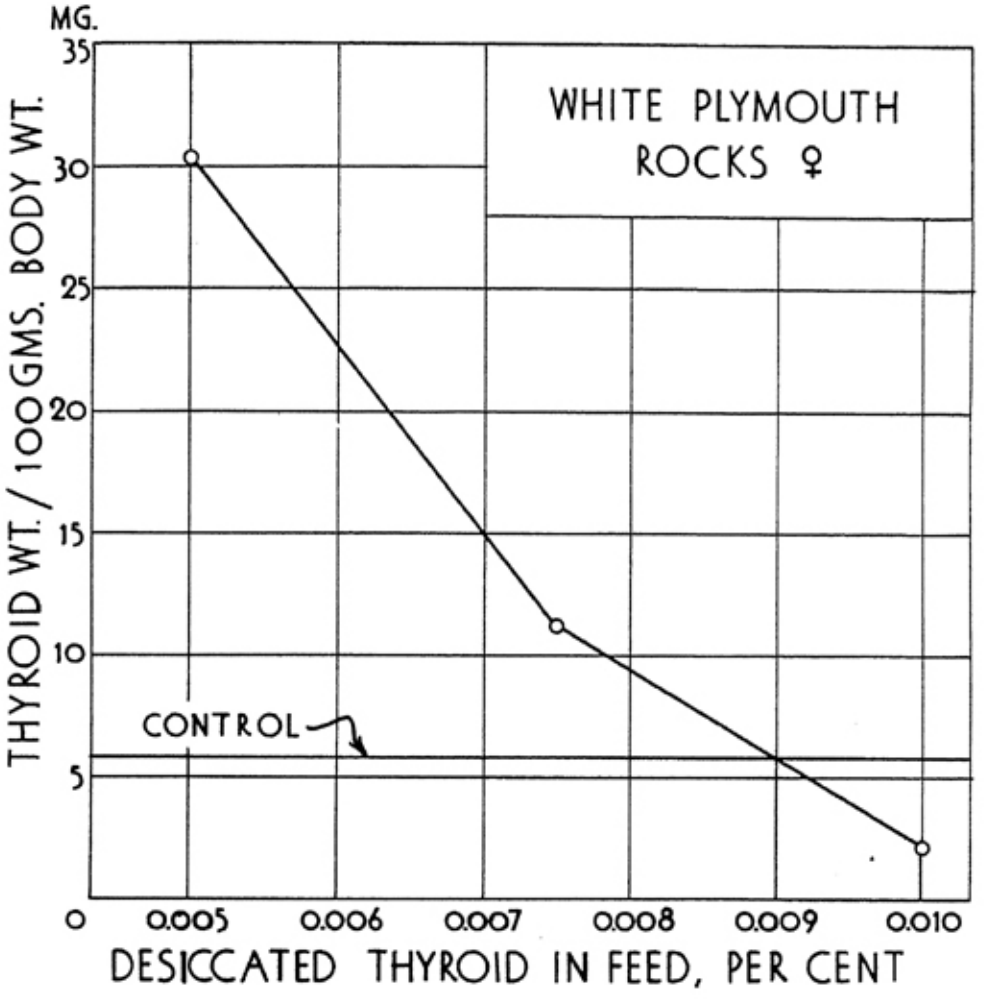

Fig. 3.—Plot of data showing the oral potency of desiccated thyroid powder.

In addition, by courtesy of Mr. E. Kauffman some data were gathered on the absorption of thyroprotein by goats on another experiment. Each of two lactating female goats was fed 1.5 grams of thyroprotein daily for three weeks. During this period their feces were collected and dried in an electric oven at 45° C. The feces were then assayed for thyroidal activity as described above, being fed at levels of 2.5, 5.0 and 10.0 percent, respectively. The difference in activity between the thyroprotein in the feed and that in the feces was taken to indicate the extent of absorption of the thyroprotein.

Results. It was found that the requirements for maintaining the thyroid glands of the thiouracil-treated male chicks at a normal weight were 0.000074 percent of crystalline D,L-thyroxine in the feed, 0.000035 percent of the monosodium salt, and 0.000038 percent of the disodium salt. Comparative figures for the female chicks were 0.000078 percent, 0.000036 percent, and 0.000039 percent, respectively, as shown in Table 1.

As was anticipated, the results of this experiment indicated some relationship between the solubility of the compound administered and its physiological activity. However, the differences noted in the compounds studied are not as marked as might be expected. For example, the difference in solubility between monosodium and disodium thyroxine would lead one to expect some difference in the activity of these salts in favor of the disodium form. On the contrary, however, they seem to be practically equal in activity. In fact, our results indicate that the disodium salt seems to be slightly less active. However, we believe that his discrepancy is negligible and within the limits of experimental error.

Also, the magnitude of the differences between the activity of crystalline thyroxine and its salts appears somewhat less than might be expected. The results obtained show that the mono- and disodium salts of thyroxine, when administered orally, are approximately twice as active as the pure crystalline form.

Although these observations do not show the exact amount of thyroxine absorbed, an approximation of this figure can be calculated. Chicks of the age used in this experiment consume, on the average, 20 to 25 grams of feed a day. The ration containing 0.000075 percent of crystalline thyroxine would therefore supply about 15 gamma of thyroxine per chick per day. This amount is about five times that required when D,L-thyroxine is injected subcutaneously in alkaline solution (Schultze and Turner, 1945). Therefore, assuming that the injected thyroxine is utilized one hundred percent, then approximately twenty percent of the orally administered crystalline thyroxine is absorbed. On the same basis, 45 percent of the sodium salts of thyroxine are absorbed. The latter calculation agrees closely with the observations of Dressler and Holling (1940) who found that disodium thyroxine is about 43 percent as effective when fed as when injected.

Clearly our results on the oral administration of thyroxine and its salts are at variance with those of Thompson *et al.* (1933), mentioned previously. There are several possible explanations for these differences. Species difference is an obvious possibility. Assay technique is another. The fact that these workers found a difference between the monosodium and disodium salts, whereas we did not, might be explained by their using thyroxine in alkaline solution as the disodium form in contrast to our use of a solid disodium salt. It must also be remembered that Thompson *et al.* observed only three patients and these had a definite myxedema. And, as these observers point out, the effect of thy-

roxine on the basal metabolism of such patients may be greater than in normal persons.

The work of Elmer and Rychlik (1934) on urinary iodine excretion shows, by inference, that thyroxine administered orally in alkaline solution is absorbed a little more than twice as readily as pure crystalline thyroxine. This observation is in agreement with our results. It must be admitted, however, that the observations of Elmer and Rychlik may be somewhat misleading. Since no thyroxine can be detected *per se* in the urine (Elmer and Scheps, 1933, 1934), the increment in urinary iodine caused by thyroxine administration must be due to some decomposition product of the hormone. And while it may be reasonable to assume that more thyroxine must be absorbed in order to promote this mode of iodine excretion, it is by no means the only excretion route for circulating thyroid hormone (Gross and Leblond, 1947).

Schittenhelm and Eisler (1932) arrived at a figure not too different from ours regarding the absorption of crystalline thyroxine. They found that 14 percent of the crystalline thyroxine was absorbed, while our calculations indicate that about 20 percent is absorbed. However, these investigators found 90 percent of the thyroxine in alkaline solution is absorbed. There is a considerable discrepancy between this figure and our calculated 45 percent for the solid disodium salt of thyroxine. Two possible explanations are suggested for this difference: (1) The thyroxine already dissolved in alkaline solution may be more readily absorbed than the solid disodium salt; and/or (2) as it is absorbed, more of the thyroxine may be inactivated or excreted and therefore would not manifest itself in our measurements. Also, Schittenhelm and Eisler introduced the alkaline solution of thyroxine directly into an isolated loop of intestine, whereas we fed the disodium salt. It is possible that not all of the thyroxine remained as the disodium salt, due to pH changes in the gastro-intestinal tract. This might also be a partial explanation for our finding no difference between the activity of the mono- and disodium salts of thyroxine.

Considering the overall picture, then, it appears that pure crystalline D,L-thyroxine is less effective when administered orally than are its more soluble sodium salts. Thyroxine already dissolved in alkali may be even more effective. Apparently, therefore, the solubility of the compound administered is an important factor—as has been suggested by several investigators—but it appears to be by no means the only factor.

It can be seen from Figure 3 that 0.009 percent desiccated thyroid in the diet was sufficient to maintain normal thyroid weight in thiouracil-treated chicks. On the basis of thyroxine content this would be equivalent to feeding 2.0 to 2.5 gamma of L-thyroxine per chick per day, or since the average body weight of this group was about 175 grams approximately 1.3 gamma thyroxine per 100-gram chick per day. If it is assumed that L-thyroxine has twice the potency of the racemic mixture (Reineke *et al.*, 1945), these results indicate that desiccated thyroid administered orally is about equally as effective as injections

of equivalent amounts of D,L-thyroxine (2.0 gamma) in alkaline solution (Schultze and Turner, 1945). Recently, however, Pitt Rivers and Lerman (1947) have claimed activity for D-thyroxine one-tenth to one-eighth that of the L-isomer, and Griesbach *et al.*, (1949) ascribe three-tenths as much activity to the D- as to the L-form. In this event, the desiccated thyroid shows somewhat more activity than would be expected from its L-thyroxine content. This calculation is in agreement with the observations of Frieden and Winzler (1948) on comparative injections of desiccated thyroid and thyroxine. It would appear, therefore, that desiccated thyroid is about equally as efficacious perorally as parenterally.

The extent of absorption of thyroprotein was measured by a more direct method than the other thyroactive compounds studied. Its measurement was based on the familiar formula

Amount ingested — amount excreted = amount absorbed.

In the present study, however, the amounts measured are not absolute quantities but relative activities, as determined by the goiter-prevention technique.

It has already been shown that thyroprotein contains about 1.5 percent true thyroxine, in terms of equivalent amounts of the racemic mixture. On this basis, then, each experimental goat received 22.5 milligrams of D,L-thyroxine equivalent per day. It can be seen from Figure 4 that feces from these goats at a 7 percent level in the feed was sufficient to maintain the thyroids of thiouracil-treated chicks at a normal weight. It will also be recalled that crystalline D,L-thyroxine was needed at a level of 0.000078 percent to perform the same task. At this level, the chicks received about 15 gamma of the disodium thyroxine per chick per day. Consequently, knowing the amount of feces consumed per chick per day on the 7.0 percent level (1.5-1.75 grams) it can be calculated that the goat feces contained the equivalent of 8.6 to 10.0 gamma of D,L-thyroxine per gram of dried feces. Each goat, therefore, excreted the equivalent of 2.6 to 5.0 milligrams of thyroxine daily. Thus, of the 1.5 grams

TABLE 2.--INFLUENCE OF FECES FROM THYROPROTEIN-FED GOATS ON THE THYROID SIZE OF THIOURACIL-TREATED CHICKS

Group	No. of Chicks	Body Weight (gm.)	Thyroid Weight (mg.)	Thyroid Weight per 100 gm. Body Wt. (mg.)
Control	25	169.8	10.3	6.1
0.1% Thiouracil alone	24	144.4	67.6	46.8
0.1% Thiouracil plus 2.5% goat feces	21	169.5	37.1	21.9
0.1% Thiouracil plus 5.0% goat feces	22	161.7	13.8	8.5
0.1% Thiouracil plus 10.0% goat feces	25	154.9	4.1	2.7

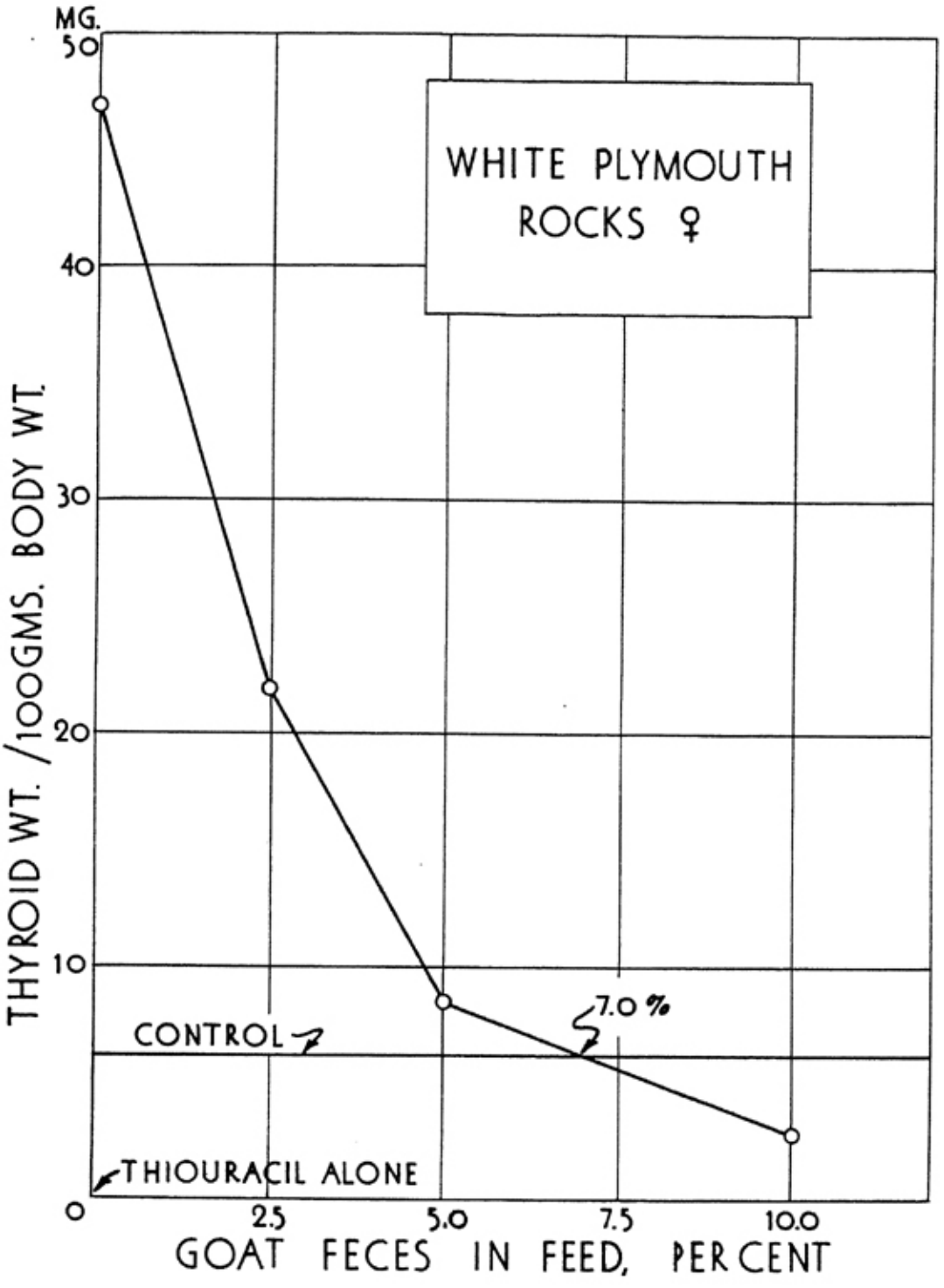

Fig. 4.—Plot of data showing the thyroidal activity in the feces of goats fed thyroprotein.

of thyroprotein fed daily to each goat, 78 to 88 percent was absorbed, in terms of equivalent amounts of the crystalline D,L-thyroxine. These figures are considerably higher than that calculated for the absorption of thyroprotein in the chick (15 percent). Indeed, they are nearly as high as the figures reported for any thyroactive compound.

A possible source of error in this experiment lies in the fact that the results are based on the assumption that all the thyroprotein (thyroidal activity) not accounted for in the feces must have been absorbed. There is no allowance made for possible destruction of the hormone in the gastro-intestinal tract. As mentioned previously, this occurrence seems unlikely, but it is a possibility which cannot be excluded at the present time.

It may well be that the active compounds in the feces are somewhat different from crystalline thyroxine in oral potency, as measured by the goiter-prevention method. There is some risk of error, therefore, in calculating the amount of hormone excreted in thyroxine equivalents. Whether or not this factor constitutes a major problem in the interpretation of the data cannot be stated with any degree of certainty at present.

Interpretation of the data is further complicated by the fact that some of the thyroprotein or its digestion products may be absorbed only to be excreted back through the intestinal wall (Schittenhelm and Eisler, 1932). Also, some of the absorbed hormone may find its way into the bile by way of the liver, whence it is emptied into the intestinal tract. Some of this hormone may be excreted directly, some reabsorbed. It is the opinion of the writers, however, that these routes do not constitute a major source of hormone in the feces, as will be shown in subsequent experiments. Indeed, the high absorption values calculated indicate, in themselves, that little of the hormone is likely to reach the feces by such devious routes.

Excretion of Thyroxine

It is distinctly evident in the review of literature that a regrettable scarcity exists on the subject of thyroid hormone excretion. However, it seems certain that no thyroxine is excreted under normal conditions in either the urine (Elmer and Scheps, 1934) or the feces (Fellenberg, 1926).

After administration of thyroidally active materials, on the other hand, fecal and urinary iodine contents are increased to a greater or lesser extent, as noted earlier, depending on such factors as dosage, route of administration and the form in which the active compound is introduced. Even under these conditions, however, the increment in urinary iodine is apparently non-thyroxine in nature (Veil and Sturm, 1925; Gross and Leblond, 1947), whereas the feces may contain considerable amounts of thyroxine (Gross and Leblond, 1947).

Since so little information is available on this subject in the literature, it seemed of primary importance to conduct an experiment to determine the extent of fecal and/or urinary excretion of thyroxine introduced parenterally. It was thought that the presence of thyroxine in the urine and/or feces could be detected by incorporating these excreta into chick rations simultaneously with thiouracil. Any material reduction of the thyroid hypertrophy caused by thiouracil would indicate the presence of a thyroidally active substance.

Methods and Materials. The urine and feces of two female lactating goats kept in metabolism stalls were collected for five days. The urine was added to ten kilograms of poultry ration and the whole mixture dried in an oven at 45° C. The feces were dried at the same temperature, then ground and mixed in the feed at a level of twenty percent by weight.

The goats were then injected subcutaneously with ten milligrams of D,L-thyroxine daily for a period of ten days. This thyroxine was dissolved in a minimum amount of N/10 sodium hydroxide and, therefore, was administered as the disodium salt.

The urine and feces were collected during the first five days of injections, and dried and mixed in the feed as described above. The same procedure was followed during the second five days of injections.

Thiouracil was added to each of these feeds at a level of 0.1 percent by weight.

Eight groups of day-old White Rock chicks were used in this assay, each group consisting of about twenty chicks. One group received normal feed and served as a control. A second group received normal feed containing 0.1 percent thiouracil. The remaining groups received the feeds containing the various samples of urine and feces described above. (See Table 3.) The assay was of three weeks' duration, starting on June 23 and ending on July 14. The chicks were kept in a basement room illuminated by incandescent bulbs and diffuse sunlight. The average daily temperature varied from 80° to 85° F.

TABLE 3.--THYROIDAL ACTIVITY IN THE FECES OF GOATS INJECTED WITH THYROXINE, AS MEASURED BY THE GOITER PREVENTION METHOD IN THE CHICK

Group	Feed	Males				Females			
		No.	Body Wt. gm.	Thyroid Wt. mg.	Thyroid Wt. /100 gm. body Wt. mg.	No.	Body Wt. gm.	Thyroid Wt. mg.	Thyroid Wt. /100 gm. body Wt. mg.
I	Control	9	148.8	4.9	3.4	12	159.3	7.9	5.0
II	Control+0.1% thiouracil	11	138.6	42.5	30.6	7	132.1	48.7	36.9
III	Control + thiouracil + 14.8 liters normal goat urine/10kg. feed	9	121.4	35.0	28.9	9	123.8	46.1	37.6
IV	Control + thiouracil + 8.4 liters goat urine* /10 kg. feed	7	140.6	30.4	21.6	12	143.2	52.0	35.8
V	Control + thiouracil + 10.3 liters goat urine** /10 kg. feed	11	161.7	48.5	31.5	8	146.4	57.0	39.3
VI	20% normal goat feces + thiouracil	12	146.8	32.2	21.9	7	143.7	49.9	35.0
VII	20% goat feces* + thiouracil	7	153.7	37.4	23.1	10	146.6	55.0	36.4
VII	20% goat feces** + thiouracil	10	145.9	8.2	5.8	8	165.8	7.4	4.1

* Collected during first five days of injections
**Collected during second five days of injections

At the end of the experimental period the chicks were killed by ether asphyxiation, their body weights ascertained, and the thyroid glands removed and weighed immediately. The sex of each chick was also determined at this time.

Results. The results of this experiment seem relatively clear cut. The addition of thiouracil to the normal chick ration, of course, caused marked thyroid enlargement (Table 3). This hypertrophy was completely counteracted by the inclusion of goat feces collected during the second five days of the injection period. By feeding this sample of feces to thiouracil-treated chicks, the thyroid glands of the chicks were maintained at normal weight.

On the other hand, the feeding of normal goat feces and feces collected during the first five days of injections to thiouracil-treated chicks seemed to cause only a slight reduction in thyroid size. However, this decrease is probably only apparent. The small decrease observed might well be accounted for by the fact that the nutritive value of the feed was probably lowered by the addition of feces. Also, the chicks ate slightly less of the feed containing feces than did the chicks given normal feed.

Likewise, the inclusion of urine, from either normal or injected goats, did not cause a reduction of thyroid hypertrophy.

Since no hormone was excreted during the first five days of injections, these observations suggest a retarded excretion of parenterally administered thyroxine. This might be the result (1) of some initial retention of the hormone, or (2) of a greater amount of hormone destruction in the first five days. In either case, one wonders whether the hormone may not be excreted for some time after the injections have been stopped. Consequently, a second experiment was conducted with this question in mind.

The injection period and dosage were the same as above. However, in this experiment only the feces were assayed, and these were collected over three-day periods. The first collection period included the fifth, sixth, and seventh days after the start of injections; the second period, the eighth, ninth, and tenth days; and the last period, the eleventh, twelfth, and thirteenth days. Unfortunately, the goats had to be removed from the metabolism stalls at this time because their feed consumption had dropped to about one-half normal, and they were being run on another (lactation) experiment. Likewise, of course, the feces output decreased proportionately so that it was necessary to include the feces of a 10 percent level in the chick ration instead of the 20 percent level fed in the preceding experiment.

It was found (Table 4) that even at the 10 percent level the feces of thyroxine-injected goats overcame the thyroid hypertrophy caused by feeding thiouracil to the chick. Apparently, therefore, the excess of thyroxine is excreted at approximately a constant rate, regardless of a lowered feed consumption.

Moreover, Table 4 shows that in the three days following the cessation of thyroxine injections considerably less hormone is excreted than during the

TABLE 4.--FURTHER STUDIES ON THE THYROIDAL ACTIVITY IN THE FECES OF GOATS INJECTED WITH THYROXINE

Group	Feed	Number	Body Weight (gm.)	Thyroid Weight (mg.)	Thyroid Weight per 100 gm. Body Weight (mg.)
I	Control	11	136.5	6.6	4.9
II	0.1% thiouracil plus 10% goat feces[1]	10	113.5	3.3	3.0
III	0.1% thiouracil plus 10% goat feces[2]	10	112.3	3.8	3.4
IV	0.1% thiouracil plus 10% goat feces[3]	10	117.1	8.3	7.1

[1]Collected during 5th, 6th, 7th days after starting thyroxine injections.

[2]Collected during 8th, 9th, 10th days after starting thyroxine injections. Injections were stopped after 10 days.

[3]Collected during 11th, 12th, 13th days after starting thyroxine injections.

preceding six days. It is unfortunate that the experiment could not be carried out for a longer period. However, these results are sufficient to indicate that the hormone is not excreted for long periods after injections are stopped; probably about a week would be a reasonable estimate. Yet it is interesting to conjecture that it takes the goat this long to dispose of the excess hormone. And an excess there must be, for no hormone is normally excreted in the feces. It is interesting, too, to calculate the percentage of hormone excreted via the fecal route.

Normally, the amount of total iodine excreted in goat feces is small—3 micrograms in 24 hours (Courth, 1931). Assuming all of this iodine to be in the form of thyroxine, the feeding of normal goat feces at a level of 20 percent by weight would be the equivalent of feeding 0.0000015 percent thyroxine. Since it takes about fifty times that amount of crystalline D,L-thyroxine in the feed to maintain the thyroid glands of thiouracil-treated chicks at a normal weight, it is not surprising that we find no thyroidal activity in normal goat feces.

On the other hand, after thyroxine has been administered, even subcutaneously, there is an appreciable amount of the hormone passed into the feces. As shown above, enough hormone is present in the feces collected during the second five days of injections to maintain the thyroids of thiouracil-treated chicks at normal weights, when the feces comprise 20 percent of the feed. As has been shown, the same result can be effected by feeding the disodium salt of D,L-thyroxine at a level of 0.0004 percent by weight. Therefore, it can be calculated that these feces contained approximately the equivalent of 0.4 micrograms of disodium D,L-thyroxine per gram of dried feces. On this basis, the daily excretion would be roughly 0.075 to 0.1 milligrams of thyroxine per goat. This amount accounts for only one percent or less of the daily injected dose.

It would appear that in the latter experiment over twice as much thyroxine was excreted, since the feces were fed at a 10 percent level and the thyroids of the assay chicks were depressed to a subnormal weight. However, in this case, the feces collected weighed less than half as much as in the former experiment. Thus, the total amount of thyroxine excreted was about the same.

It is evident that some hormone continues to be excreted for a short period after thyroxine injections have been stopped. But even taking this fact into consideration, it is inconceivable that more than 5 percent of the injected dose could be accounted for in the feces.

At any rate, the amount of thyroidally active material found in the feces in these experiments is much smaller than the amounts reported by other workers (Krayer, 1928; Gross and Leblond, 1947). Krayer (1928), of course, based his findings on the increment in fecal iodine after the injection of thyroxine; so we have no way of knowing, from his work, how much biologically active material was actually excreted. Gross and Leblond (1947), used thyroxine labelled with I^{131} and found that 80 percent of the injected dose of radioactive thyroxine appeared in the feces in 24 hours. About a half of this amount was butanol-soluble; i. e., was present as thyroxine. This recovered thyroxine, however, was found to be somewhat less active biologically than thyroxine prepared by the investigators or commercial preparations.

Concerning the mechanism of fecal excretion of thyroxine, there seem to be two major possibilities; (a) the liver probably excretes some unchanged thyroxine into the bile, and hence into the digestive tract; and (b) the intestine may actively excrete some thyroxine. The former route is doubtless the more important (Gross and Leblond, 1947).

It must be remembered, however, that the gastro-intestinal tract also absorbs thyroxine. Probably the actual, overall picture is a complicated combination of excretion, reabsorption, and metabolism, the extent of each of these processes depending on various unknown factors.

Rate of Disappearance of Thyroxine

The studies thus far have shown that the various thyroxine compounds are absorbed from the gastro-intestinal tract into the general circulation to a greater or lesser extent, depending on the form in which the material is fed. The fate of the circulating hormone would seem to be limited, in general, to three possible directions: (1) retention, (2) excretion, and (3) inactivation or destruction.

In our experiments, excretion of the active substance seems not to be the chief mode of hormone disposal. Indeed, even the investigations which show higher excretion rates (Gross and Leblond, 1947) indicate that a considerable amount of the hormone must be accounted for in some other way. Thus, a consideration of the other two possibilities seems mandatory.

One means of studying the extent of retention of the thyroid hormone or,

conversely, the rate of its destruction is to measure the rate of disappearance of the hormone. Most of the earlier investigations on this subject lead one to believe that administered thyroactive substances are retained in the body for considerable periods. In reviewing the literature it has been noted that administration of thyroxine compounds stimulates the basal metabolic rate for periods of several weeks. This type of observation has led to the common conception of the thyroid hormone as comparatively slow in its action and capable of acting over considerable periods.

Recent evidence, however, gained from studies utilizing radioactive iodine has proved this view to be false. Taurog and Chaikoff (1947), it will be remembered, estimated that the total amount of thyroxine in the rat thyroid is used up in one day. Likewise Taurog, Chaikoff and Entenman (1947) calculated that the turnover time for plasma protein-bound iodine in the dog was only 4 to 7.5 hours.

At this point it becomes advisable to emphasize the distinction between the duration of the hormone and the duration of its *effect*. The most recent studies cited show conclusively that the hormone itself disappears quite rapidly from the organism. On the other hand, there seems to be no reason to doubt the earlier investigations, which show that thyroxine has an extremely prolonged effect on the basal metabolic rate. It would appear, therefore, that although the hormone itself may disappear rapidly from the body, it may effect general cellular changes (especially in the pituitary gland) which manifest themselves for a considerable time in an altered metabolic rate.

Hughes (1945) devised an ingenious technique for studying the duration of single doses of thyroid hormone. Rats were injected with thiouracil 48 hours before hormone administration. Thereafter the rats received thiouracil as a 0.1 percent solution in the drinking water. Thus, the thyroid hypertrophy ordinarily caused by thiouracil was inhibited for a time after the thyroid hormone was administered. The point at which hypertrophy began was taken to indicate that the quantity of hormone in the body had fallen below normal level. Hughes concluded that duration time increased with the size of the dose —three days for a single injection of ten micrograms of thyroxine in solution to six days for a 1.0 milligram dose. It was also found that desiccated thyroid powder administered orally exhibited effects as long as thyroxine injected subcutaneously or intravenously in solution.

A modification of Hughes' technique was used in the present studies on the rate of disappearance of thyroxine from the organism. It seemed advisable, for the purpose of these experiments, to study the duration of orally administered thyroxine. Moreover, it was thought that a more nearly physiologic dose could be used if administered over a period of time. Consequently, the assay should then be more sensitive. And, finally, it was thought that once the proper dosage was established, the effect of various factors on the duration of action of thyroxine could be studied.

Methods and Materials. Day-old, female White Plymouth Rock chicks were kept in a basement room and fed solely the basal ration for one week. At the end of this time, thiouracil was incorporated into the feed at a level of 0.1 percent by weight simultaneously with graded doses of crystalline D,L-thyroxine, the doses ranging from a level of 0.0001 percent to 0.00125 percent by weight. The chicks were fed this ration for one week. Then they received feed consisting of the basal ration and 0.1 percent thiouracil.

The chicks were then killed at intervals, and the thyroid weights of each group were plotted on coordinate paper against the time after the cessation of thyroxine treatment. The point at which the thyroids returned to normal weight was taken arbitrarily as indicative of the duration time of the administered thyroxine.

It must be realized, of course, that measurements made by this technique are not absolute, but relative. Such factors as the time lag between the disappearance of thyroid hormone and the onset and rate of compensatory thyroid hypertrophy cannot accurately be taken into account. Hence, this technique is limited in that regard. However, it is believed that the relative duration times under different conditions can be measured with considerable acuity.

Results. Effect of graded oral doses. It can be seen from Figure 5 that the lowest dose fed (0.0001 percent) was not sufficient to bring the thyroid weights below normal weight, and thus the rate of disappearance of this dose could not be measured. The next higher dose, 0.00015 percent, lasted about 2.5 days, and the 0.0002 percent dose, 5 days. Increasing the dosage as much as four times the latter amount did not prolong the duration time. However, this high dose apparently approached the lower limits of toxicity, since a few of the chicks died within a day or so after being fed thyroxine at this level. On the highest level studied (0.00125 percent) the duration period was extended slightly to 6.5 days. This level was found to be definitely toxic, and it was necessary to increase the dosage gradually over a 4-day period until the desired level was reached, in order to limit fatalities to a minimum.

There comes to mind two possible explanations for these results. First, a limit of absorption may be reached at the 0.0002 percent level. On this basis, any amount fed beyond this level would be directly excreted, and no increase would be effected in the amount of circulating thyroxine. Consequently the duration period would necessarily remain the same. On the other hand, the amount of thyroxine absorbed might be greater with increasing doses and the animal stimulated in some way to dispose of the hormone at an increased rate. The fact that the higher doses proved to be toxic indicates that more thyroxine was being absorbed. Consequently, we are forced to the conclusion that the latter theory is the more plausible. It should be remembered, also, that Kellaway *et al.* (1945) are of the opinion that with larger doses of thyroxine the liver is stimulated to decompose more of the hormone than normally is the case.

The slightly prolonged duration period on the 0.00125 percent level could be taken at face value to indicate that, at this higher level, the thyroxine lasted longer. It seems more probable, however, that either (a) the fact that the chicks were fed thyroxine for 3 days longer than the other groups resulted in a greater accumulation of the hormone, or (b) the toxicity of this dose resulted in a diminished ability to dispose of the increased amount of hormone.

These results agree with the recent concept of a rapid turnover of thyroid hormone as opposed to the older idea of duration times of the order of several weeks. Part of the explanation, as Hughes (1945) points out, may be due to the fact that most of the earlier studies were carried out on myxedematous patients, which are known to differ from normal in their response to thyroid hormone. Equally likely, however, is the possibility that the thyroid hormone, in the course of its action, may cause general cellular changes which might well outlast the actual presence of the hormone. Quite probably measurements of the basal metabolic rate would reflect this state of affairs.

Apparently, at any rate, thyroxine disappears at a fairly rapid rate from the general circulation. Moreover, it seems that beyond a certain point the length of time that the hormone is present is independent of the amount of hormone administered. This is in agreement with the suggestion of Kellaway, Hoff and Leblond (1945), mentioned above, that amounts of thyroxine above the normal level stimulate an inactivation mechanism in the liver. It may be, too, that, as Winkler *et al.* (1934) suggest, myxedematous patients lack the ability to inactivate thyroxine. This also would serve as partial explanation of the discrepancy between our results and those of earlier workers. The suggestion of Winkler *et al.* that the site of such inactivation might be the thyroid gland itself, however, is contrary to the finding that thyroxine *per se* does not enter the thyroid gland (Gross and Leblond, 1947).

Possibly the rate at which the hormone is excreted from the circulation, depending on the amount present, may have a bearing on this problem. It is the opinion of the writers, however, that of the amount of active hormone which must be disposed of, the amount excreted is not a major fraction.

It might be mentioned, in connection with these results, that the tolerance for thyroxine is apparently much lower in chicks than in rats. At the 0.00125 percent level, the chicks were receiving 0.25 milligrams thyroxine per chick per day. If 20 percent of this thyroxine was absorbed, as was calculated earlier, only 50 micrograms of thyroxine actually reached the bloodstream daily. Even if this quantity were entirely accumulated every day for the week it was fed, it would amount to only 0.35 milligram. This amount is considerably less than the 1.0 milligram thyroxine injected in rats by Hughes (1945) without apparent ill effect. This finding is interesting, in that Zawadowsky and Asimoff (1927), on rather flimsy evidence, suggested that birds metabolize thyroxine more slowly and less completely than do mammals.

Factors Affecting the Rate of Disappearance of Thyroxine

Having established the standard response of chicks to graded oral doses of thyroxine, it was decided to study the effect of various factors on the rate of disappearance of thyroxine. Among the factors studied were sex differences, the effects of several vitamins, partial hepatectomy, and a comparison of subcutaneous, intraperitoneal and intrasplenic injections of thyroxine.

It was hoped that if a difference were exhibited between the sexes as to the duration of action of thyroxine, a suggestion might be offered concerning interrelationships between the thyroid hormone and such sexual differences as comparative growth rates.

On the other hand, the effects of the various vitamins were studied not only to determine the possibility of increasing the effectiveness of thyroxine feeding, but also to attempt to gain an insight into some of the possible biochemical mechanisms of thyroxine metabolism, especially with regard to the reactions involved in the catabolism of thyroxine.

Finally, the experiments on partially hepatectomized chicks, and chicks injected directly into the spleen with thyroxine were conducted in an attempt to determine the importance of the liver, so strongly implicated in the literature, in the process of thyroxine catabolism.

Sex Differences in the Disappearance of Thyroxine. *Methods and Materials.* The methods used in this determination were identical with those described above. Male chicks, starting at one week of age, were fed thyroxine at 0.00015 and 0.0002 percent levels, and the figures obtained were compared with those for females at the same levels (Figure 6).

Results. It was found that, at both levels, the thyroxine exhibited effects in the male chicks which lasted for approximately five days, or about the same length of time as for the 0.0002 percent level in female chicks. In other words, when administered at the 0.0002 percent level, thyroxine disappeared at an equal rate in both male and female chicks. At the 0.00015 percent level, on the other hand, the thyroxine lasted nearly twice as long in males as in female chicks.

It would seem, therefore, that male chicks are as capable of disposing of an excessive amount of hormone as are the females. On dosages nearer the normal level, however, the males apparently do not use up the hormone as rapidly.

Now, the thyroid secretion rates of male and female chicks three weeks of age do not differ appreciably (Schultze and Turner, 1945). If, however, the hormone is not so rapidly metabolized in the male chick, the result would be, in effect, the presence of more hormone available for the tissues of the male chick. In other words, the males would have a higher *effective* thyroid secretion rate even though the *apparent* secretion rate were the same as that of the females.

At first sight it seems difficult to reconcile these two facts; that the male

and female thyroid secretion rates remain equal while in the latter case the hormone disappears more rapidly. It would seem that an accumulation of the hormone would occur in the male, causing a depression of secretion by the thyroid. However, it is quite possible that a higher hormone level is brought about in the male up to a certain point. Beyond this level, the male chick would be stimulated to metabolize and/or execrete the excess hormone. Worded differently, the male would have a higher threshold tolerance for thyroid hormone. This hypothesis implies, of course, that, for some reason, the male anterior pituitary (thyrotrophic hormone) is less sensitive to the presence of thyroxine in the blood, perhaps because of some antagonistic endocrine balance.

At any rate, the evidence presented (Figure 5, page 46, and Figure 6, page 47) indicates that beyond a certain thyroxine level the duration time remains remarkably constant and independent of dosage.

The results presented here are provocative of speculation. For example, if males have a higher *effective* thyroid secretion rate than females, as calculated, it is readily conceivable that the increased amount of utilizable hormone might be a factor in the superior growth rate of young male chicks. In this connection, it is interesting to note that Rupp *et al.* (1949) have observed that, in the thyroidectomized rat, small, physiologic doses of thyroxine decrease nitrogen excretion—i. e., exert a protein anabolic effect—whereas, large doses have a protein catabolic effect.

It is obvious that a complete picture of the causes and effects of the thyroid hormone level must await much more research.

The Effect of Added Vitamins on the Disappearance of Thyroxine. Many reports have appeared in the literature indicating a functional relationship between thyroid activity and some of the vitamins, especially vitamin A and vitamins of the B complex. Some of the vitamins are considered antagonistic to the thyroid hormone and some synergistic. Although the mechanisms of this synergism or antagonism are unknown, some suggestions are offered in the literature.

As early as 1923 McCarrison found that the administration of cod liver oil would delay the metamorphosis of tadpoles receiving a high iodine intake. A decade later Eufinger and Gottlieb (1933) found that vitamin A would depress the accelerating effect of thyroxine on the metamorphosis of tadpoles. Thus, it is evident that an antagonistic relationship exists between vitamin A and the thyroid hormone.

Many workers have confirmed this viewpoint in various ways. Freudenberger and Clausen (1935) observed that vitamin A feeding reduced the thyroid weight of young rats, a fact noted more recently by Sadhu and Brody (1947). Moreover, the loss of weight caused by experimental hyperthyroidism can be partially offset by vitamin A administration (Drill, 1938; Logaras and Drummond, 1938). Further, Sheets and Struck (1942) found that in rats pretreated

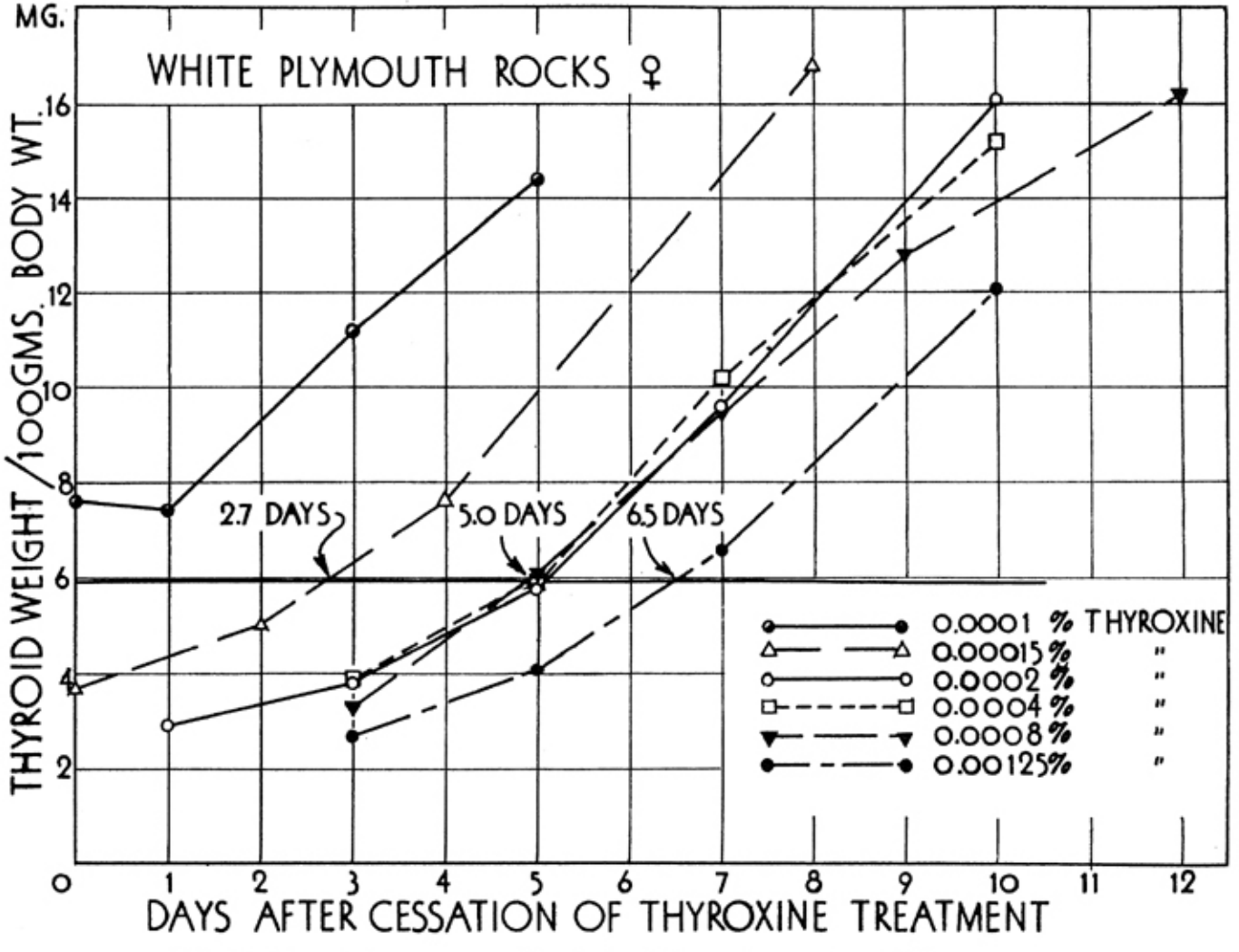

Fig. 5.—Rate of disappearance of graded oral doses of crystalline D,L-thyroxine.

with vitamin A the feeding of desiccated thyroid raised the basal metabolic rate only 25 percent, as compared with 58 percent for rats which received no vitamin A. However, after the BMR had increased in the latter rats, vitamin A administration had no significant effect. Likewise, it was found that vitamin A had no effect on oxygen consumption of normal rats, confirming the observation of Belasco and Murlin (1940). Thus, it seems that vitamin A can prevent a rise in BMR due to thyroid administration, but is powerless to reduce the BMR once it has been raised.

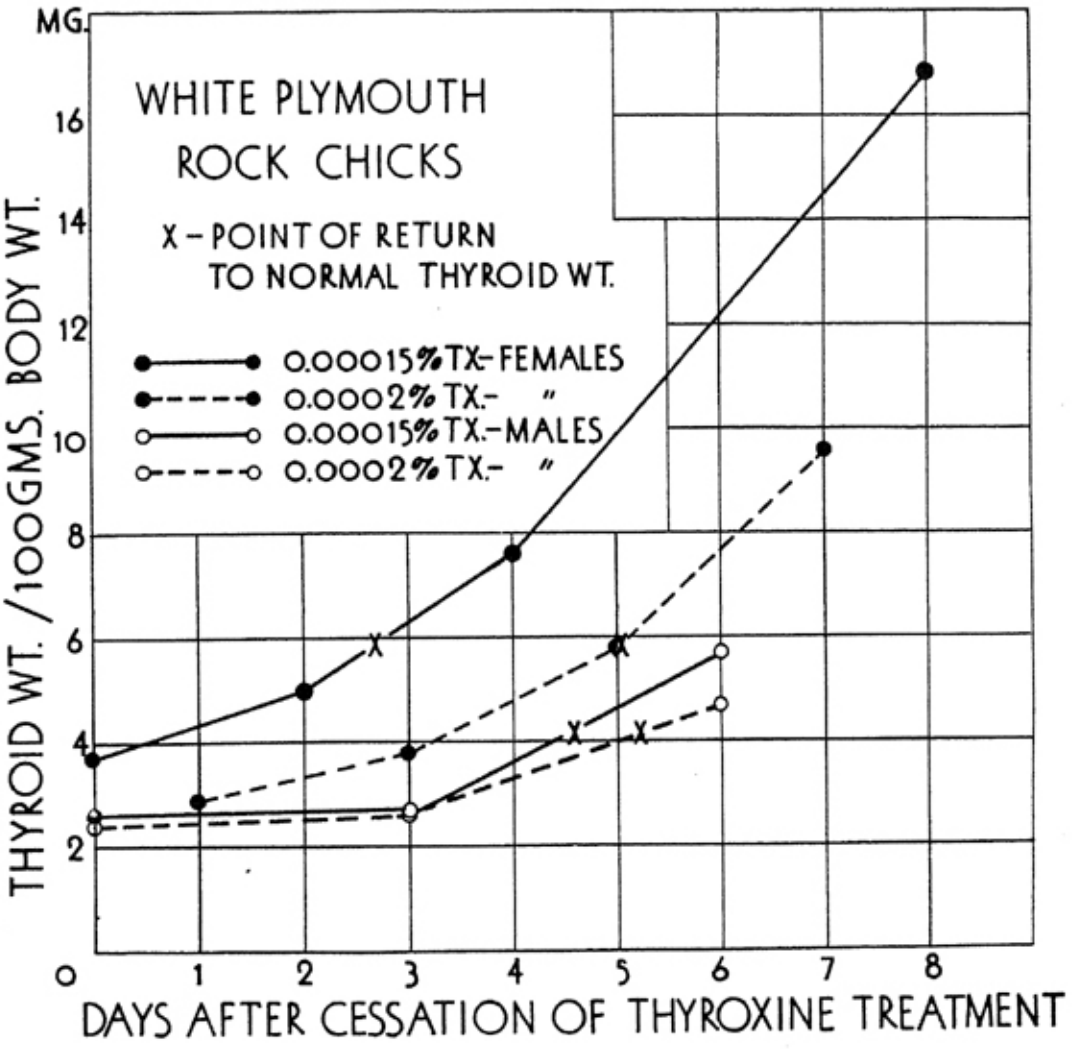

Fig. 6.—Comparative rates of disappearance of thyroxine in male and female chicks.

It is puzzling to try to conceive by what means vitamin A acts as an antagonist to thyroxine. Sadhu (1948) is of the opinion that the vitamin acts through the pituitary as an inhibitor of the thyrotrophic hormone. Indeed, Elmer *et al.* (1935) reported that vitamin A will partially prevent the histological changes in the thyroid glands of guinea pigs produced by injection of thyrotrophic hormone. And recently Couciero *et al.* (1947) have shown that vitamin

A causes a diminished response to the accelerating effect of the thyrotrophic hormone in the fixation of iodine by the thyroid, although the vitamin did not change the ability of the normal gland to fix iodine.

Thus, it is difficult to say with certainty just what part vitamin A plays in thyroxine metabolism. One would be led to believe that under normal conditions it does not play a significant part, in view of the fact that, in all the investigations mentioned, large doses of the vitamin were needed to evoke the observed effects.

The situation concerning the interrelationship of thyroid function and vitamins of the B complex is even more confused than is the case with vitamin A. There can be no doubt that a relationship exists, but whether it is an intimate, casual one or due to secondary effects is problematical.

A number of workers have shown that the requirements of the B vitamins are increased by feeding thyroid (Cowgill and Palmieri, 1933; Himwich *et al.*, 1932; Mouriquand *et al.*, 1939). It has also been shown that the loss in body weight associated with experimental hyperthyroidism can be prevented by administration of a high B vitamin diet (Sure and Smith, 1934). In hyperthyroid rats which had sustained some loss in weight, a further loss was prevented by administration of thiamin (Drill and Sherwood, 1938; Peters and Rossiter, 1939), but none of the weight could be regained until a rich source of riboflavin was administered (Drill and Sherwood, 1938). Later, it was found that a combination of pyridoxine and calcium pantothenate could replace the riboflavin (Drill and Overman, 1942).

More recently, Allardyce *et al.* (1947) have found that riboflavin, thiamine, and pyridoxine were effective in countering the effect of desiccated thyroid feeding in rats (weight loss and elevation of BMR), riboflavin proving to be the most effective. Also, these workers found that the rats, even without vitamin supplements, developed a tolerance to thyroid feeding, as evidenced by the regression of BMR to normal levels despite continued thyroid administration. However, vitamin supplements brought about a more rapid recovery. The effectiveness of the vitamins was in the following order, from best to poorest: riboflavin, thiamine, para-aminobenzoic acid, nicotinic acid amide, calcium pantothenate, and pyridoxine.

A partial explanation for the above observations may lie in the fact that excessive thyroid feeding causes liver damage which can be prevented by a high B vitamin diet (Drill and Hays, 1940). However, once an abnormal liver function had been produced in hyperthyroid dogs, treatment with large doses of B vitamins did not improve the abnormal condition (Drill and Hays, 1942). Furthermore, Drill *et al.* (1943) fed desiccated thyroid to dogs over long periods and found that a high B vitamin diet will delay but not prevent the eventual appearance of an abnormal liver function. Apparently, therefore, the deficiency of B vitamins brought about by thyroid feeding is one of the causes of liver damage in experimentally hyperthyroid animals. This is not to say,

however, that it is the only or even the most important factor. Nevertheless, since it is assumed that the liver plays an important role in the metabolism of thyroxine, this is a possibility which should be borne in mind.

Some workers have suggested interrelationships between thyroid function and vitamins C, D, E, and K. However, they are so problematical as to warrant omission from this discussion. (See review by Drill, 1943).

With reference to the above observations, it should be mentioned that they ought to be viewed with caution. The fact that requirements for many of the vitamins are increased in hyperthyroidism does not necessarily imply an antithyroid effect. Yet the effect may be considered antagonistic to the extent that many of the symptoms seen in hyperthyroidism may be due to secondary deficiencies of these vitamins. A definite antithyroid effect has been shown in only a few cases. Other effects noted are merely suggestive and must await further clarification.

In view of the unsettled status of the interrelationship between the thyroid gland and the various vitamins, and since an antagonism is suggested in some cases, it was thought that some light might be shed on the problem by measuring the effect of some vitamins on the duration of thyroxine action. Such a study should yield empirical information on the beneficial or detrimental effects of the vitamins on the action of thyroxine and, in addition, since some of the vitamins are known to be important constituents of enzyme systems, the evidence thus gained might be suggestive of the mechanism responsible for the shortening or lengthening of the time required for the disappearance of thyroxine.

Methods and Materials. The method used to determine the disappearance of thyroxine in chicks was identical with the technique already described, except for one fact. During the first week, when the chicks ordinarily were fed the basal ration only, a supplementary amount of the vitamin to be studied was added. The chicks, of course, received this supplementary vitamin during the week the thyroxine was administered (0.00015 percent crystalline D,L-thyroxine in the feed) and during the period following the cessation of thyroxine treatment. The following vitamins were fed to the various groups at the level indicated:

Vitamin	Amount per 100 gm. Feed	Normal Requirement per 100 gm. Feed
A	100,000 U.S.P. Units[1]	350-400 U.S.P. Units
Thiamin HCl (Merck)[2]	4.0 mg.	0.4 mg.
Riboflavin (Merck)	8.0 mg.	0.8 mg.
Pyridoxine HCl (Merck)	6.0 mg.	0.6 mg.
Niacin (Merck)	50.0 mg.	5.0 mg.
Ca Pantothenate (Merck)	20.0 mg.	2.0 mg.

[1]Vitamin A ester concentrate generously supplied by the Distillation Products, Inc. At this level the chicks received approximately 20,000 U.S.P. units per chick per day.

[2]The writers are indebted to Merck and Company, Inc., for the supply of B vitamins used in this experiment.

Results. The results of this experiment are shown in Figure 7. It is evident that only three of the vitamins fed—calcium pantothenate, niacin, and thiamin—had a pronounced effect on the rate of disappearance of thyroxine.

Calcium pantothenate decreased the duration time of thyroxine to a striking extent. The thyroids of chicks fed thyroxine at the 0.00015 percent level did not return to normal until two and a half days after the cessation of thyroxine treatment. When calcium pantothenate was added to the ration the thyroids returned to normal in one day. Presumably, therefore, the administration of excessive pantothenate hastens the inactivation of thyroxine. This finding is in accord with the observation of Glanzmann and Meier (1945) who found that pantothenic acid exercises a greater protective effect in thyrotoxicosis than does any other known vitamin.

The means by which pantothenic acid exerts its effect is unknown. It is generally thought that this vitamin is concerned in amino acid metabolism, but

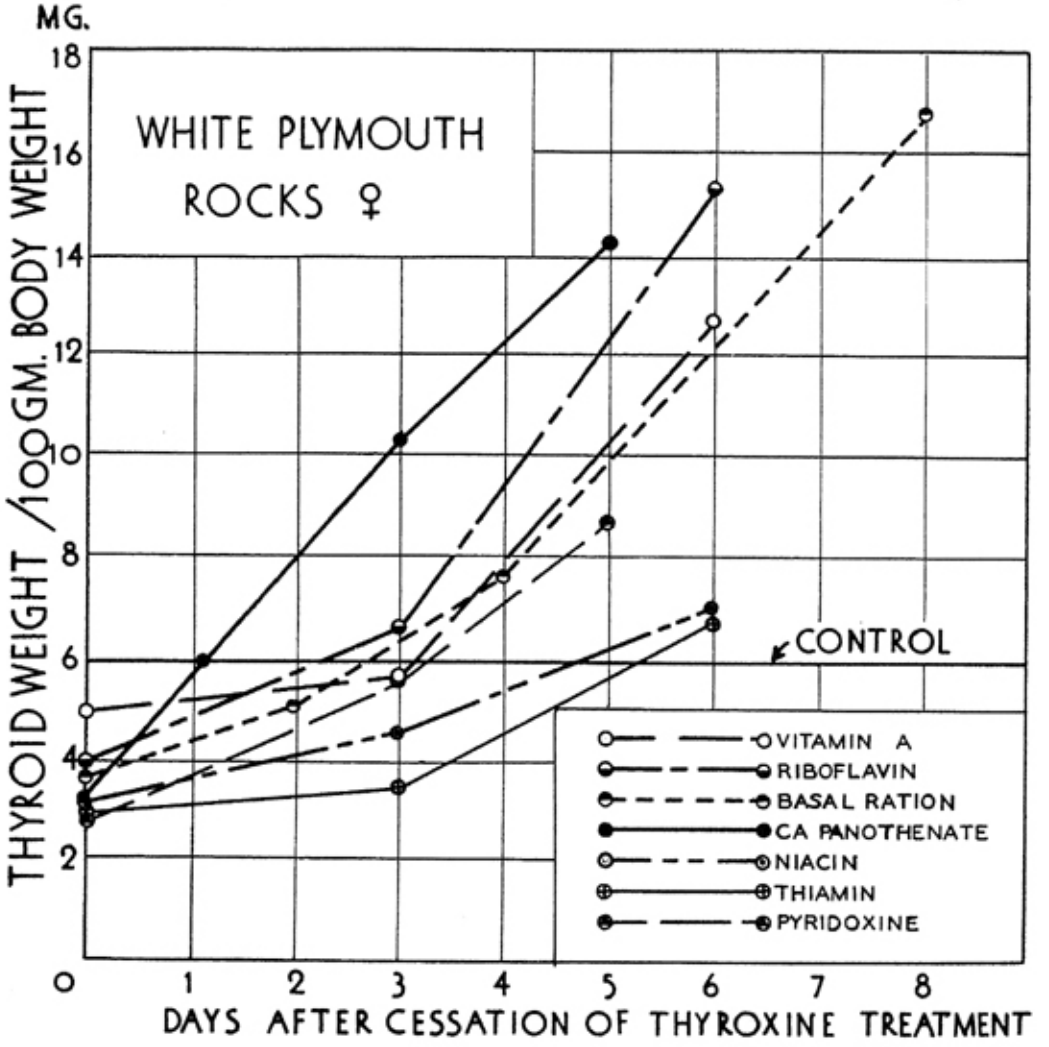

Fig. 7.—Effect of various vitamins on the rate of disappearance of thyroxine fed at 0.00015 percent level.

its mode of action has not been demonstrated. Recently, however, it has been suggested that pantothenic acid may be concerned in acetylation reactions (Shils, 1949). If true, this could account for a considerable diminution of thyroxine activity, since it has been shown that N-acetyl-thyroxine is much less active than the parent compound (Pitt Rivers and Lerman, 1947).

It should be mentioned that Glanzmann and Meier (1945) in contradiction to their findings reported above found that in hypothyroidism pantothenic acid works synergistically with thyroxine. This observation might be accounted for by the finding of Pitt Rivers (1948) that the blocking of the amino group by acetylation results in a greater conversion of diiodotyrosine to thyroxine at the pH of body tissues. An enzyme system containing pantothenic acid as the prosthetic group might well be involved in such an acetylation.

In this connection, one wonders if the postulated acetylation of thyroxine in the somatic tissues might be a step preparatory to conjugation with some other compound, much like glycine is combined with benzoic acid, the well-known detoxication reaction for accumulated benzoic acid. On this basis, pantothenic acid may be involved in both the synthesis of thyroxine and its inactivation, depending, presumably, on the state of activity of the thyroid and on the concentration of the circulating hormone.

If pantothenic acid is concerned with the acetylation of thyroxine, another interesting possibility in regard to thyroxine inactivation should be pointed out. Du Vigneaud and Irish (1937) have shown that levo-rotatory amino acids may be converted to their dextro-isomers in the body. Furthermore, they believe that this conversion may involve acetylation. Consequently, it is possible that pantothenic acid may play a part in the conversion of the highly active L-thyroxine to its much less active stereo-isomer. It would be interesting to know whether any D-thyroxine is excreted in the urine or feces.

The actions of niacin and thiamin in prolonging the duration are not easily explained, or even speculated upon. Niacin is an important constituent in the pyridinoprotein enzyme complex (coenzymes I and II) which is concerned in protoplasmic oxidations and, in particular, in the oxidation of sugar by liver enzymes. The pyridinoprotein enzymes may be thought of as the first in a series of catalytic substances necessary for the oxidation of substrate by molecular oxygen. Electrons are removed from the substrate by pyridinoprotein enzymes and passed on, in order, to the flavoprotein enzymes (which contain riboflavin), cytochromes, and cytochrome oxidase. The latter substance can react directly with molecular oxygen. For a general discussion of these enzymes and many references, see Heilbrunn (1945).

Thiamin, after it has been phosphorylated to diphosphothiamin, becomes the coenzyme for the carboxylase system concerned in the decarboxylation of pyruvic acid (end product of sugar oxidation) to form carbon dioxide and acetaldehyde. Whether it is also the coenzyme for other decarboxylations is

not known. Diphosphothiamin can also act as a coenzyme for the oxidation of pyruvic acid to carbon dioxide and acetic acid.

The manner in which these enzymes can act to exert a thyroxine-sparing action is not readily understandable, and must await clarification by further research.

At any rate, the effect observed for niacin and thiamin, a two-fold increase in thyroxine duration time, is contrary to what would be expected from the reported observations of a protective action by these vitamins against the effects of administered thyroid hormone. These results, too, must go unexplained at the present time.

Likewise, it is curious, in the light of past observations, that no effect on the disappearance of thyroxine could be detected after feeding riboflavin or pyridoxine. Especially was the lack of effect of pyridoxine surprising, since this vitamin is considered to be closely associated with amino acid metabolism (Woods, 1949). Most probably, pyridoxine (as pyridoxal phosphate) is an essential prosthetic group for amino acid decarboxylases (Umbreit *et al.*, 1945; Gunsalus *et al.*, 1945). The theory has been set forth that pyridoxal phosphate is also a coenzyme in transaminase systems (Green *et al.*, 1945). The results presented here might be taken to indicate that neither of these reactions is concerned in the inactivation of thyroxine. However, such an assumption would be dangerous. In the first place, other, more specific enzymes might be involved. Secondly, certain inherent difficulties in the experimental method complicate the interpretation of the observations.

It must be remembered that all of these explanations concerning the observations on the B vitamins present some difficulties at the outset. It is far from certain that these vitamins, fed in excessive amounts, are necessarily incorporated into the enzyme systems as they ordinarily would be. Moreover, even if an increased concentration of enzyme systems did result, it is questionable as to the extent to which the rate of cellular reactions would be affected. Thus, the results presented are subject to certain reservations. Of course, where an effect was observed it may be assumed that the vitamins function as constituents of enzymes. But where no effect was noted these reservations must be borne in mind.

The lack of effect of vitamin A on the disappearance of thyroxine was also interesting, in view of the rather definite antagonism reported in the literature. It should be noted, in this regard, that the thyroid weight of thyroxine-treated chicks receiving added vitamin A were depressed to a considerably lesser degree than the thyroids of chicks receiving thyroxine, but no vitamin A. Thus, perhaps some antagonism between the two substances is indicated. But whatever the mechanism involved, the antagonistic effects are apparently not due to an increased rate of disappearance of thyroxine.

The site of these postulated reactions cannot be identified with certainty, of course. But considering the well-known fact that the liver is a rich source

of vitamins and that the evidence in the literature points to the liver as an important organ in thyroxine catabolism, it must be considered a strong possibility that many vitamin-thyroid interrelationships are mediated, in one way or another, in the liver.

The Effect of Partial Hepatectomy on the Disappearance of Thyroxine.

As early as 1920, Blum and Grutzner showed that the liver has the power to decompose the thyroid hormone. Since that time, as seen in the review of literature, many workers have shown that the liver picks up thyroxine from the blood and releases it into the bile, after first partially destroying it.

Kellaway *et al.* (1945) observed that excessive doses of thyroxine caused a greater response in heart rate in thyroidectomized, partially hepatectomized rats than in thyroidectomized rats. But partial hepatectomy had no effect on the response to small, physiological doses. From these observations the investigators conclude that the liver does not play a large part in the destruction of thyroxine under normal conditions, but that with larger doses the liver is stimulated to decompose the hormone.

It was thought that the method used in the preceding experiments for measuring the duration of action of thyroxine could also be used to good advantage in studying the role of the liver in the metabolism of thyroxine. One drawback to this method for such a study was the possibility of some regeneration of liver tissue during the experimental period. However, it was felt that the attempt would be worthwhile.

Methods and Materials. Male White Plymouth Rock chicks of 300 to 400 grams body weight were partially hepatectomized by the following procedure.

The chicks were partially anaesthetized with nembutal; and complete anaesthesia was attained by the use of ether. A ventral incision was made to the left of midline one and one-half inches long. The small portal vessel running between the two major lobes of the liver was ligated and the entire left lobe was passed through a loop of silk thread. This ligature was then pulled taut around the hilus of the left lobe of the liver. The entire left side of the liver was removed with an electric cautery. Finally, a loop of thread was passed around the tip of right lobe so that about a quarter to a third of the visible portion of this lobe was ligated. This portion of the right lobe was then removed with an electric cautery, care being taken not to injure the gall bladder.

The injured parts were swabbed with adrenalin (diluted 1:1500) to stop superficial bleeding. The wound was closed with an uninterrupted suture.

In all, approximately one-half of the liver (wet weight) was removed.

These partially hepatectomized chicks were then used for measurements of the rate of disappearance of thyroxine fed at a level of 0.00015 percent. A group of intact male chicks of the same body weight underwent the same thyroxine treatment and served as controls.

Results. As was feared, the results were complicated by the occurrence of liver regeneration. In fact, at the end of a week of thyroxine treatment the livers of the partially hepatectomized chicks were appreciably larger than those of normal chicks or hepatectomized controls (Figure 8).

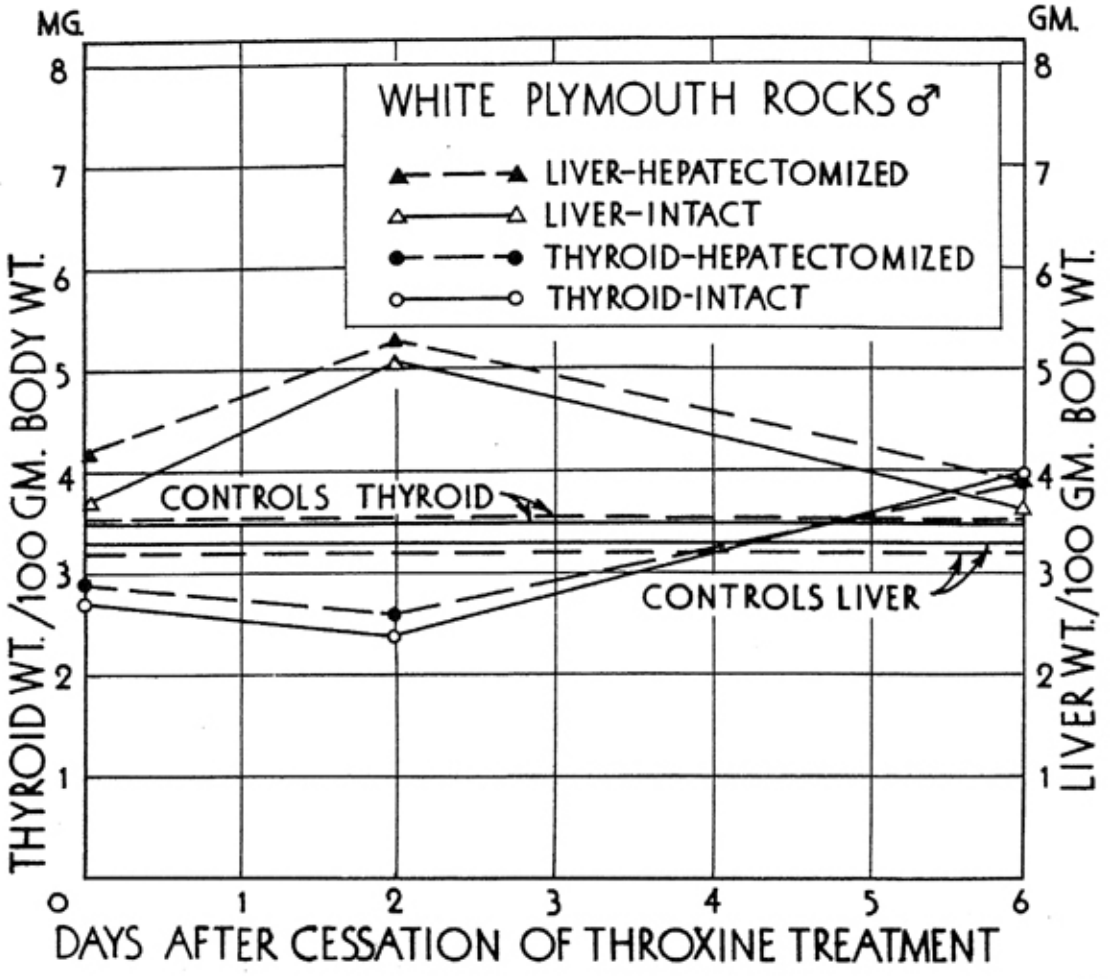

Fig. 8.—Plot of data showing the effect of partial hepatectomy on the disappearance of thyroxine and the effect of thyroxine treatment on liver regeneration. Thyroxine fed at a level of 0.00015 percent.

It was found, as might be expected under these conditions, that no difference could be observed in the duration of thyroxine action between the operated and intact chicks. Apparently, therefore, the livers of the operated animals had regenerated to the point where they were able to metabolize thyroxine in a normal manner; or only a portion of the liver is necessary to carry out a normal catabolism of thyroxine.

Despite the failure of this experiment to accomplish its original purpose, however, some curious observations were noted which should be reported. First, the regenerated livers of the operated, thyroxine-treated chicks were severely

blanched and somewhat edematous appearing. Their gall bladders were smaller than normal and the bile reddish brown in color immediately after cessation of thyroxine treatment (zero days in Figure 8, page 54). After 2 days the bile contained some green color, but was still predominantly reddish. After 6 days the gall bladders were normal in size and the bile had regained its normal dark green color. None of the other groups showed this effect in the bile.

The intact, thyroxine-treated animals showed livers somewhat lighter in color than normal and the hepatectomized control's livers were still lighter in color. In neither case, however, did the livers approach the semi-translucent appearance of the operated, thyroxine-treated chicks.

At present, no explanation can be put forth regarding these results. Further research on this point should prove interesting.

Disappearance of Thyroxine Injected Subcutaneously, Intraperitoneally and Intrasplenically. Since it was found impossible to study the role of the liver in thyroxine metabolism by partial removal of the liver, it was thought that an indirect approach to the problem might prove enlightening. Therefore, it was decided to measure the effects of an intrasplenic injection of thyroxine on duration time as compared with subcutaneous and intraperitoneal injections.

Methods and Materials. Week-old White Plymouth Rock chicks, weighing seventy-five to one hundred grams, were used in this experiment. Each experimental chick was injected subcutaneously with 10.0 milligrams thiouracil in slightly alkaline solution. From this time on, the experimental chicks received 0.1 percent thiouracil in the feed.

One group was injected subcutaneously with thyroxine in alkaline solution at the rate of 2.5 micrograms per chick. A second group was injected at the same level intraperitoneally, and a third group, intrasplenically. The intrasplenic injections were done by making a small opening in the abdominal wall and pushing the liver aside so that the syringe needle could be entered directly into the spleen, which was in plain view. It was found unnecessary to close this opening with a suture, wound clips being sufficient.

The dilutions of thyroxine for injection were made up so that 2.5 micrograms of the active substance was contained in each 0.05 milliliter of solution.

Results. It can be seen from Figure 9 that no difference can be observed in the disappearance of thyroxine injected by any of the three routes, each lasting about 2.5 days. It seems, therefore, that little or none of the thyroxine is decomposed in its first trip through the liver.

Effect of Varying the Vitamin B_{12} Content of the Diet on the Disappearance of Thyroxine. In view of the reports in the literature which ascribe a protective action to various vitamins against the effects of hyperthyroidism, it is not surprising that the feeding of liver powder or extracts has been found to exert similar effects. Some recent observations, however, indicate that a new factor or factors must be considered. Retarded growth and reduced sur-

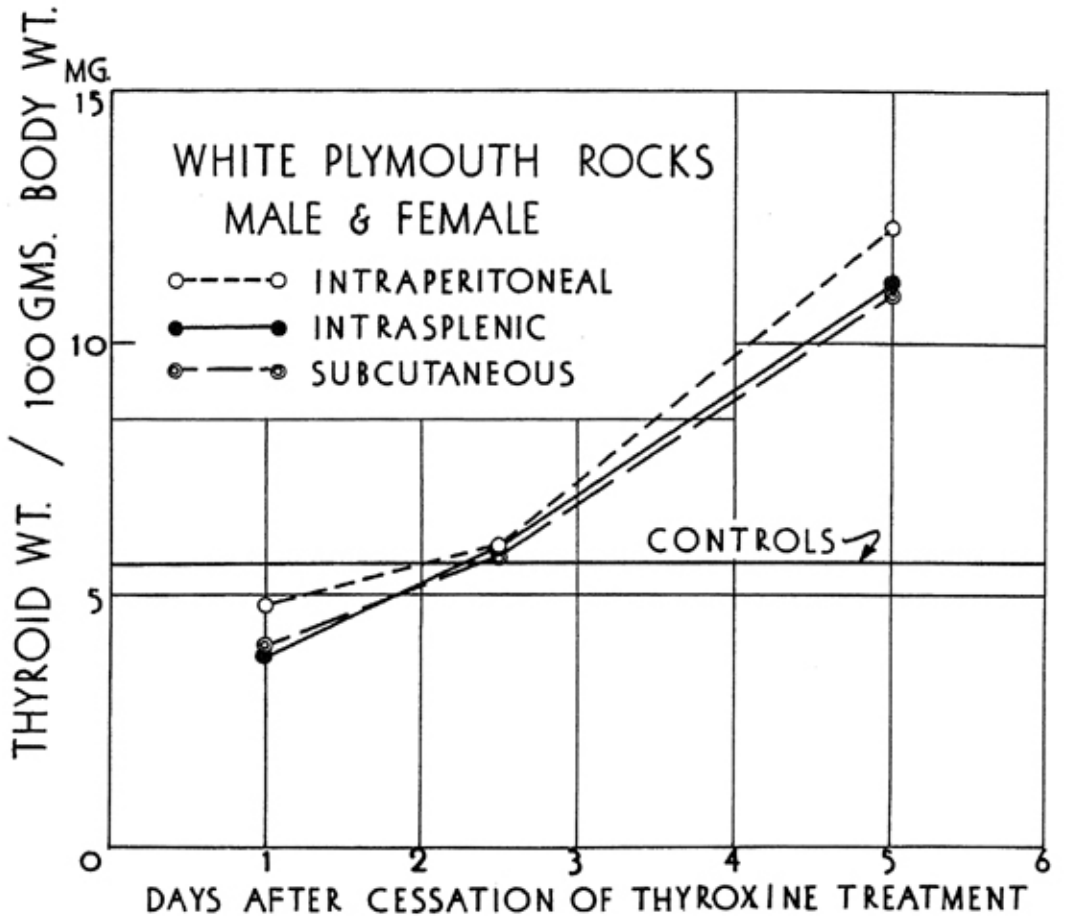

Fig. 9.—The duration of single doses of thyroxine injected subcutaneously, intraperitoneally, and intrasplenically. Thyroxine injected at the level of 2.5 micrograms per chick.

vival have been observed in young rats (Ershoff, 1947a, 1947b; Betheil *et al.*, 1947), mice (Ershoff, 1949a), and chicks (Roblee *et al.*, 1948a; Nichol *et al.*, 1948) made hyperthyroid by the feeding of desiccated thyroid, especially in animals fed a diet devoid of protein from animal sources. These investigators found that the thyrotoxic effects could be completely counteracted by the inclusion in the diet of fish solubles (Roblee *et al.*, 1948b), whole liver powder (Betheil *et al.*, 1947; and others), and certain other liver extracts (Nichol *et al.*, 1948). And since no other known nutrients could be shown to possess this "antithyrotoxic" activity, it is evident that the protective factor(s) in this case must be considered a distinct entity.

Recently it has been found that Vitamin B_{12}, a crystalline compound isolated from liver (Rickes *et al.*, 1948), exhibits antithyrotoxic properties, as shown by a neutralization of the growth-inhibiting effect of desiccated thyroid administration to young rats fed a diet free of animal protein (Emerson, 1949a). A similar effect was observed in immature mice fed liver extracts rich in vitamin B_{12} (Bosshardt *et al.*, 1949). In contradiction, Ershoff (1949a), found

that a liver concentrate containing vitamin B_{12} failed to counteract the growth retardation of immature mice fed desiccated thyroid or iodinated casein. He did observe, however, a growth-promoting effect of vitamin B_{12} in the mice fed thyroxine.

Naturally, a question came to mind regarding the possibility of an effect of vitamin B_{12} on the rate of disappearance of thyroxine. Fortunately, some chicks[1] were available which afforded an excellent opportunity to undertake just such a study.

Methods and Materials. The chicks used in this experiment were hatched from groups of White Leghorn hens fed on rations which varied in vitamin B_{12} content. The exact composition of the various rations is listed below (Table 5). It can be seen that, basically, four different rations were fed—rations I, III, V, and VII. Rations II, IV, VI, and VIII, respectively, were identical with the odd numbered rations except for the addition of thyroprotein (Protamone).

TABLE 5.--COMPOSITION OF RATIONS FED TO LAYING HENS

Ingredients	Ration No.							
	I	II	III	IV	V	VI	VII	VIII
Yellow Corn	38.7	38.7	38.7	38.7	38.2	38.2	41.0	41.0
Bran	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Shorts	15.0	15.0	15.0	15.0	15.0	15.0	15.0	15.0
Alfalfa Meal	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Soybean Oil Meal	30.0	30.0	30.0	30.0	30.0	30.0	14.0	14.0
Steamed Bone Meal	3.0	3.0	3.0	3.0	3.0	3.0	1.5	1.5
Ca Carbonate	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Salt	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mn Sulfate. $4H_2O$	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
A D Oil Nopco xx	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Fish Meal							10.0	10.0
Skim Milk							5.0	5.0
KI Mg/lb.	0.45	0.45	0.45	0.45	0.45	0.45		
Riboflavin Mg/lb.	1.8	1.8	1.8	1.8	1.8	1.8	1.8	1.8
B_{12} Conc. (Merck) %					0.5	0.5		
Ca Pantothenate Mg/lb.			9.0	9.0	9.0	9.0		
Niacin Mg/lb.			9.0	9.0	9.0	9.0		
Folic Acid Mcg/lb.			0.45	0.45	0.45	0.45		
Biotin Mcg/lb.			45.0	45.0	45.0	45.0		
Choline Gm/lb.			0.45	0.45	0.45	0.45		
Protamone Gm/lb.		0.1		0.1		0.1		0.1
% Protein	20	20	20	20	20	20	20	20

Ration I contained the basal constituents plus a supplementary amount of riboflavin only. Ration III contained supplements of the other necessary vitamins of the B complex except for vitamin B_{12}. Ration V was identical to ration III except for the addition of vitamin B_{12}. Ration VII contained fish meal and skim milk powder as a source of vitamins, and was the only ration to contain protein from animal sources. Consequently ration VII was considered as the experimental control diet, against which the effects of the other rations were compared.

[1]The chicks used in this study were made available through the courtesy of Mr. J. E. Savage.

For the purpose of this experiment, chicks hatched from hens receiving rations III, V, and VII were used. The chicks, after hatching, were placed on the same diet as their respective mothers. After a week on this feed, crystalline D,L-thyroxine and 0.1 percent thiouracil were added to the respective rations, and these rations fed for one week. At the end of this period, thyroxine was withdrawn from the feed and the rate of disappearance of thyroxine determined for each group by the method already described.

Since it was found that the chicks on these rations consumed only five to seven grams of feed daily, and since the thyroid secretion rate of the White Leghorn chick is slightly higher than that of the White Plymouth Rock chick (Schultze and Turner, 1945), it was calculated that, in order to be within the dosage range where effective measurements can be made, it would be necessary to feed thyroxine at a level of 0.0006 percent. This level was fed to the chicks in all three groups.

Results. The results of this experiment are shown in Table 6 and Figure 10. It is evident that the amount of thyroxine fed was only slightly in excess of the amount which would be necessary to maintain a normal thyroid weight, since the thyroids of the chicks receiving ration VII (fish meal) required only one day after the cessation of thyroxine treatment to return to normal. Yet, in the chicks receiving ration III (B_{12} free) thyroxine lasted for a comparatively long period. The increased susceptibility to thyroxine of the chicks on Ration III was also manifested by a generally weakened condition, apathetic behavior, and increased mortality rate, typical symptoms of normal chicks fed an overdose of thyroxine.

TABLE 6.--EFFECT OF VITAMIN B_{12} ON THE RATE OF DISAPPEARANCE OF THYROXINE

Ration	Days after cessation of thyroxine treatment					
	0		3		6	
	Body Wt. gm.	Thyroid Wt. /100 gm. Body Wt. mg.	Body Wt. gm.	Thyroid Wt. /100 gm. Body Wt. mg.	Body Wt. gm.	Thyroid Wt. /100 gm. Body Wt. mg.
			MALES			
III (B_{12} free, control)	99.5	5.9				
III (exp.)	75.3	3.7	95.8	4.9	101.5	9.1
V (B_{12}, control)	137.7	6.3				
V (exp.)	100.2	3.8	144.8	9.3	157.0	17.3
VII (fish meal, cont.)	149.9	8.5				
VII (exp.)	119.4	4.9	169.4	14.0	180.4	37.1
			FEMALES			
III (control)	94.8	6.4				
III (exp.)	71.9	4.7	81.4	6.9	81.6	11.5
V (control)	130.3	7.2				
V (exp.)	92.5	3.8	126.5	11.9	145.3	38.5
VII (control)	140.5	9.0				
VII (exp.)	105.8	6.0	152.6	16.7	160.4	29.1

Some mortality was observed in all experimental groups due to the unfortunate necessity of keeping the chicks under overcrowded conditions. In chicks fed rations V and VII, however, the mortality rate tapered off, whereas the chicks receiving ration III increased in mortality rate upon initiation of thyroxine treatment. Unmistakably, therefore, at the level fed thyroxine exerted a toxic effect in the vitamin B_{12} deficient chicks, and this toxicity was overcome by the addition of vitamin B_{12} concentrate or fish meal to the diet. This is in agreement with the observations of other investigators (Emerson, 1949a; Bosshardt *et al.*, 1949).

In addition to counteracting the toxic effects of thyroxine, addition of vitamin B_{12} to the diet devoid of animal protein exerted a shortening effect on the duration of thyroxine action. Indeed, it is possible that this is the means by which vitamin B_{12} exerts its antithyrotoxic action, either directly, by reason of its possible role in some as yet unknown enzyme system, or indirectly, through some effect on other factors concerned in thyroxine catabolism.

A sex difference was noted in the rate of disappearance of thyroxine in vitamin B_{12}-deficient chicks. Females fed ration III were able to dispose of the administered thyroxine in 2 to 2.5 days, as compared with one day for chicks receiving fish meal or vitamin B_{12} supplements. And the duration time of thyroxine action in vitamin B_{12}-deficient male chicks was about four days. This difference is comparable to that found between normal male and female White Plymouth Rock chicks.

The fact that no sex difference was observed in the disappearance of thyroxine in chicks fed rations supplemented with vitamin B_{12} or fish meal leads one to wonder if, above and beyond a counteraction of the effects of vitamin B_{12} deficiency, the vitamin and/or the factors in fish meal do not actively hasten the disappearance of thyroxine. Further research on this point should prove interesting.

Obviously, as shown in Figure 10, no difference could be detected in the present experiment between the activities of vitamin B_{12} and fish meal in diets devoid of animal protein. However, the fact that the thyroids of the control chicks fed ration VII were larger per unit body weight than those of the ration IV controls, and that both were larger than those of ration III controls may indicate some difference (Table 5). Also, it will be noted, the thyroids of the ration VII chicks were depressed to a lesser extent by thyroxine feeding than were those of the chicks on ration V, again indicating a possible difference. However, because of the non-specificity of these observations, the question of whether vitamin B_{12} can exert, in the chick, all of the "antithyrotoxic" effects attributed to such animal protein sources as liver and fish solubles (fish meal, in this experiment) must await further research.

The literature is confused on this point. Some workers, as noted above, indicate that vitamin B_{12} is the "antithyrotoxic" factor in liver. Our experiments would seem to lend weight to this view. Yet Ershoff (1949b) maintains

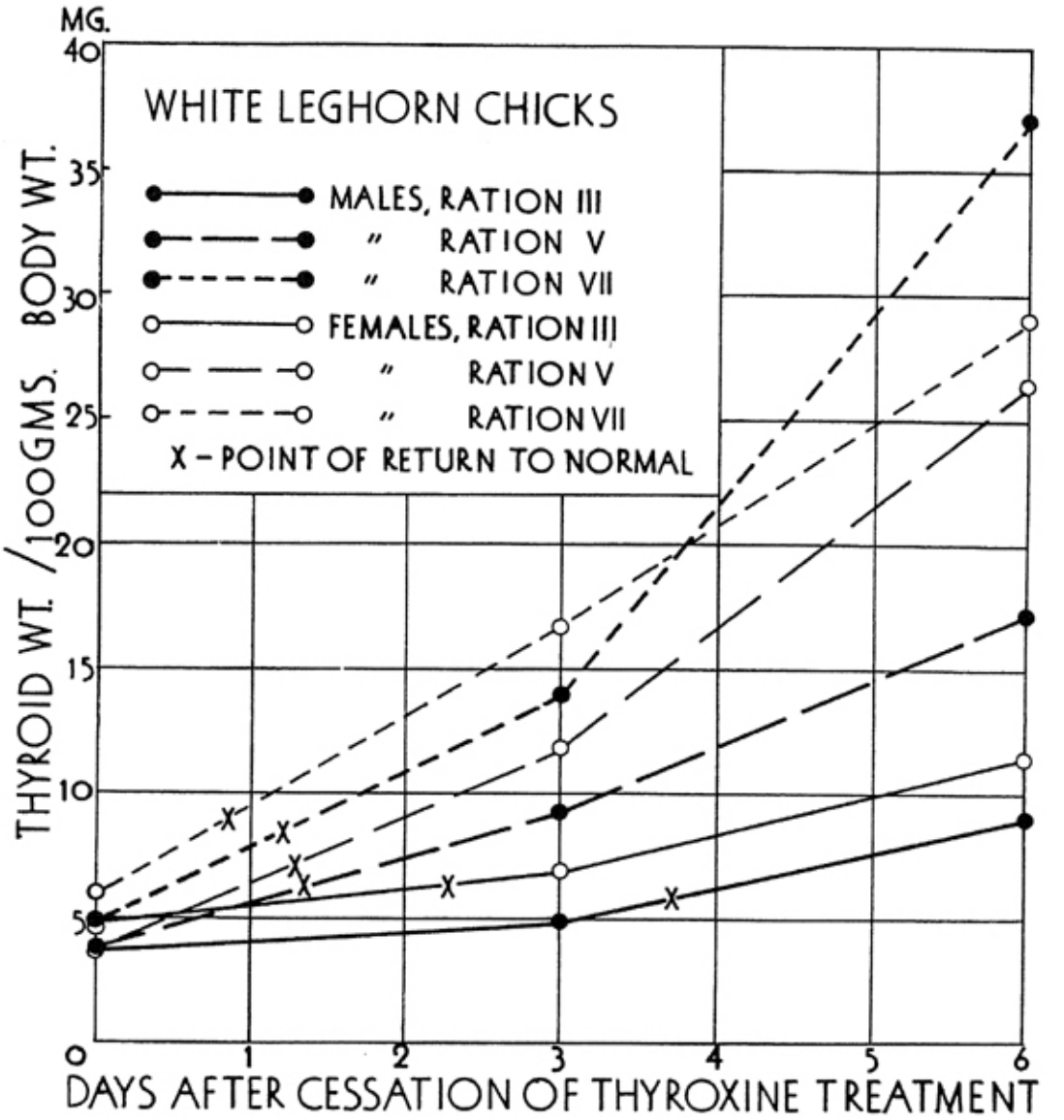

Fig. 10.—Effect of diets containing no vitamin B_{12}, containing vitamin B_{12}, and containing fish meal, respectively, on the rate of disappearance of thyroxine.

that the protective factor in liver is distinct from vitamin B_{12}, while Betheil and Lardy (1949) found that vitamin B_{12} *partially* counteracts the retardation of growth caused by feeding excess desiccated thyroid. Perhaps some of the confusion can be resolved by the fact that vitamin B_{12} is stored in the young animal from amounts accumulated during gestation and lactation (Emerson, 1949b). It must be remembered, also, that all the reported tests of antithyroid activity involve the administration of massive doses of thyroid hormone and are based on growth rate measurements, a non-specific criterion at best. Quite possibly two separate effects are involved; one, an "antithyroid" effect, and the other an effect on the growth process. It would appear that vitamin B_{12} is not the only factor (if, indeed, it is a factor) in the latter instance, but it

apparently does play a part in the catabolism of thyroxine and, hence, can be considered "antithyrotoxic" from that point of view. The extent to which this effect would manifest itself in animals fed massive doses of thyroid is problematical.

In view of the above discussion, it is of interest to note the effect of these rations on the growth of the chicks. Although no periodic measurements of growth were made, it is evident (Table 6) that at the time they were killed the chicks on ration VII were considerably heavier than those on ration V, which, in turn, were heavier than those on ration III. It seems, therefore, that vitamin B_{12} is a factor essential for proper growth, but that fish meal may contain other essential factors in addition to vitamin B_{12}.

In this connection, it should be noted that, in all cases, growth was retarded in the chicks fed thyroxine. However, insofar as this experiment is concerned, this fact cannot be taken to mean that the vitamin B_{12} and fish meal diets failed to overcome the growth-retarding effect of thyroxine. The crowded quarters of the thyroxine-fed chicks, a complication not encountered with the controls, could account for most, if not all, the difference observed. Hence, this point must be left unsettled for the present time.

Effect of the Diet of Hens on Thyroid Size and Metabolism of Newly Hatched Chicks

It has been known for some time that when thiouracil is administered to pregnant rats (Goldsmith, Gordon and Charipper, 1945) or to hens (Andrews and Schnetzler, 1945) the offspring have enlarged thyroid glands. On the other hand the finding that chicks hatched from thyroprotein-fed hens also have enlarged thyroids (Wheeler and Hoffman, 1948; McCartney and Schaffner, 1949) comes as a distinct surprise, since thyroprotein fed directly to chicks produces an involution of the thyroid.

Consequently, it was thought that the availability of chicks like those used in the preceding experiment afforded an excellent opportunity to attempt a confirmation of the observations above, and, at the same time, to observe the effects, if any, of the various diets on the influence of thyroprotein.

Methods and Materials. White Leghorn chicks hatched from hens fed rations I to VII (described above) were used in this experiment. Within 24 hours after hatching the chicks were killed, sexed, and the thyroids removed and weighed immediately. Comparative rates of metabolism of the various groups of chicks were determined by the closed-vessel method proposed by Smith, Emmens and Parkes (1947). Twenty-five to fifty chicks in each group were suffocated in this manner. Each chick was placed in a pint fruit jar, the cover closed tightly, and the survival time of the chick recorded.

Results. The thyroids of chicks hatched from thyroprotein-fed hens were larger in all cases than those hatched from hens receiving the same ration without thyroprotein (Table 7). Although the differences observed were less

TABLE 7.--EFFECT OF DIET OF HENS ON THYROID SIZE OF NEWLY HATCHED CHICKS

Ration	No. Chicks	Body Wt. gm.	Thyroid Wt. mg.	Diff. in Thyroid Wt. mg.
		MALES		
I	86	36.6	3.5	1.6
II	43	34.2	5.1	
III	36	31.9	5.2	1.4
IV	29	34.5	6.6	
V	41	36.6	5.2	1.3
VI	25	36.2	6.5	
VII	51	35.8	4.1	1.9
VIII	30	35.4	6.0	
		FEMALES		
I	63	36.0	4.3	1.4
II	60	33.5	5.7	
III	19	30.8	5.9	1.3
IV	18	33.1	7.2	
V	47	36.4	5.9	3.3
VI	22	34.3	9.2	
VII	37	35.8	4.7	2.6
VIII	19	35.7	7.3	

marked than those reported by Wheeler and Hoffman (1948) and McCartney and Shaffner (1949), they were none the less definite.

There seem to be no differences in the response of the chick embryos to thyroprotein feeding in the mother hens which can be attributed to dietary variations except that rations V *versus* VI and VII *versus* VIII fed to the hens, cause wider differences in the thyroid weights of the female chicks than do other rations. The same rations did not cause a similar response in male chicks. A similar sex difference in responsiveness was noted by Wheeler and Hoffmann (1948b). The significence of the lack of sex difference in response to the other rations is not clear.

At any rate, it is evident that thyroprotein feeding in hens causes an enlargement of the thyroids of the chicks in all cases. Metabolism measurements by the closed-vessel technique led McCartney and Shaffner (1949) to believe that these enlarged thyroids were indicative of a hypothyroid state. In general, the present experiment seems to be in agreement with this view (Table 8).

In viewing these results, the variations in temperature must be taken into account. Some data calculated by Brody (1948) show that a difference of 10°

TABLE 8.--EFFECT OF DIET OF HENS ON METABOLISM[1] OF NEWLY HATCHED CHICKS

Ration	No. Chicks	Time (min.)	Temperature (F.)
I	50	128.9	78°
II	50	113.6	81°
III	25	105.6	89°
IV	25	106.2	81°
V	25	91.2	88°
VI	25	110.5	81°
VII	25	94.9	87°
VIII	25	122.9	80°

[1]Comparate metabolic rates measured by survival time in a closed vessel.

F. within the 70° to 95° F. range could raise or lower the heat production of the chick by some 30 percent. With this correction in mind, the apparent difference between groups I and II fades to insignificance, and the apparent similarity of groups III and IV changes to an appreciable difference. Likewise, the differences in metabolism noted between the other groups become even more exaggerated. Therefore, it seems that in all cases except that of ration II, thyroprotein feeding in hens resulted in a reduced metabolic rate in their chicks.

No very clear explanation is apparent for these results. The possibility that thyroprotein acts differently in the chick embryo than it does in the adult must be ruled out, since McCartney and Schaffner (1949) have shown that thyroxine injected directly into the egg caused a depression of thyroid weight in the chick. About the only alternative explanation, therefore, is that apparently not enough thyroprotein enters the egg to maintain the necessary thyroid-pituitary balance.

It is suggested that thyroprotein feeding in the hens would result, through the pituitary, in a virtual cessation of thyroid activity in the hen. All of the circulating "thyroid" hormone would, then, be derived from thyroprotein. If it be assumed that the active entity derived from thyroprotein differs in its permeability characteristics from the "natural" hormone, so that none of the thyroprotein derivative can penetrate the egg, the above observations could be explained. In favor of this hypothesis is the observation of McCartney and Schaffner (1949) that no thyroid enlargement was found in chicks hatched from thyroxine-injected hens. It would be interesting to study the effects of feeding desiccated thyroid or thyroglobulin to hens on the thyroid size of their chicks.

DISCUSSION

The chemical processes which determine the functional well-being of the organism are dependent to no mean extent upon the presence of the thyroid hormone in proper concentration. That physiological processes in general are stimulated by the thyroid hormone is indicated not only by an increased oxidation rate, but also by the wasting of tissues and increased sensitivity of the nervous system. Thus, it becomes evident that the thyroid hormone is undoubtedly one of the most important of the manifold inter-related factors which make possible a sufficiently constant physiological equilibrium to permit efficient adjustment of the organism to a constantly changing external environment.

As a consequence, the thyroid gland, and the factors which regulate its activity, have been the subjects of intensive research, since, after all, the thyroid produces the thyroid hormone, and, subject to regulation by many factors, releases it into the bloodstream. It has been well established that, first and foremost, thyroid function is dependent upon the thyrotrophic hormone of the anterior pituitary which, in turn, is regulated by the thyroid hormone level of the blood. Thus, a delicate balance mechanism, the thyroid-pituitary axis, is set up for the regulation of the thyroid activity.

But it must be emphasized that the rate of hormone secretion by the thyroid is not the only factor regulating the concentration of thyroid hormone in the blood. The rate at which the hormone is removed from the circulation is also of utmost importance in the regulation of the level of circulating hormone. Such mechanisms include the excretion of the hormone, destruction of its activity, and the binding of the hormone and its utilization by the tissues. It is conceivable, therefore, that a thyroid gland might be hyperactive, and yet not relieve the symptoms of hypothyroidism. Likewise, a condition of hyperthyroidism would not necessarily imply an overactive thyroid gland. In fact, DeRobertis (1948) has reported observations which support this contention.

A homeostatic control of the blood concentration of thyroid hormone by peripheral mechanisms is also suggested by the finding, in the present investigation, that beyond a certain dosage no increase could be found in the length of time that thyroxine remained in the organism. These observations are in accord with the suggestion of Kellaway *et al.* (1945) that dosages of thyroxine above the normal level stimulate mechanisms in the liver to destroy increasing amounts of the hormone. Indeed, the finding that thyroxine disappears rapidly from the organism, a fact observed by several other investigators, is indicative in itself that the possibility of peripheral control must be considered in relation to any study concerning thyroid function and/or its regulation.

The strength of evidence in the literature must be regarded as proof that the liver plays an important part in the destruction of thyroxine, the inconclusive observations reported herein notwithstanding. Due to limitations of the technique used, no difference could be detected in the rate of disappearance of thyroxine in partially hepatectomized chicks as compared to unoperated con-

trols. It is plain, however, from the results obtained (Figure 8, page 54) that a relationship exists between thyroxine administration and liver function. However, it is not yet known to what extent the liver exerts its action in this direction either under normal or abnormal physiological conditions. Doubtless, also, such factors as the dietary regimen play an important part.

The literature contains many reports which attribute some of the vitamins with activities either synergistic or antagonistic to thyroid hormone activity. Many of these reports are confusing and almost none are definite. Consequently, it is difficult to form a picture which fits all of the pieces of the puzzle together. A great many of the reports describe the effects of the various vitamins (especially those of the B complex) in counteracting the effects of experimentally induced hyperthyroidism. It must be remembered, in this connection, that thyroid hormone administration increases the requirements for most of the vitamins. Consequently, the fact that administration of the various vitamins counteracts this induced deficiency should not be mistaken for a true thyroid-vitamin interrelationship.

The findings, in this study, that calcium pantothenate hastened the disappearance of thyroxine is in accord with the observation of Glanzmann and Meier (1945) that pantothenic acid exerted a greater protective effect in thyrotoxicosis than any other known vitamin. In this case, at least, the effect of the vitamin apparently is actually antagonistic to thyroxine, in the sense that thyroxine does not act over as long a period. A possible explanation of the effect of pantothenic acid is a postulated role in acetylation reactions (Shils, 1949). Possibly, then, the activity of thyroxine is affected directly, N-acetyl thyroxine being less active than thyroxine (Pitt Rivers and Lerman, 1947), or indirectly, through the formation of an inactive compound.

Likewise, the "antithyrotoxic" action of vitamin B_{12} and the factor(s) in fish meal are due to a shortening effect on the duration of action of thyroxine.

Thiamin and niacin were found to prolong the action of thyroxine, in contradiction to most reports in the literature. At present, no explanation can be offered concerning these results.

Thus, some of the vitamins may be directly concerned in the metabolism of thyroxine. Others, such as vitamin A, must exert their antagonistic effects in some other manner. Much more research is needed to clarify the picture concerning the interrelationship between thyroid function and the various vitamins.

The oral administration of thyroidally active compounds presents another problem. In order to get into the bloodstream, the compounds must first pass through the wall of the gastro-intestinal tract. As indicated in the literature, the form in which the active compound is fed is an important determining factor in the absorbability of the compound. Thompson *et al.* (1933) suggested that the solubility in water of the material fed is probably the most important factor in its absorption. In this investigation, too, it was found that the solu-

bility of the orally administered compound is apparently a determining factor. Thus, desiccated thyroid and thyroprotein were the most readily absorbed and pure crystalline thyroxine the most poorly absorbed of the substances fed. The mono- and disodium salts were found to be about twice as effective orally as the crystalline compound. That solubility is not the only factor, however, is indicated by the fact that monosodium thyroxine was found to be equally as effective as the disodium form, which is many times the more soluble of the two, and several hundred times as soluble as crystalline thyroxine.

In summing up, then, the orally administered thyroid hormone is absorbed from the gastro-intestinal tract according, but not in direct proportion, to the water solubility of the form fed. The absorbed portion of the hormone then resides very briefly in the blood, the rest being excreted in the feces. From the blood it may take either of two routes.

Some of the active hormone may be excreted in the feces, probably by way of the liver and bile. Apparently none of the active hormone is excreted through the kidney. And since only a fraction of the thyroxine can be found in the feces, most of it must be accounted for by its inactivation. Otherwise an accumulation of the hormone would take place.

Presumably most of this inactivation occurs in the liver, although some of the hormone may be degraded through use in the peripheral tissues. The biochemical mechanisms of thyroid hormone destruction are unknown, but deiodination and perhaps deamination and acetylation are among the possibilities. The inactive end product of these reactions could then be excreted in the feces and/or the urine.

The problem is complicated, however, by at least two factors:

1. The iodine liberated in the destruction of thyroxine would be trapped by the thyroid and incorporated into a new thyroxine molecule, which would, itself, go through the cycle outlined above.
2. A portion of the thyroxine may escape destruction in the liver and, hence, be released through the bile into the gastro-intestinal tract, where some of it would doubtless be reabsorbed, to run the gamut of the destruction cycle again.

Admittedly, this brief outline of the metabolic circuit of the thyroid hormone is tremendously oversimplified. It is evident from the reservations, stated or implied, in the earlier discussions on the various phases of the problem that an understanding of thyroxine metabolism is hampered at the outset by a multiplicity of complicating factors. The problem will not be easily solved. Much painstaking investigation is needed before a concept even approaching integrality can be reached concerning the fate of thyroxine in the body.

SUMMARY

1. An investigation has been conducted in order to gain an insight into the fate of thyroactive substances in the organism, with special reference to compounds administered preorally to the chick. The problems studied include the absorption of thyroidally active compounds fed to the chick and the goat, excretion of thyroxine injected subcutaneously in the goat, the rate of disapperance of orally administered thyroxine in the chick, and the effect of several factors on the rate of disappearance of thyroxine in the chick.

2. In a study on the comparative oral effectiveness of crystalline D,L-thyroxine and its mono- and disodium salts it was found that male White Plymouth Rock chicks, three weeks of age, required 0.000074 percent of the crystalline compound, 0.000035 percent of the monosodium salt, and 0.000038 percent of the disodium salt of thyroxine in the feed to maintain the thyroids at a normal weight when thiouracil was fed. Comparable figures for the female chicks were 0.000078 percent crystalline, 0.000036 percent monosodium, and 0.000039 percent disodium thyroxine, respectively.

On this basis, it was calculated that crystalline D,L-thyroxine was 20 percent, and the salts 45 percent, as effective orally as by subcutaneous injection in the form of the disodium salt of thyroxine.

3. In a similar study it was found that 0.009 percent desiccated thyroid in the diet was sufficient to maintain normal thyroid weight in thiouracil-treated White Plymouth Rock chicks. This amount of desiccated thyroid is equivalent to about 2.5 micrograms D,L-thyroxine per 100-gram chick per day. Thus, it was concluded that desiccated thyroid is about equally as active preorally as parenterally.

4. Thyroprotein (iodinated casein, Protamone) was fed to goats and its absorbability calculated by comparing the crystalline D,L-thyroxine equivalents (relative activities) of the feed and the feces. On this basis, it was calculated that thyroprotein was absorbed to the extent of 78 to 88 percent.

5. The excretion of parenterally administered thyroxine was studied by determining the thyroidal activity of goat feces and urine after ten daily subcutaneous injections of ten milligrams of D,L-thyroxine in alkaline solution. Only 1 percent of the thyroxine injected could be accounted for in the feces during the period of injections, all of this amount being excreted during the second five days of injections. Even in the period following the cessation of thyroxine injections a calculated maximum of only 5 percent of the amount injected could be accounted for via the fecal excretion route. No thyroidal activity could be detected in the feces of normal goats or in the urine of either the normal or injected goats.

6. The rate of disappearance of thyroxine in the female White Plymouth Rock chick was determined by the oral administration of crystalline D,L-thyroxine in sufficient amount to depress the thyroid weight of thiouracil-treated chicks below a normal weight and then measuring the length of time necessary

for a return to normal thyroid weight under the influence of thiouracil alone. When thyroxine was fed at a level of 0.00015 percent, return to normal occurred in approximately 2.5 days. Increasing the dosage level to 0.0002 percent prolonged this duration time to 5 days. Higher dosage levels did not increase the duration time beyond this point until the 0.00125 percent level was fed, when the return to normal was extended to 6.5 days. This increased duration time can be explained, however, by the possibility of toxic damage, resulting in a reduced capacity to dispose of the hormone, or by the fact that, due to the toxicity of this level of thyroxine, the dosage had to be increased gradually, with the result that thyroxine was fed to this group for three days longer than for other groups. It was emphasized that the mechanism(s) for hormone disposal is probably one of destruction rather than simple excretion.

7. At the 0.00015 percent level, thyroxine disappeared in half the time (2.5 days) in the female chick than was required for the male chick (5 days). At the 0.0002 percent level, however, the duration time was 5 days for both sexes. These observations were taken to indicate that while the male chick can dispose of excess amounts of thyroxine equally as well as the females, the males do not normally use up the hormone as rapidly.

8. The administration of vitamin A in massive amounts did not affect the rate of disappearance of thyroxine (0.00015 percent level) in the female chick. Whatever the antagonistic effect of vitamin A for the thyroid, as reported in the literature, it apparently is not through an effect on thyroxine metabolism.

9. Calcium pantothenate administration was found to hasten the disappearance of thyroxine. It was postulated that calcium pantothenate may exert its effect through an influence on acetylation reactions.

10. Niacin and thiamin hydrochloride were found to prolong the duration of action of thyroxine. No explanation was offered for this effect.

11. Neither riboflavin nor pyridoxine hydrochloride affected the rate of disappearance of thyroxine in the chick.

12. Due probably to certain limitations in the technique employed, partial hepatectomy in the chick could not be detected as having any influence on the rate of disappearance of thyroxine. However, thyroxine was found to aid in the regeneration of liver tissues. Other effects noted, although their significance is not clear, tend to indicate a correlation between thyroid and liver functions.

13. Thyroxine injected directly into the spleen showed no difference in duration time from thyroxine injected subcutaneously or intraperitoneally, indicating that no appreciable amount of thyroxine is destroyed in its first passage through the liver.

14. Despite the above observations, it is felt that the liver plays an important part in the metabolism of thyroxine.

15. Groups of White Leghorn chicks fed vitamin B_{12} and fish meal supplements, respectively, in diets free of protein from animal sources, and hatched from hens on identical diets, were able to metabolize thyroxine more rapidly than were similar chicks without these supplements. The latter chicks also showed symptoms of thyrotoxicosis. Apparently vitamin B_{12} or the factor(s) in fish meal (possibly identical in this case) are necessary factors in the metabolism of thyroxine.

16. Thyroprotein feeding in hens caused thyroid enlargement in their chicks. Moreover, this enlargement was accompanied by a decrease in metabolic rate. The roles of vitamin B_{12} and animal protein in this phenomenon are not clear.

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