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The Relation of the Route of Administration of Thyroxine, Thyroprotein, and Intermediate Products Upon Their Utilization by Ruminants

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FOREWORD

During the past fifteen years great progress has been made in the study of the influence of the hormones of the various glands of internal secretion upon growth, reproduction, lactation, egg production and the fattening process of domestic animals. Much of this information can be put to practical use as hormones become available which can be administered orally to domestic animals and which are sufficiently low priced.

Of the various hormones now being investigated, the hormone of the thyroid gland called thyroxine holds promise of early utilization in the feeding of domestic animals. For more than fifty years, dried thyroid gland tissue from domestic animals has been administered orally by clinicians for the treatment of thyroid deficiencies in man. Since the human requirements are small, the cost of medication has been moderate. However, when Graham (1934) demonstrated the effectiveness of thyroid gland tissue and thyroxine in stimulating increased milk and fat secretion in dairy cattle, the need of cheaper sources of thyroidally active material became apparent if full advantage of this important discovery were to be made.

For the past five years the writers have been studying the factors involved in the conversion of various proteins containing the amino acid, tyrosine, into thyroidally active compounds which we have called thyroproteins. The thyroprotein preparations were at first of rather low potency, but as further knowledge of the chemical conditions promoting the formation of thyroxine have been discovered, preparations of rather high potency have been formed. To what extent further progress in this field may be made is unpredictable. With casein containing about 5.6% tyrosine the maximum theoretical yield of thyroxine would be 10.6%. The maximum thyroxine content of 3.36% observed in these preparations constitutes slightly more than 30% of the theoretical conversion. Since the cost of the product is essentially the same whether of low or high thyroidal potency, methods of further increasing the thyroidal potency of thyroprotein are being sought.

The active hormone in both desiccated thyroid gland tissue and thyroprotein is absorbed from the digestive tract. This is an advantage over many hormones which are almost if not entirely destroyed by the enzymes secreted in the stomach and intestines. However, the evidence presented in this bulletin indicates that the absorption of the thyroid hormone from the digestive tract of ruminants is of a rather low order. If the absorption rate of the thyroid hormone in thyroprotein could be increased from the present 5% to even 10 or 15%, the cost of effective thyroid treatment would be materially reduced.

The Relation of the Route of Administration of Thyroxine, Thyroprotein, and Intermediate Products Upon Their Utilization by Ruminants

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The effectiveness of desiccated thyroid tissue administered orally (Graham, 1934; Herman, Graham and Turner, 1937) or of the thyroid hormone, thyroxine, injected subcutaneously (Graham, 1934; Jones, 1935; Folley and White, 1936; Herman et al., 1937) or intravenously (Jack and Bechdel, 1935) in substantially stimulating an increased rate of milk and milk fat secretion has been confirmed by several investigators (Hurst, Reece, and Bartlett, 1940; Ralston et al., 1940; Smith and Dastur, 1940). When from 5 to 10 mg. daily of thyroxine was injected subcutaneously into dairy cows, milk yields were observed to increase rather consistently. The oral administration of desiccated thyroid (U.S.P.) at a level of 2 oz. (56.7 gm.) per day by Herman, Graham and Turner (1938) failed to increase milk and fat production comparable to that of the above level of thyroxine, but did maintain milk secretion. They discussed the apparent low oral effectiveness of the thyroid hormone and advanced several theories as to the cause.

With the development of synthetic thyroprotein by Reineke and Turner (1942-3), the low oral effectiveness of this product by ruminant animals in comparison with its biological (Reineke and Turner, 1942; Reineke, 1943; Reineke et al., 1944) or chemical (Reineke et al., 1945) thyroxine equivalent again emphasized the need of study of the possible reasons for the low rate of utilization of thyroprotein when fed. It was hoped that such knowledge might lead to the development of methods by which the effectiveness of thyroprotein fed to ruminant animals might be increased.

This report will describe a number of experiments indicating the relative biological effect of thyroprotein and related products when administered to ruminant animals (sheep) by various routes. Attempts to increase the efficiency of orally administered thyroprotein will be described.

BIOLOGICAL ASSAY OF THYROPROTEIN IN RUMINANTS

The thyroid hormone manifests its physiological effect in many ways in animals. Greatest interest naturally centers in the effect on the rate of milk and fat secretion. While the superior value of this type of response is evident, for many reasons it seemed desirable to

use body weight reduction as a measure of thyroidal action instead. The number of available assay animals were thus greatly increased so that a series of samples could be assayed under similar conditions:

(a). **Animals.** Mature sheep were selected of rather uniform type and body weight. Unless otherwise indicated, all lots consisted of 4 animals.

(b). **Ration.** A good ration consisting of mixed grain and alfalfa hay was fed to each lot ad libitum during the assay period. A similar ration was fed during the period between assays to restore the body weight depleted by thyroid administration. Water was available at all times except during the weigh days. To attain uniform weights, the water was removed from noon the day before the weight was taken until immediately after weighing which took place about 9 a. m.

(c). **Body Weight Groups.** The weights of about 24 sheep were obtained on the first weigh day. They were listed in decreasing order of body weight. Usually six lots of four sheep were selected as follows: The heaviest sheep was placed in lot I, the next in lot II, etc., down to lot VI. Then the distribution was reversed and the animals were allotted to lot VI, lot V, lot IV, in decreasing order of body weight. By this plan, fairly even weight lots were selected.

(d). **Decline in Body Weight.** The sheep were weighed three successive days at the beginning of the assay period and three days at the close of the 14-day assay period. The average body weight of the first three days was compared with the average body weight of the final three days and the percentage difference determined. The hormone administration was started on the second day of the initial weigh period and the final administration of hormone was made on the middle day of the final weigh period. *The average percentage decline in body weight of the group during the two week period was used as the index of biological activity of the thyroprotein preparation.* It should be emphasized that the dosages of thyroprotein administered in these experiments to cause a decrease in body weight are far above the amounts required to favorably influence growth or milk production.

(e). **Modes of Administration.** Powdered thyroprotein was administered in No. 12 veterinary capsules, each holding about 4 grams. The animal's head was elevated and the capsule

placed at the back of the tongue with a capsule ejector. Each animal was watched until the capsule was swallowed. Solutions or suspensions of thyroprotein or their hydrolysates were administered slowly in metal syringes with care being taken to insure regular swallowing.

(f). **Thyroprotein.** All samples of thyroprotein used in these experiments were supplied by Dr. W. R. Graham, Jr., Cerophyl Laboratories, Inc., Kansas City, Missouri. All subsequent treatments of the various samples either by coating or hydrolysis were conducted in our laboratory.

EXPERIMENT I. ROUTE OF ADMINISTRATION OF THYROPROTEIN

A thyroprotein preparation numbered FD1-63 was used in the present series of experiments. Chemical analysis (Reineke et al., 1945) showed this product to contain 1.42 per cent thyroxine. Using the assay method already described, the effect of increasing amounts of this preparation was first determined. As will be noted in Table 1, it appeared to require about 8 gm. of this preparation per day to produce a decline in body weight of 6 to 8 per cent. In six subsequent trials the variability of the response upon the 8 gm. dosage will be noted. The average reduction in body weight of 28 sheep fed this amount per day for 14 days was 6.8 per cent.

To compare parenteral with oral administration of thyroprotein, the same sample was dissolved in a slightly alkaline solution such that 10 ml. contained the daily dosage. In the first trial 200 and 400 mg. per animal were injected subcutaneously daily in two lots of sheep. The average body weight reduction in three trials in which 400 mg. was injected subcutaneously was 6.9 per cent. This reduction is essentially the same as observed following the oral administration of 8.0 gm. Thus it appears that this thyroprotein preparation was 20 times as effective when administered subcutaneously as when fed orally. In other words, only 5 per cent of the biologically active material in this preparation was absorbed from the digestive tract of ruminant animals (sheep).

A comparison of the oral and subcutaneous administration of preparation FD1-39 showed an even wider difference between the two methods. In single assays similar responses were obtained with 8 gm. orally and 249 mg. subcutaneously, a difference of 32 times or about 3 per cent absorption by oral as compared with subcutaneous dosage.

Instead of administering the thyroprotein in dry form in a cap-

TABLE 1. - BIOASSAY OF THYROPROTEIN - ROUTE OF ADMINISTRATION

Route of Administration	Daily Dosage	Thyroxine Equivalent* mg.	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay was Conducted
Preparation FD1-63					
Oral by capsule	2 gm.	28.4	126	-1.7	Aug.
	4 gm.	56.8	122	-5.2	Aug.
Oral by capsule	8 gm.	113.6	119	-8.3	Aug.
	8 gm.	113.6	123	-6.5	Sept.
	8 gm.	113.6	131	-3.8	Sept.-Oct.
	8 gm.	113.6	132	-8.2	Oct.
	8 gm.	113.6	138	-8.9	Nov.
	8 gm.	113.6	122	-8.7	Dec.
	8 gm.	113.6	129	-3.6	April
Ave. 7 trials 8 gm. with 28 sheep				-6.8	
Subcutaneous Inj. (10 c.c.)	200 mg.	2.8	129	-3.9	Sept.-Oct.
Subcutaneous Inj. (10 c.c.)	400 mg.	5.7	125	-5.9	Sept.-Oct.
	400 mg.	5.7	124	-7.9	Oct.
	400 mg.	5.7	121	-6.9	Feb.
Average 3 trials with 11 sheep				-6.9	
Oral by drench	8 gm.	113.6	131	-0.9	Oct.-Nov.
(Followed by 10 c.c. $CuSO_4$)	8 gm	113.6	128	-2.0	Oct.-Nov.
(Preceded by 10 c.c. $CuSO_4$)	8 gm.	113.6	120	+3.5	Oct.-Nov.
(In phosphate buffer)	8 gm.	113.6	138	-4.1	Feb.
	16 gm.	227.2	144	-10.8	Nov.-Dec.
	16 gm.	227.2	136	-7.4	Feb.
Preparation FD1-39					
Oral by capsule	4 gm	128.4	110	-2.2	Dec.-Jan.
	8 gm.	256.8	109	-9.0	Dec.-Jan
Subcutaneous Inj.	0.125 gm.	4.0	128	-4.7	Dec.
	0.249 gm.	8.0	127	-6.5	Dec.

*Computed from the chemical analysis (Reineke et al., 1945). Preparation FD1-63 contained 1.42 per cent and FD1-39 contained 3.21 per cent thyroxine as determined by this method.

sule, the thyroprotein was suspended and partially dissolved in a slightly alkaline solution. In three trials in which 8 gm. were thus administered, the response appeared definitely lower than with equal amounts of thyroprotein by capsule (Table 1). Since a copper sulphate solution has been shown to close the esophageal groove and thus cause solutions to pass directly into the abomasum (Mönnig & Quin, 1935), 10 c.c. of a copper sulphate solution was administered to one lot immediately before the thyroprotein drench was administered and in the second case immediately after. Although the copper sulphate may have been effective in causing the solution to pass directly into the abomasum, it appeared non-effective in increasing the biological activity of the thyroprotein. When 16 gm. of thyroprotein was ad-

ministered either suspended in a slightly alkaline solution or in a phosphate buffer solution, the body weight reduction appeared only slightly better than 8 gm. administered in a capsule. These observations were interpreted as indicating that the suspension of thyroprotein in water, rather than improving the utilization, depresses the oral biological value. No explanation for these observations are offered. However, they were substantiated by subsequent observations on the low biological effect of incompletely hydrolyzed (4 hours) preparations at the 8 gm. level administered both into the rumen and abomasum (Tables 1 and 4).

EXPERIMENT II. EFFECT OF COATING THYROPROTEIN

To account for the low oral effectiveness of thyroprotein when fed to ruminant animals, it was suggested that the thyroxine in thyroprotein might be inactivated in part by the rumen flora or fauna since it has been shown that much of the protein fed to ruminant animals is broken down by the rumen micro-organisms and reconstructed into micro-organismal protein, which in turn is digested in the abomasum and intestinal tract. If thyroprotein were digested by the enzymes of the rumen micro-organisms and the thyroidally active fraction were then metabolized into a biologically inactive compound, it was suggested that this destruction might be reduced by coating the thyroprotein particle with some substance which would resist the chemical

TABLE 2 - BIOASSAY OF THYROPROTEIN - COATING OF PARTICLE

Composition of Coating	Daily Dosage gm.	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay was Conducted
(Preparation FD1-63)				
Stearic acid, 20%	10	128	-3.5	Sept.-Oct.
Paraffin, 20%	10	128	-1.3	Sept.-Oct.
Beeswax, 20%	10	132	-5.0	Oct.
Formaldehyde	8	138	+2.7	Oct.
Formaldehyde + Na_2CO_3	8	139	-0.9	Oct.
Calcium carbonate	8	130	-4.4	Oct.
Rosin	8	131	-2.3	Oct.-Nov.
Clarite	8	137	-1.3	Oct.-Nov.
Vinylite	8	133	-0.8	Oct.-Nov.
Linseed oil (then dried)	8	132	-4.2	Dec.-Jan.
Uni-Lac (sub. for shellac)	8	130	-4.9	Dec.-Jan.
(Preparation 3D-101)				
Water glass coating (9.5 gm. = 8 gm. original)	9.5	138	-0.7	Nov.-Dec.
50% Beeswax + 50% Rosin (20% coating)	10.0	139	-3.3	Nov.-Dec.
50% Stearic acid + 50% Rosin (20% coating)	10.0	138	-2.8	Nov.-Dec.

conditions in the rumen but would permit the thyroprotein to be digested in the true digestive system.

The standard preparation, FD1-63, used in Experiment I was used in the first part of this work. To this preparation 20 per cent by weight of stearic acid was added and mixed thoroughly. The mixture was then heated to above the melting point of stearic acid (69.3° C.), and stirred while cooling to form small aggregates of the material. Since 20 per cent of the total consisted of stearic acid, the daily dosage was increased to 10 gm. and was administered to the sheep by capsule as before. In a similar way 20 per cent of paraffin and beeswax was added to thyroprotein. As will be seen in Table 2, the use of these substances as coating materials appeared to depress the biological activity of the thyroprotein rather than to increase it.

In the next trial, thyroprotein was treated with formaldehyde alone and in combination with Na_2CO_3. Here again the biological activity was partially or entirely suppressed. In further trials, rosin, clarite and vinylite (plastic materials) linseed oil, and a substitute for shellac called Uni-Lac were used as coating materials. In every case the use of coatings for thyroprotein decreased the weight reduction obtained with preparation FD1-63.

With the idea that the effectiveness of the coating might be demonstrated more effectively using a preparation of lower biological value, preparation 3D-101 was coated with water glass, a mixture of equal parts of beeswax and rosin and of stearic acid and rosin. As noted in Table 7, this preparation uncoated showed only slight weight depressing effect. The coatings of beeswax and rosin and stearic acid and rosin were ineffective in increasing the biological activity of this preparation to any extent (Table 2).

EXPERIMENT III. ADMINISTRATION OF THYROPROTEIN INTO ABOMASUM

Since the methods and substances employed in coating the thyroprotein particle proved to be valueless in improving the oral effectiveness of thyroprotein, some other approach to the problem was indicated. The most obvious method would be to by-pass the rumen system entirely and administer the thyroprotein directly into the abomasum.

Two methods were suggested. One would be to stimulate the closure of the esophageal groove by means of copper sulphate by the technique of Mönnig and Quin (1935), then drench the animals with a suspension of thyroprotein. Since this technique involved the administration of considerable amounts of copper sulphate over a two

week assay period, the amount to be administered daily would necessarily be minimal and the assurance that the esophageal groove would close each time raised serious doubts as to the usefulness of this method. One such trial was conducted (Table 1) but as indicated, if closure of the esophageal groove was effected, the biological value of the thyroprotein was not enhanced by direct passage into the abomasum.

The second method adopted in order to administer the thyroprotein directly into the abomasum of the experimental sheep, was to make an abomasum fistula. Plastic tubes described by Quin, Van der Wath and Myburgh (1938) were inserted directly into the abomasum and brought out to the surface of the skin. Rubber stoppers placed in the end of the tubes prevented the outflow of the abomasal contents. At the time of administration of the thyroprotein, the animals were laid on their sides, the stopper removed and the end of the syringe placed into the tube and its contents discharged directly into the abomasum.

In the first trial, 400 mg. and 800 mg. of FD1-63 in phosphate buffer solution was administered to two groups of three sheep each directly into the abomasum since the smaller amount injected sub-

TABLE 3 - ADMINISTRATION OF THYROPROTEIN INTO ABOMASUM

Type of Preparation	No. of Animals in Lot	Daily Dosage	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay was Conducted
FD1-63 in phosphate buffer solution 40 mg./ml.	3	400 mg.	105	+3.1	Feb.
"	3	800 mg.	106	+6.9	Feb.
"	2	4 gm.	118	+3.0	Feb.-Mar.
"	2	8 gm.	118	-2.5	Feb.-Mar.
FD1-63 (alkaline hydrolysis)*	4	4 gm.	119	+3.2	Mar.
FD1-63 (4 hrs. acid hydrol.)**	4	8 gm.	125	-1.9	Apr.
FD1-63 (mixture of 8+13 hrs. hyd)**	3	8 gm.	117	-7.5	Apr.

*Hydrolyzed for 4 hours in 5 N sodium hydroxide solution.
**Hydrolyzed in 30 per cent sulfuric acid solution.

cutaneously produced an average weight reduction of 6.9% (Table 1). As will be seen in Table 3, these sheep gained in weight on this treatment. Then 4 and 8 gm. of this preparation was administered into the abomasum. On the 8 gm. level, the response in weight reduction was essentially the same as that observed upon oral administration of the same amount of thyroprotein into the rumen. While these

observations are limited, it appeared that the rumen was not responsible for the low oral biological activity of thyroprotein. These data would thus explain the observed lack of value of coating thyroprotein in order to "protect" it from rumen action.

As an alternative theory to explain the low oral utilization of thyroprotein by ruminant animals, it was suggested that possibly the digestive juices were incapable of digesting the thyroprotein molecule (in this case iodocasein) to thyroxine or to a state where the thyroxine-containing protein fragment could be absorbed from the intestinal tract. If this were true, then preliminary chemical hydrolysis of thyroprotein might be helpful.

As indicated in Experiment IV, several trials were conducted with hydrolyzed preparations. It is interesting to note that at the 8 gm. level, the results obtained on the 4 hour hydrolysis in both normal sheep and in sheep with abomasal fistulas, the biological effect was low. However, when the hydrolysis was complete, in the 8 and 13 hour acid hydrolysates, the weight reducing effect was markedly increased. This would indicate a beneficial effect of the hydrolysis of thyroprotein when administered either into the rumen or into the abomasum.

EXPERIMENT IV. EFFECT OF CHEMICAL HYDROLYSIS UPON THYROPROTEIN

In a consideration of the favorable effects which might be obtained by chemical hydrolysis of thyroprotein, there are certain fundamental problems which must be considered. The thyroxine present in thyroprotein has been shown to be present in the levorotatory (l-) form (Reineke and Turner, 1943). When thyroprotein is acted upon by the digestive enzymes, the thyroxine is not racemized. Similarly, chemical hydrolysis with acids does not cause racemization. However, hydrolysis in alkaline solutions such as with barium hydroxide causes racemization, with the formation of d, l-thyroxine (Reineke and Turner, 1943).

The importance of the above lies in the fact that the biological activity of thyroxine resides only in the levorotatory (l-) form; racemized thyroxine, (the d, l-form) possesses only half the biological activity of l-thyroxine (Reineke and Turner, 1945). Thus, where racemization occurs in the hydrolysis of thyroprotein, the biological activity would be expected to be reduced by one-half in the process. On the other hand, acid hydrolysis may result in some destruction of the free thyroxine.

All thyroprotein preparations used in this experiment were hydrolyzed in 30% H_2SO_4 with heat for variable periods of time. After cooling they were neutralized with 20% NaOH solution. The insoluble humin was dispersed by grinding in a Waring blendor and added to the hydrolysate. Finally, the pH was adjusted slightly to the alkaline side of phenolphthalein, and the volume adjusted so that 30 ml. contained the equivalent of one gram of thyroprotein. The hydrolysate was then administered orally by syringe in 4 oz. units which would be the equivalent of 4 gm. of dry thyroprotein.

TABLE 4 - ACID HYDROLYSIS OF THYROPROTEIN

Description of Preparation	Daily Dosage gm.	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay was Conducted
FD1-63	4	122	-0.1	March
FD1-63 (4 hrs. Biuret test +)	8	125	-0.7	April
FD1-63 (8 hrs. Biuret test sl. +)	8	131	-6.3	April
FD1-63 (13 hrs. Biuret test -)	8	123	-6.6	April

Upon the acid hydrolysis of preparation FD1-63 for four hours, 4 gm. and 8 gm. were found to be ineffective in depressing the body weight (Table 4). Since the Biuret reaction was positive, it was evident that the hydrolysis was not complete. After 8 and 13 hours of acid hydrolysis, the Biuret test changed from slightly positive to negative. These hydrolysates both gave positive biological responses of greatly increased magnitude. This would indicate that acid hydrolysis improved the oral absorption of thyroprotein. These data are also supported by the observations upon the administration of hydrolysates directly into the abomasum (Table 3).

In Experiment I, it was observed that the administration of FD1-63 suspended as a drench at the 8 gm. level was definitely less effective than when administered dry in capsule form. The two assays with 4 hour hydrolysates (which were incomplete) with both normal sheep and sheep with abomasal fistulas at the 8 gm. level caused body weight reduction comparable with the earlier observations (Table 1). In fact, 16 gm. given as a drench was only slightly more effective than 8 gm. administered dry.

These data appear to indicate that while the complete hydrolysis of thyroprotein increased the effectiveness of thyroprotein administered as a drench, it did not increase its biological value above that of the same preparation administered dry in a capsule.

These observations might indicate that while acid hydrolysis increased the oral absorption of the thyroidally active part of thyroprotein, the chemical treatment may have destroyed part of the biological activity in the process so that the over-all gain by hydrolysis was reduced.

Experiments on the absorption of the whole thyroprotein in comparison with that of hydrolysates were conducted with preparation FD1-38. The undigested thyroprotein and the acid hydrolysate were prepared for administration as described earlier, except that the latter was hydrolyzed for only 2 hours in an attempt to effect partial breakdown of the thyroprotein without destruction of thyroxine.

The acid-insoluble precipitate was prepared by first hydrolyzing the thyroprotein in 40 per cent barium hydroxide solution for 20 hours. After removal of the excess barium hydroxide by crystallizing and filtration, the thyroxine-containing fraction was precipitated by adjusting the reaction of the filtrate to pH 5.0 with dilute hydrochloric acid. From 800 gm. of preparation FD1-38, containing 3.36 per cent thyroxine by chemical analysis (Reineke et al., 1945), 89.9 gm. of

TABLE 5 - EFFECT OF HYDROLYSIS AND ROUTE OF ADMINISTRATION ON UTILIZATION OF THYROPROTEIN
(FD1-38)

Route of Admin.	Type of Preparation	Daily Dosage gm.	Thyroxine Equivalent* mg.	Ave. Body wt. of Group lbs.	Change in Body wt. %	Season Assay was Conducted
Subcut.	undigested thyroprotein	0.238	8.0	127	-8.8	Jan.
"	acid hydrolysate**	0.238	8.0	112	-8.3	Feb.
"	Acid-insol. ppt.***	0.028	8.0	128	-8.3	Jan.
Oral	" " "	0.056	16.0	129	+1.75	Jan.
"	" " "	0.112	32.0	129	+1.05	Jan.
"	" " "	0.225	64.0	128	-0.75	Jan.
"	" " "	0.450	128.0	114	-7.2	Feb.

*Computed from the chemical analysis (Reineke et al., 1945). Preparation FD1-38 contained 3.36 per cent thyroxine as determined by this method.
**Hydrolyzed for 2 hours in 30 per cent sulfuric acid.
***Prepared subsequent to hydrolysis of thyroprotein in 40 per cent barium hydroxide, as explained in the text.

acid-insoluble precipitate was recovered. Analyzed by the same method, this fraction contained 28.43 per cent thyroxine. Thus 90.5 per cent of the thyroxine in the starting material was recovered.

When injected subcutaneously in an amount sufficient to supply 8 mg. of thyroxine in each case, body weight losses of 8.8, 8.3 and 8.3 per cent were produced by the undigested thyroprotein, the acid hydrolysate, and the acid-insoluble precipitate (Table 5). However, the thyroxine in the first two preparations would occur as the pure

l-compound and in the last one as a d, l-mixture due to racemization during alkaline hydrolysis. Thus if complete utilization were to occur, the acid-insoluble precipitate should exert only one-half the thyroidal effect of the first two preparations instead of showing an equal response. A similar discrepancy can be observed by comparing the response to thyroxine injected as thyroprotein (Table 1) and as the free d, l-compound (Table 6). It appears probable that this apparent

TABLE 6. - COMPARISON OF d, l-THYROXINE ADMINISTERED SUBCUTANEOUSLY AND ORALLY

Route of Administration	Daily Dosage*	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay was Conducted
Subcutaneously (as monosodium salt)	4 mg.	126	-5.3	Feb.
" "	4 mg.	130	-5.9	Dec.
" "	8 mg.	129	-7.5	Feb.
" "	8 mg.	128	-9.8	Feb.
Orally (as monosodium salt)	16 mg.	123	-3.1	Dec.
Orally (as disodium salt)	16 mg.	110	-0.5	Dec.
" "	32 mg.	111	-4.4	Feb.

*4 mg. thyroxine per millileter.

discrepancy is due to differences in the rate of absorption of undigested or only slightly hydrolyzed thyroprotein and free thyroxine from the injection site. In guinea pigs (Reineke et al., 1945), thyroprotein is utilized much more completely when given by intraperitoneal than by subcutaneous injection.

When given orally, the acid-insoluble precipitate was only about 6 per cent as effective as by subcutaneous injection. This low response as compared to that of free thyroxine (Table 6) is believed to be due to the fact that small amounts of barium remaining in this fraction would combine with the thyroxine to form the insoluble barium salt, thus interfering with its absorption from the gut.

EXPERIMENT VI. COMPARISON OF d, l-THYROXINE ADMINISTERED BY VARIOUS ROUTES

If it were assumed that chemical hydrolysis of thyroprotein made available to the animal all of the l-thyroxine present, the next problem would be to determine to what extent crystalline thyroxine or its various derivatives would be absorbed when administered orally as compared to subcutaneous administration. Since rather large amounts of thyroxine were required for this comparison, our supply became exhausted before sufficient comparisons could be conducted. While the results are considered only preliminary, they may indicate the trend.

The thyroxine was either given in alkaline solution as the disodium salt, or in a slightly alkaline suspension as the monosodium salt.

In two trials with 4 mg. per day of d, l-thyroxine, the body weight reduction averaged 5.6% when administered subcutaneously (Table 6). On 8 mg. daily the average reduction in body weight was 8.7% In comparison, in two trials when 16 mg. daily of thyroxine was given orally, the body weight reduction averaged 1.8% and in one trial on 32 mg. daily, there was a reduction of 4.4%. Since three of the 4 sheep in this latter group averaged 5.9%, it is believed that this dosage (32 mg.) actually produced a biological effect very close to that produced by 4 mg. injected subcutaneously. On this basis the oral absorption of d, l-thyroxine is about 1/8 or 12½% as effective as subcutaneous administration. It would thus appear that even the amino acid thyroxine is absorbed rather poorly from the sheep intestinal tract. However, in comparison with the oral and subcutaneous utilization of thyroprotein (Table 1), these data are believed to indicate that incomplete digestion of the latter was also involved in its low biological value when given orally.

BIOLOGICAL AND CHEMICAL COMPARISON OF VARIOUS THYROPROTEINS

A large variation can occur in the potency of thyroprotein, depending upon its method of preparation, as shown by the authors in a series of publications (Reineke, Williamson and Turner, 1942; 1943; Reineke and Turner, 1942; 1945). In the present report (Table 7) similar differences in potency of various preparations were observed, whether measured by their biological effectiveness or their chemical analysis for thyroxine. It is thus imperative that a measure of the thyroidal activity of such preparations be obtained before they are used in large scale animal experiments.

Because of greater rapidity, and repeatability as compared to

TABLE 7 - COMPARISON OF THE BIOASSAYS AND THYROXINE ANALYSIS OF VARIOUS THYROPROTEINS

Description of Preparation	Daily Dosage gm.	Thyroxine by Chemical Analysis %	Body Weight of Group Ave. lbs.	Change in Body Weight %	Season Assay Was Conducted
FD1-38	4	3.36	110	-5.2	Dec.-Jan.
"	8	3.36	115	-14.2	Feb.
FD1-39	4	3.21	110	-2.2	Dec.-Jan.
"	8	3.21	109	-9.0	Dec.-Jan.
FD1-55	4	2.68	134	-2.6	Dec.
"	8	2.68	131	-10.2	Dec.
FD1-56 (Dried at 120° C.)	4	1.57	129	-2.3	Sept.
" "	8	1.57	125	-4.1	Sept.
" "	8	1.57	131	-3.3	Sept.-Oct.
FD1-63 (Ave. 7 trials)	8	1.42		-6.8	All seasons
3D-100 (greater than 20 mesh)	8	0.76	133	+0.4	Dec.
3D-100 (unscreened)	8	0.76	133	-1.3	Dec.
3D-101	8	0.62	134	-2.3	Nov.-Dec.

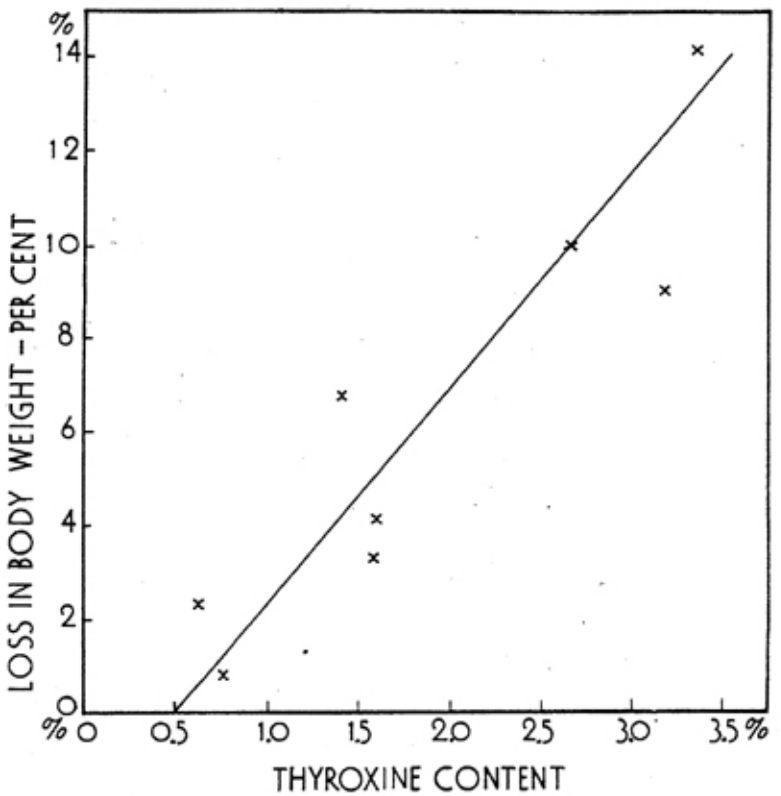

Figure 1.—Comparison of the body weight loss response and the thyroxine analysis as measures of the activity of thyroprotein given at the 8 gm. level.

biological assays, a chemical method would offer many advantages for this purpose, provided that it will accurately predict the biological response.

When the chemical thyroxine analyses of the preparations investigated in the present work are compared with the bioassays (Fig. 1), fairly good agreement between the two measures is obtained. While it cannot be stated with certainty that the chemical method employed would be specific for all types of thyroprotein, it appears to show considerable promise for use in the standardization and control of such preparations.

DISCUSSION

The experiments discussed in this bulletin were initiated with the object of determining more accurately the relation of the route of administration of thyroproteins to their utilization by ruminants and to discover, if possible, methods of increasing the oral absorption. Using the effectiveness of thyroprotein when administered subcutaneously as a standard, it was observed that only about 5 per cent of that activity was obtained following oral administration. This observation presents a real challenge to investigators to determine the sources of loss of the hormone from the digestive tract of ruminants and to develop methods of preventing these losses to the greatest possible extent.

Our data show clearly that attempts to "protect" the thyroprotein particle from rumen micro-organisms were without benefit. These observations are confirmed by the work of Blaxter (1945) who showed that stearic acid coated thyroprotein elicited no greater response in either milk production or metabolism than did an equal amount of the uncoated material when fed to dairy cattle.

That the rumen is not an important source of loss of thyroidal activity was demonstrated by by-passing this section of the digestive tract entirely. The physiological activity of thyroprotein was not increased when it was administered directly into the abomasum or true ruminant stomach.

Since casein is generally considered to be highly digestible, one might expect that iodinated casein would also be completely hydrolyzed to its constituent amino acids by the enzymes of the digestive tract and the free thyroxine readily absorbed from the gut. Under these conditions, the l-thyroxine in the thyroprotein would not be racemized and the full physiological activity would be preserved.

Our data, however, suggests the possibility that upon the iodination of casein, the peptide linkages may be less readily broken by the

digestive enzymes, resulting in the production of peptides of various lengths as well as free amino acids. The elimination of these peptides containing thyroxine in the feces might account in part for the low oral effectiveness of thyroprotein.

Upon the chemical hydrolysis of thyroprotein, the peptide linkages are broken and the thyroxine may be absorbed in larger amount from the digestive tract. However, in alkaline medium, racemization of the thyroxine occurs with a loss of one-half of the physiological activity while in acid medium, there may be considerable losses of thyroxine during the course of the hydrolysis.

While these data are not extensive, the observations on the oral effectiveness of thyroxine do not offer great promise for improvement of the oral absorption rate of thyroprotein. Our data indicate that only about 12½ per cent of free thyroxine administered orally is active in comparison with subcutaneous absorption. However, great differences have been reported in the rate of oral absorption of thyroxine depending upon the form in which it is administered. It is quite possible that the form of the thyroxine in these experiments is quite different from the form present in the digestive tract either when hydrolyzed or when normal thyroprotein is fed.

If the feeding of thyroprotein as part of the ration of farm animals is to be successful, it will be necessary to prepare preparations of uniform physiological activity. Since even with a standardized manufacturing procedure, some variation will occur, it will be necessary to determine the thyroidal activity of each batch of material. At best, biological methods such as were used in these experiments are time consuming. Chemical methods offer many advantages in speed and repeatability. Since the active biological principle found in thyroprotein is thyroxine, a method of determining chemically the amount of thyroxine present in thyroprotein has been developed (Reineke et al., 1945).

In the course of our experiments a number of preparations have been assayed biologically with sheep. The same preparations have been analyzed chemically for their thyroxine content. It will be seen that there is fair agreement between the thyroxine content of the various preparations and their ability to reduce the average body weight of groups of sheep.

While the biological activity of thyroprotein administered orally to ruminants will always be the final criterion of potency, the chemical method may prove of considerable value in the control and standardization of such preparations.

SUMMARY

1. The effect of the route of administration of thyroxine, thyroprotein, and intermediate products upon their utilization by ruminant animals (sheep) was investigated.
2. The physiological effect of the various thyroidally active preparations was measured by the decline in body weight of the assay animals during a two week period.
3. The subcutaneous administration of a sample of thyroprotein was found to be 20 times as effective as the same sample when fed. Thus only 5 per cent of the biologically active material absorbed from a subcutaneous injection site was absorbed from the digestive tract of sheep.
4. The administration of thyroprotein orally in dry form in a capsule was found almost twice as effective as when the same preparation was either suspended in a slightly alkaline or in a phosphate buffer solution and given as a drench.
5. The coating of the thyroprotein particle by stearic acid, paraffin, beeswax, rosin, clarite, vinylite, and linseed oil in every case reduced the biological effect.
6. To eliminate the rumen as a possible source of destruction of thyroprotein, preparations were administered directly into the abomasum of sheep. Since the body weight reduction was the same as when the preparation was administered by mouth, it was concluded that the low oral biological value of thyroprotein was not due to destruction in the rumen.
7. The hydrolysis of thyroprotein with 30% H_2SO_4 with heat for 8 hours or more caused the Biuret test to become negative, indicating the absence of peptide linkages. Such hydrolysates given as a drench gave increased biological responses when administered by mouth or directly into the abomasum. However, hydrolysis did not increase the biological value above that of the same preparation administered in a capsule. The observations are interpreted as indicating that the chemical treatment may have destroyed part of the biological activity in the process so that the over-all gain by hydrolysis was reduced.
8. Following the hydrolysis of thyroprotein for 20 hours in 40 per cent barium hydroxide, the acid-insoluble precipitate was recovered. This fraction contained 28.43 per cent thyroxine as compared to 3.36 per cent in the starting material. This represented a recovery of 90.5% of the thyroxine in the starting material. When given orally, the acid-insoluble fraction was only about 5 per cent as effective as by subcutaneous injection.

9. Comparing the biological effect of d, l-thyroxine administered orally with the subcutaneous route, it was observed that the former was only about 1/8 or 12½ per cent as effective as the latter. These data indicate that even the free amino acid, thyroxine, is absorbed rather poorly from the sheep intestinal tract.
10. The preparations of thyroprotein used in these experiments were observed to contain thyroxine varying from about 0.5 per cent to about 3.3 per cent as determined by a chemical method. Fairly good agreement between the biological assays and the chemical determination of the thyroxine content of thyroprotein was obtained.

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