FIG. 3. Beginning at 2 min of fatty acid perfusion and continuing for 60 min (as shown here) there appears to be an increase in granules, morphologically similar to those in the space of Disse, within the cisternae of the Golgi system (G) and the endoplasmic reticulum (at the arrows). 29,000 ×.

FIG. 4. An electron micrograph of a portion of the pellet obtained after fixation and centrifugation of the free fatty acid perfusate after 1 hr of liver perfusion. The electron dense granules shown are of the same dimension as those in the liver and space of Disse. At slightly higher magnification (see insert) they appear to have an irregular outline and a stippled substructure. 44,500 × ; Insert, 62,500 ×.

bodies are very numerous in the endoplasmic reticulum and Golgi apparatus. They are first seen in significant numbers in the space of Disse at 5 minutes. Similar granules are present in the final media of livers perfused with fatty acid and the d $<$ 1.006 fraction of human and rat sera. The data suggest that these bodies are very low density lipoprotein and indicate that the isolated perfused liver is an excellent experimental system for morphological study of lipoprotein metabolism.

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Comparison of Biological Activity of Orally Administered and Injected L-Thyroxine, L-Triiodothyronine and Thyroprotein* in Rats. (31389)

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Oral administration of thyroid hormones as salts, desiccated thyroid, or thyroactive iodinated casein (thyroprotein) is the most practical method of administration of these hormones. The reduced effectiveness of thyroxine, or thyroxine-containing preparations by oral administration has been recognized for some time.

Sturnick and Lesses(1), using PBI levels

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as an index in man, found that 0.1 to 0.2 mg of L-thyroxine per day produced normal PBI levels, whereas 195 mg of desiccated thyroid was required for the same effect. Lavietes and Epstein(2) reported that 60 mg of thyroid USP, 0.1 mg of L-thyroxine and 0.025 mg L-triiodothyronine were equally effective orally in man.

Frieden *et al*(3) were first to present data on the comparison of oral and subcutaneous administration of thyroid preparations, DL-T_4, and thyroprotein using the goitrogen prevention method of assay in rats. He reported that DL-T_4 orally was 49% as active as by injection. Two samples of desiccated thyroid were about 70% as active orally as by injection, and thyroprotein only 13 to 14% as active orally.

Littrel *et al*(4), also using the goiter prevention method in rats, reported that L-thyroxine, sodium-L-thyroxine and disodium-L-thyroxine were 50% as effective orally as by subcutaneous administration.

Albert *et al*(7) injected L-T_4-^{131}I into the stomach, small and large intestine of rats. From 20 to 25% of the thyroxine was absorbed from the isolated small and large intestine.

Chung and Van Middlesworth(8) reported that 20 to 50% of L-T_4-^{131}I was absorbed from intestinal loops of the rat intestine during the first hour with little additional absorption during 3 subsequent hours. They suggested that the continued absorption of thyroxine was prevented by its binding by a large molecule, possibly plasma albumin.

The present study was undertaken to determine quantitatively the relative efficiency of orally administered L-thyroxine (L-T_4), L-triiodothyronine (L-T_3), and thyroprotein in male rats as compared to their subcutaneous administration. The thyroid secretion rate (TSR) technique and the same groups of animals were used for successive assays.

Materials and methods. Two groups of mature male Sprague-Dawley-Rolfsmeyer rats were housed in an animal room maintained at 78 $\pm 1°$F, artificially illuminated during daylight hours and fed Purina Lab Chow and water. The TSR of the rats was determined by a method previously described(5) in which graded doses of thyroxine were injected to suppress TSH release, thus inhibiting release of thyroidal ^{131}I. The amount of L-T_4 required completely to suppress TSH discharge would necessarily be slightly in excess of the rat's equivalent endogenous secretion of thyroid hormones. Since the level of thyroxine is increased by 0.25 μg/100 g bw every 48 hours and the dose previous to the final one does not completely block the release of thyroidal ^{131}I, the error must be less than 0.25 μg/100 g bw. In this study, 0.4 mg methimazole/100 g bw was injected daily to prevent recycling of ^{131}I from metabolized thyroxine-^{131}I(10). It has been reported that methimazole appears to have no extrathyroidal effects in the rat(6).

Group I consisted of 48 rats which survived the initial TSR estimation and the oral assay of L-T_4 and L-T_3, each spaced one month apart (94% survival of rats). Normal TSR was determined by injecting ^{131}I, then 48 hours were allowed for maximum uptake, and L-T_4 at the level of 0.75 μg/100 g bw and methimazole were injected concurrently after the uptake count was made. L-T_4 and methimazole were injected daily, the L-T_4 dosage being raised 0.25 μg/100 g bw every 2 days at which time thyroidal ^{131}I counts were made. The dose of L-T_4 that inhibited thyroidal ^{131}I release (95-100% of previous count) was used as the animal's estimated TSR. This end-point was used in all of the assays.

Na L-T_4 was dissolved in 0.1 N NaOH brought up to approximate volume with distilled water, precipitated with HCl, and final volume adjusted.

For the assay of its oral effectiveness, the L-T_4 was diluted in a slightly alkaline saline solution (pH approx. 7.5-8.0) and administered at the rate of 0.1 ml/100 g bw through a stainless steel tube designed for oral administration. L-T_3 was administered in the same manner, but given on an equimolar basis in comparison with L-T_4.

Group II consisted of 39 rats that survived the initial TSR determination with L-T_4 and the oral and injected thyroprotein assay (88% survival of rats).

Thyroprotein is a synthetic thyroactive

iodinated casein with the commercial name of Protamone. It is standardized by the manufacturer to contain 1% thyroxine by a counter-current distribution fractionation system and differential spectrophotometric method. Its possible L-T_3 content is not known at this time. On this basis 100 μg thyroprotein would contain 1 μg L-T_4. To determine the oral effectiveness of thyroprotein, it was homogenized in a 50% solution of saline and propylene glycol. The initial level was adjusted to contain sufficient thyroprotein (250 μg thyroprotein) in 0.1 ml to be the equivalent of 2.5 μg L-T_4. After this, the doses were increased every 2 days to contain 0.50 μg L-T_4/100 g bw (50 μg thyroprotein) more than the preceding dose. It was administered orally by a stainless steel tube.

Thyroprotein for subcutaneous injection was prepared in the same manner, but doses were adjusted to contain sufficient thyroprotein to be the equivalent of 0.75 μg L-T_4/100 g bw (75 μg thyroprotein) and were increased every 2 days by an amount equal to 0.25 μg L-T_4 (25 μg thyroprotein) more than the preceding dose.

Results. The normal TSRs of the rats in Group I ranged from 1.25 to 1.75 μg L-T_4/100 g bw, with a mean of 1.35 $\pm$ .53 μg/100 g bw (Table I). When L-T_4 was administered orally, the mean dose required to block release of thyroidal ^{131}I was 3.56 $\pm$ .53 μg/100 g bw, with a range from 2.5 to 4.5 μg. Using the subcutaneous administration of L-T_4 as a standard the mean oral effectiveness was 37.9%. L-T_3 administered orally required a mean of 1.41 $\pm$ .16 μg/100 g bw, with a range of 1.25 to 1.75 μg/100 g bw. Thus L-T_3 was 95% as effective orally as an equimolar equivalent of L-T_4 by injection (Table I).

The rats in Group II had a normal TSR of 1.65 $\pm$ .64 μg L-T_4/100 g bw by injection with a range from 1.25 to 2.0 μg/100 g bw (Table I). When thyroprotein was injected, subcutaneously, it required a mean amount of thyroprotein that contained 1.40 $\pm$ .56 μg L-T_4 equivalent (141 μg thyroprotein)/100 g bw. The range varied from 1.0 to 1.75 μg L-T_4 (100 to 175 μg)/100 g bw. The efficiency of absorption when injected ranged from 94.3 to 132.0% as compared to injected L-T_4. The mean was 111.8%.

When thyroprotein was administered orally, the mean thyroidal ^{131}I blocking dose was 4.56 $\pm$ .64 μg L-T_4 (or 456 μg thyroprotein)/100 g bw with a range from 3.0 to 5.5 μg L-T_4 (or 300 to 550 μg thyroprotein)/100 g bw. In comparison with injected thyroprotein, the oral effectiveness was 30.7%, or in comparison with injected L-T_4, 36.2%.

The effect of the carriers alone on TSR was studied but no significant differences were noted (Table II).

Discussion. In a previous study(9) a comparison of the biological activity of L-T_3 and L-T_4 by subcutaneous administration in male rats was reported using as an end point, the amount of hormone required to block the release of TSH and, in turn, the release of thyroidal ^{131}I. By this method L-T_3 was shown to be 2.6 times as active as L-T_4 by subcutaneous injection.

In the present study, using the same method, the oral effectiveness of L-T_3, L-T_4 and thyroactive iodinated casein (thyroprotein, Protamone) were compared with the subcutaneous method.

The mean TSR of 48 male rats injected subcutaneously with L-T_4 was 1.35 $\pm$ .53 μg/100 g bw. When the same rats were given L-T_4 orally, the mean TSR equivalent was 3.56 $\pm$ .53 μg/100 g bw. This indicates that orally, L-T_4 is 37.9% as effective as L-T_4 by injection. Or stated in another way, 2.5 times as much L-T_4 must be administered orally to be as effective as by injection.

When L-T_3 was administered orally on an equal molar basis to these same rats the mean amount required to block thyroidal ^{131}I release was 1.41 $\pm$ .16 μg/100 g bw. In comparison with L-T_4 by injection, oral administration of L-T_3 was 95% as effective. On the basis of the previous study indicating that L-T_3 is 2.6 times as effective as L-T_4 by injection, the oral effectiveness of L-T_3 in comparison with injected L-T_3 is 36.9% Thus the oral effectiveness of L-T_3 and of L-T_4 is nearly the same.

Up to date, the increased potency of L-T_3, as compared to L-T_4, is attributed to the

fact that L-T_3 is bound less firmly than thyroxine to plasma proteins, thus making it more readily available to the body cells. If L-T_3 has the same efficiency of absorption as L-T_4, then these data suggest that the plasma proteins that may migrate into the intestine and bind the thyroid hormones(8), bind them with equal intensity.

In a continuation of the study a second group of 38 male rats was observed to have a mean TSR of 1.65 ± .64 μg L-T_4/100 g bw by injection. When thyroprotein was injected, it required only a mean of 1.41 ± .56 μg L-T_4 equivalent (or 141 μg thyroprotein)/

TABLE I.

Group I: Biological Activity of L-Thyroxine and L-Triiodothyronine when Administered Orally and by Subcutaneous Injection.

No. of rats	Mean TSR* (μg/100 g bw) L-T_4	Range of TSR's (μg/100 g bw) L-T_4	Mean oral dose* (μg/100 g bw)	Range of oral doses (μg/100 g bw)	Mean % efficiency (as compared to inj L-T_4)	Range of % efficiency (as compared to inj L-T_4)	Hormone used
48	1.35 ± .53	1.25–1.75	3.56 ± .53	2.5 –4.0	37.9	30.0– 54.0	L-T_4
48	1.35 ± .53	1.25–1.75	1.41 ± .16	1.25–1.75	95.7	77.1–108.0	L-T_3

Group II: Biological Activity of Thyroprotein when Administered Orally and by Subcutaneous Injection.

No. of rats	Mean TSR* (μg/100 g bw) L-T_4	Range of TSR's (μg/100 g bw) L-T_4	Mean dose of thyroprotein* (L-T_4 equivalent in μg/100 g bw)	Range of doses of thyroprotein (L-T_4 equivalent in μg/100 g bw)	Mean % efficiency (as compared to inj L-T_4)	Range of % efficiency (as compared to inj L-T_4)	% Efficiency of oral thyroprotein as compared to inj thyroprotein	Route of administration of thyroprotein
39	1.65 ± .64	1.25–2.00	1.40 ± .56†	1.00–1.75	111.8	94.3–132.0		injected
39	1.65 ± .64	1.25–2.00	4.56 ± .69	3.0 –5.5	36.2	12.1– 65.8	30.7	oral

* ± S.D.

† Example: It took a mean of 1.65 μg L-T_4/100 g bw to determine normal TSR. When giving thyroprotein, doses were based on manufacturer's guarantee of 1% thyroxine. Here a dose of thyroprotein was given that had the equivalent of only 1.40 μg L-T_4 /100 g bw, but was as effective as 1.65 μg L-T_4/100 g bw. Thus, the thyroprotein was more potent than 1% thyroxine.

TABLE II. Effect of Oral Administration of Saline and Propylene Glycol on TSR.

Treatment	Control	Oral saline (.1 ml/100 g bw)	Oral propylene glycol and saline (a 50% solution of each) at .1 ml/100 g bw
No. of rats	30	30	30
Mean TSR ± S.D.	1.30 ± .30	1.38 ± .28	1.35 ± .26

100 g bw to inhibit thyroidal ^{131}I release. This indicates that the thyroprotein contained 11.8% more biological activity than was indicated by the manufacturer's guarantee of 1% L-T_4.

When thyroprotein was administered orally, the mean requirement was 4.56 ± .64 μg L-T_4 or 456 μg thyroprotein/100 g bw. In comparison with injected thyroprotein, the oral effectiveness was 30.7%, but in comparison with L-T_4 by injection, the oral effectiveness was 36.2%. It appears that the oral effectiveness of the thyroprotein is slightly decreased. This may be due to less efficient digestion of thyroprotein as compared to the absorption of the free amino acid, L-T_4.

While this study and previous observations indicate that thyroidal preparations are orally effective, quantitative data on the relationship has been lacking. The present study indicates that L-T_4 and L-T_3 are about 37% as effective orally as by injection and that thyroprotein is about 31% as effective. Since the oral effectiveness of thyroprotein is slightly less than L-T_4 or L-T_3, but exceeded its guaranteed potency of 1% L-T_4 by 11.8%, its percentage of oral effectiveness was about the same as L-T_4 or L-T_3.

The quantitative determination of the oral effectiveness of L-T_4, L-T_3 and thyroprotein in rats makes possible the administration of these hormones as a constituent of the ration rather than by injection. Since the individual animals vary from the mean, both in their thyroid secretion rate (TSR) and in feed consumption, it would not be possible to administer to each animal, in a feed, the exact amount of hormone required to equal their TSR. However, it would be possible to feed amounts which would be in their physiological range and would be equal to the TSR of the higher L-T_4 secretors.

In normal rats of this strain the TSR may range from .5 μg to 2.0 μg/100 g bw with a mean of about 1.0 μg. To equal 1 μg L-T_4/100 g bw, it would require 2.63 μg L-T_4 given orally in 5.7 g of feed (normal food intake per 100 g bw). Thus the incorporation of 461.3 μg L-T_4/kg of feed would provide the L-T_4 sufficient to equal the mean TSR. The incorporation of double this amount, 922.6 μg/kg would provide L-T_4 equal to the highest TSR.

If L-T_3 were to be fed, it would require 1.04 μg/100 g bw in 5.7 g of feed (or 182.4 μg/kg feed) to equal the mean TSR. By feeding double this amount, or 364.8 μg/kg feed it would provide L-T_3 equal to the highest TSR.

In the case of thyroprotein containing 1% L-T_4, 278 μg/100 g bw in 5.7 g of feed (or 48.8 mg/kg feed) would provide hormone equal to the mean TSR. By doubling this amount, 97.6 mg/kg feed, it would provide L-T_4 equal to 2.0 μg/100 g bw, the highest TSR observed in this strain of normal rats.

Summary. A comparison of the biological activity of L-T_4, L-T_3, and thyroprotein (a synthetic thyroactive iodinated casein guaranteed to contain 1% L-T_4), when administered orally and by subcutaneous injection, is reported. The comparative activity was measured by the thyroid secretion rate (TSR) technique using the same groups of Sprague-Dawley-Rolfsmeyer strain of male rats for each comparison. Using the subcutaneous administration of L-T_4 as a standard, it was shown that L-T_4 given orally was 37.9% as effective and L-T_3 was 95% as effective as L-T_4 on an equimolar basis when administered orally. Since L-T_3 is 2.6 times as active as L-T_4 by injection, its oral effectiveness would be 36.9%. Thyroprotein, on the basis of 1% L-T_4, was 36.2% effective orally.

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Oxidation of Infused Acetate to Body Pool CO_2. (31390)

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Experiments with constant infusion of C^{14}-sodium bicarbonate into the jugular vein of sheep indicated turnover time for body pool bicarbonate of about 30 minutes(4). The turnover time of infused C^{14}-acetate to expired CO_2 has been shown to be 60 minutes for 1-C^{14} and 120 minutes for 2-C^{14} labeled acetate(9). This indicates a turnover time of approximately 30 minutes from pool acetate to pool bicarbonate for 1-C^{14} labeled acetate.

The present experiments were planned to study the turnover time involved in the oxidation of pool acetate to pool CO_2 and its turnover to expired CO_2. By determining the turnover time from the acetate pool to the bicarbonate pool, a better indication of the actual oxidation rate of acetate is obtained, since the bicarbonate pool turnover time is eliminated. In addition, the distress to the animal involved in the collection of CO_2, via a tracheal cannula, is obviated. 1-C^{14} and 2-C^{14} labeled acetate were used in these experiments.

Methods. Four yearling wethers were used for 4 infusions of sodium acetate. 1-C^{14} or 2-C^{14} sodium acetate dissolved in physiological saline was infused by means of a constant infusion pump through a polyethylene catheter (I.D. 0.062″-O.D. 0.082″) inserted into right jugular vein. Blood samples were withdrawn through a catheter inserted in the left jugular vein.

The amount of CO_2 released by acid in whole blood was determined as previously reported(4). For radioactivity assay, the CO_2 released by acid was trapped in 2N sodium hydroxide and an aliquot removed and counted in the scintillation solution described by Harlane(3).

Expired CO_2 was collected from a tracheal cannula by the method of Sabine and Johnson (9) and its specific activity determined by the method of Oppermann *et al*(7).

Blood acetate was measured by the method of Ramsey(8) modified slightly as follows: to one volume of heparinized blood were added 2 volumes of water, one volume of 10% sodium tungstate and one volume of 0.66N sulphuric acid. The mixture was centrifuged to remove the protein, the supernatant made alkaline with potassium hydroxide and evaporated to dryness at about 60°C under reduced pressure. The residue was dissolved in one to two ml of water, made acidic with sulphuric acid, mixed with silicic acid and transferred quantitatively to the top of a silicic acid column, previously prepared. The column was eluted with benzene, chloroform and 1% tert-butanol in chloroform. Ten ml fractions were collected in scintillation vials, titrated with 0.01 N alcoholic potassium hydroxide in a stream of nitrogen and dried at 60-70°. The residue was dissolved in 1 ml of absolute ethanol, 14 ml of scintillation fluid (5g of 2.5 diphenyloxazole (PPO) per liter of toluene) were added and the solution counted in a Tricarb liquid scintillation counter.

The specific activity, turnover time, turn-