necessary to counteract growth-inhibition induced by injections of 1 mg of cortisone daily. These data also suggest that large amounts of thiamine have beneficial effects on cortisone-treated rats, apart from any effect on appetite. Rats given cortisone and thiamine, but limited in food intake to that of cortisone-injected, thiamine-deficient rats, at least survived and failed to show typical deficiency symptoms. Data reported by Schultz *et al.*(7) suggest that large doses of cortisone may also aggravate a pre-existent deficiency of pantothenic acid in rats.

Summary. (1) A total of 128 young male albino rats were used in 2 experiments to determine the effects of large doses of cortisone on thiamine requirements. They were fed a semi-synthetic ration from which thiamine was omitted for preliminary periods of either 10 or 18 days, followed by injections of 1 mg of cortisone daily with or without several levels of thiamine for another 18 or 12 days. (2) Pre-existent deficiency symptoms were aggravated by cortisone, resulting in decreased body growth and survival rate, priapism, cannibalism and loss of muscular coordination. The rats which were initially deficient in thiamine for the longer period, showed the most severe reactions to cortisone administration. Cortisone decreased the efficiency of converting food into body weight gains in all cases. (3) When 1 mg of thiamine per kilo of ration was fed, representing normal requirements for growing rats, there was little or no increase in body weight although most deficiency symptoms disappeared. When 5, 10 or 20 mg of thiamine per kilo of ration were fed, each level was equally effective in counteracting growth inhibition by cortisone.

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Calorigenic and Antigoitrogenic Actions of L-Triiodothyronine and L-Thyroxine in Thyroidectomized and Intact Rats.* (20520)

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3:5:3′-L-triiodothyronine has been reported to be 3 to 4 times as effective as L-thyroxine in preventing goitre development in thiouracil-treated rats(1). Also, in the treatment of 2 myxedematous patients, it appeared to be effective at doses lower than those required with thyroxine administration(2). On the contrary, Gemmill(3), using a small number of intact rats, has recently reported that the two agents produce quite similar calorigenic responses. This latter apparent discrepancy might possibly be the result of variability in the individual animal or strain, differences in experimental approach or in the type of animal preparation (intact or thyroidectomized) used. To further an understanding of these dissimilarities of response, a comparison has been made in intact and thyroidectomized rats with reference to calorigenic and antigoitrogenic potencies of these two agents.

Experimental. Male rats (Albino Farms), weighing between 125 and 175 g at the beginning of the experiment, were used. Purina Dog Chow Checkers and tap water were given *ad libitum* unless otherwise indicated. Both the triiodothyronine (3:5:3′-L-triiodothyronine) and thyroxine (L-thyroxine sodium pentahydrate) were given by subcutaneous injec-

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tion in a vehicle of 0.9% saline adjusted to pH 8.5. The concentration was such that each rat received 1 ml solution/kg/injection.

The rate of oxygen consumption of the individual rats was determined following a 24-hour fast, by measuring the oxygen consumed by each rat during three consecutive 15-minute periods. The expired carbon dioxide was collected over soda lime. Mean oxygen consumption of the three 15-minute periods was calculated, corrected to standard temperature and pressure, and converted to l/sqm/hr utilizing the Hill and Hill(4) formula of wt $^{2/3}$ x 10.

Thyroidectomies were performed surgically using ether anesthesia. Following surgery, the thyroidectomized rats were maintained on 1% calcium gluconate as the drinking fluid continuously, including the 24-hour fasting periods which preceded the oxygen consumption measurements. At least 30 days intervened between thyroidectomy and the first testing period. To assure completeness of extirpation, only those rats with an oxygen consumption of less than 5.5 l/sqm/hr were used. The pre-treatment mean oxygen consumption of the thyroidectomized rats was 4.7 l/sqm/hr (range 4.1 to 5.4 l/sqm/hr).

In the antigoitrogenic test, there were 8 groups of rats. One of these 8 groups served as a normal control and received tap water as the drinking fluid, while the other 7 groups received 0.1% thiouracil. Three of these 7 groups received daily injections of 1.25, 2.5, and 5.0 γ/kg/day of L-triiodothyronine, respectively; another 3 groups received 6, 12, and 24 γ/kg/day of L-thyroxine sodium pentahydrate, respectively; and the one remaining, which served as thiouracil-treated control, received injections of only the alkaline saline vehicle. Following 10 days of such treatment, the rats were sacrificed, and the thyroid glands were removed and weighed.

The calorigenic test in thyroidectomized rats consisted of measuring the response to these two compounds: 1) following chronic daily administration, and 2) after a single injection. For the former, 6 groups of 5 or 6 rats each were observed concurrently. One group was composed of intact control rats, and the other 5 groups consisted of thyroidectomized rats. One of these 5 groups remained untreated and served as the thyroidectomized control group. Following initial control observations, 4 of the 5 thyroidectomized groups were injected daily with 6, 12, 24, and 60 γ/kg/day of L-thyroxine sodium pentahydrate, respectively, for a 17-day period. This was followed, first, by a 17-day recovery period during which no drug was administered, and then a 17-day period of L-triiodothyronine administration at doses of 1.25, 2.5, 5.0 and 12.5 γ/kg/day respectively. The molar ratio between the respective doses of L-triiodothyronine and L-thyroxine sodium pentahydrate was maintained at 1:3.5 throughout all of these experiments. This ratio was chosen on the basis of the suggested activity ratio reported by Gross and Pitt-Rivers(1).

In studying the response to a single injection, 3 groups of 5 or 6 thyroidectomized rats per group were injected simultaneously at zero time with 1500 γ/kg of L-thyroxine sodium pentahydrate. Each group was observed at two different intervals during the ensuing 100-hr period so that the response was observed at about 4, 8, 24, 52, 75 and 98 hours after the time of administration of the drug. Nine days after the thyroxine administration, the rats were given a single injection of 312 γ/kg of L-triiodothyronine and tested at intervals similar to those following the thyroxine administration. The doses of both of these agents were 25 times larger than the highest doses used in the previously described study involving chronic daily administrations.

For the study in intact, non-thyroidectomized rats, a somewhat different procedure was employed. Here, the rats were injected for 3 days and the rate of oxygen consumption was determined on the fourth day. In this case, 4 groups of 6 rats each were observed concurrently. One was treated with only the alkaline saline vehicle, one with 0.1 mg/kg/day of L-thyroxine sodium pentahydrate, one with 0.073 mg/kg/day of L-triiodothyronine (molar ratio of triiodothyronine:thyroxine = 1:1), and one with 0.021 mg/kg/day of L-triiodothyronine (molar ratio of triiodothyronine:thyroxine = 1:3.5).

Results. The results of the antigoitrogenic test are shown graphically in Fig. 1. As judged by the relative thyroid weights, ex-

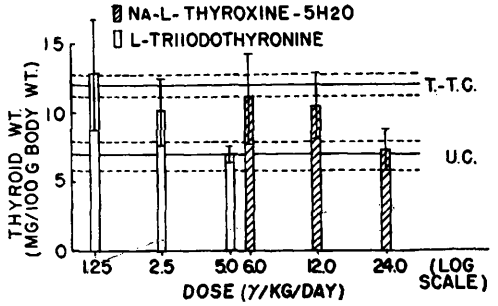

FIG. 1. Thyroid gland weights (mean ± S.D.) of thiouracil-treated rats given subcut. injections of 3 dose levels of either sodium-L-thyroxine pentahydrate or L-triiodothyronine. The mean (solid line) and stand. dev. (broken line) of the thyroid gland weights of both the untreated control (U.C.) and thiouracil-treated control (T.-T.C.) rats is given for reference purposes.

pressed as mg/100 g body weight, both the 1.25 γ of L-triiodothyronine and the 6 γ of L-thyroxine sodium pentahydrate failed to produce a significant change in weight of the thiouracil-induced goitre. On the contrary, the rats treated with the 5 γ dose of triiodothyronine, or the 24 γ dose of thyroxine sodium pentahydrate, had thyroid glands equivalent in weight to those of normal, untreated control rats. The goitrogenic effect of the thiouracil was, therefore, completely inhibited by these doses.

In Fig. 2 is shown the comparative mean change in oxygen consumption induced by the various respective doses of L-thyroxine sodium pentahydrate and L-triiodothyronine, when given chronically by daily injection. The values as plotted represent the mean change in oxygen consumption of the treated rats, using the mean oxygen consumption of thyroidectomized control rats as the baseline. The mean difference in oxygen consumption between the intact control rats and thyroidectomized control rats was 1.15 l/sqm/hr. Neither 6 γ of thyroxine sodium pentahydrate nor 1.25 γ of triiodothyronine elevated the oxygen consumption of the treated thyroidectomized rats to the level of the intact controls.

The rats with 2.5 γ of triiodothyronine reached the intact control level after 14 days of treatment, but those treated with 12 γ of thyroxine sodium pentahydrate attained this same level only by the seventeenth day. In the next higher dosage level, it was again evident that the animals treated with 5 γ of triiodothyronine reached the oxygen consumption level of the intact control rats earlier (10 days) than the ones treated with 24 γ of thyroxine sodium pentahydrate (14 days). The highest doses of both agents (12.5 γ of triiodothyronine and 60 γ of L-thyroxine sodium pentahydrate) quickly increased the oxygen consumption into the hyperthyroid range. Furthermore, with this 1:3.5 dosage ratio of triiodothyronine to thyroxine, the triiodothyronine produced a definitely greater response ($P = <0.05$ by the "t" test(5) for the 10th, 14th, and 17th days) than the thyroxine. These data indicate, therefore, that within the hypothyroid and euthyroid ranges, the molar

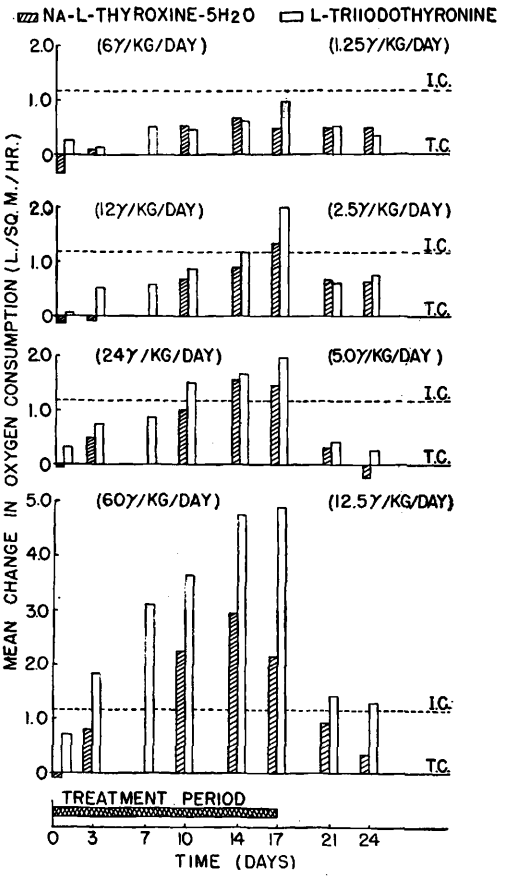

FIG. 2. Mean change in oxygen consumption induced by 4 dose levels of either sodium-L-thyroxine pentahydrate or L-triiodothyronine, when administered by daily inj. The mean oxygen consumption of each thyroidectomized control (T.C.) and intact control (I.C.) rats is given for reference purposes.

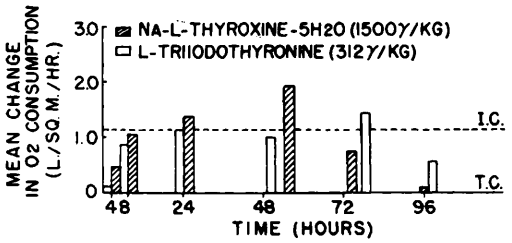

FIG. 3. Mean change in oxygen consumption induced by either sodium-L-thyroxine pentahydrate or L-triiodothyronine when administered by a single inj. at zero time.

potency ratio of L-triiodothyronine to L-thyroxine is close to 3.5:1, but that for the hyperthyroid range the ratio may be greater.

The calorigenic effect produced by a single injection of either agent is shown in Fig. 3. The response to 1500 γ/kg of L-thyroxine sodium pentahydrate and to 312 γ/kg of L-triiodothyronine was of a similar magnitude. The thyroxine gave a greater response 52-56 hours after the injection ($P = <0.05$ by the "t" test), but this relationship was reversed after about 75 hours.

Table I summarizes the results obtained when these two agents were injected into intact rats. The rats which received the triiodothyronine in a 1:1 molar dosage ratio of thyroxine sodium pentahydrate to triiodothyronine (Group 3) showed a mean oxygen consumption of 9.1 l/sqm/hr. This is not much different from the 8.4 l/sqm/hr ($P = <0.3; >0.2$) of the thyroxine-treated animals (Group 2). Furthermore, the mean oxygen consumption (8.0 l/sqm/hr) of rats treated with triiodothyronine at the 3.5:1 molar ratio of thyroxine sodium pentahydrate to triiodothyronine (Group 4), was only slightly less than that of the thyroxine-treated rats.

Discussion. The antigoitrogenic test indicates that, on a molar basis, triiodothyronine is 3½ times as potent as thyroxine. These results are in agreement with the original publication of Gross and Pitt-Rivers(1). Observations on the effect of these agents on oxygen consumption indicate that the respective calorigenic responses induced by these agents are again 3.5:1 when they are not above the euthyroid range, and therefore are in agreement with the results of the antigoitrogenic test. However, when the oxygen consumption rises to the hyperthyroid range, the activity ratio is greater than 3.5:1. Intact rats apparently react differently (quantitatively) to triiodothyronine than to thyroxine for the 1:1 molar dosage ratio of thyroxine to triiodothyronine gave results not too far different from the 3.5:1 ratio. The results obtained with the 1:1 dosage ratio confirm in part, the work reported by Gemmill(3) in which he stated that there was little difference in the response induced by the two agents in intact rats. However, since in the intact animal, neither dose of triiodothyronine, gave results too dissimilar from those produced by thyroxine, one must consider possible conversion of triiodothyronine to a less active derivative by the thyroid gland. In such a case measurements in the intact animal would not be a true indication of the inherent calorigenic potency of triiodothyronine itself. This latter possibility is in agreement with the suggestion of Roche(6) that triiodothyronine and thyroxine may both be synthesized by the thyroid gland. It is also in

TABLE I. Effect of L-Thyroxine Sodium Pentahydrate and 3:5:3'-L-Triiodothyronine on Oxygen Consumption of Intact Rats.

Group No.	Treatment	Molar dosage ratio*	No. of rats	Oxygen consumption† ($l/m^2/hr$)
1	Control		6	6.5 ± 0.4
2	L-thyroxine sodium pentahydrate (0.1 mg/kg/day)		6	8.4 ± 0.9
3	3:5:3'-L-triiodothyronine (0.073 mg/kg/day)	1:1	6	9.1 ± 1.0
4	3:5:3'-L-triiodothyronine (0.021 mg/kg/day)	3.5:1	6	8.0 ± 0.3

* Thyroxine : triiodothyronine.
† Mean ± S.D.

accord with the work of Keating and Albert (7) who state that following radioactive triiodothyronine treatment, there is a greater accumulation of I^{131} in the thyroid than after radioactive thyroxine treatment.

Summary. Antigoitrogenic tests in rats showed L-triiodothyronine to be about 3.5 times as active as L-thyroxine on a molar basis. In addition, in thyroidectomized rats, the L-triiodothyronine had a more potent calorigenic effect than L-thyroxine; in this case, the molar potency ratio was not less than 3.5:1. When the response was in the hyperthyroid range, the ratio was even greater than 3.5:1. Calorigenic tests in normal, intact rats showed a somewhat different effect in that both the triiodothyronine at a 1:1 molar dosage ratio and the triiodothyronine at 1:3.5 molar dosage ratio (triiodothyronine:thyroxine) produced changes not too far different from that obtained with the thyroxine.

The 3:5:3′-L-Triiodothyronine used in this study was prepared by Glaxo Laboratories Ltd., England.

We thank Miss Mary F. Sax for performing the surgical thyroidectomies, and Mr. Alfred Boris for his valuable technical assistance throughout the entire course of this study.

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Dextran, an Antigen in Man.* (20521)

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Kabat and Berg have recently reported(1)† that various dextran preparations are antigenic in man. Quantitative immunochemical studies were presented on the reaction of various native and partially hydrolized dextrans with homologous human antidextran sera. As the implications of these original findings to the use of dextran as a blood substitute are manifold, it was agreed to have the results checked independently in another laboratory. Dextrans have been shown to cross react with types II, XII, and XX horse and rabbit antipneumococcal sera(2-4). Therefore, it is conceivable that the individuals who had high concentrations of precipitins and showed skin sensitivity to dextran may have produced antibodies to a cross reacting pneumococcal polysaccharide. A complete analysis for antibodies to these polysaccharides was performed in both the pre- and post-immunization sera.

The persistence of antibodies to many pneumococcal polysaccharides has been shown by Heidelberger, *et al.*(5-8). However, the pneumococcal polysaccharides(9), in contrast to the dextrans(10,11), are not metabolizible and may persist for some time. It was of great interest, therefore, to see what the concentration of antibodies against a metabolizible polysaccharide would be a year after the antigen was injected.

Experimental dextrans. Five samples of native dextran were used. Three of these, B 1254, B 742, and B 512, were obtained from the Northern Regional Research Laboratory, U. S. Department of Agriculture, Peoria, Ill.

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