Comparative Methods for Determining Biological Half-Life ($t_{1/2}$) of L-Thyroxine in Normal, Throidectomized and Methimazole Treated Female Rats.* (30550)

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The biological half-life ($t_{1/2}$) of a hormone refers to the time required for the disappearance from blood of ½ of the hormone present at any one time. Maintenance of a constant level of hormone in the blood depends upon the rate of its secretion and the $t_{1/2}$ in the blood. If the $t_{1/2}$ is rapid, then the secretion rate must be rapid to compensate for the rapid passage of hormone from the blood to the body tissue.

In this laboratory, the thyroxine secretion rate (TSR) of a number of groups of Sprague-Dawley-Rolfsmeyer female rats has been determined(1,2). The purpose of the present study was to determine the short term effects of thyroidectomy as well as the effects of comparatively long term 1-methyl-1-2-mercapto-imidazole (methimazole) treatment on the $t_{1/2}$ of L-thyroxine-I^{131} (L-T_4-I^{131}) in the rat. The effects of route of administration of the L-T_4-I^{131} on the $t_{1/2}$ was compared also in 2 control groups of rats.

Materials and methods. Female Sprague-Dawley-Rolfsmeyer rats weighing approximately 240 g were used throughout these studies. One group of 23 animals was surgically thyroidectomized and injected subcutaneously with 1.0 μg L-T_4/100 g bw/day which was the approximate mean TSR of the group. A second group of 19 animals which was also thyroidectomized received no L-T_4 replacement therapy. The $t_{1/2}$ of L-T_4-I^{131} was determined in these 2 groups of rats 7 days following thyroidectomy. The procedure was to inject 15 μc of L-T_4-I^{131} subcutaneously.

Twelve hours were allowed for absorption of the L-T_4-I^{131} after which time 0.1 ml blood samples were obtained intracardially. Blood samples were taken thereafter at 12-hour intervals for 72 hours.

In the second experiment using rats with intact thyroids, 10 rats were held as controls and 10 rats were injected subcutaneously with methimazole at a level of 400 μg/100 g bw/day for 30 days. This level of methimazole has been shown to be effective in blocking thyroidal-I^{131} uptake in the rat(3).

At the end of the 30-day period of methimazole administration, the $t_{1/2}$ of L-T_4-I^{131} was determined in these groups of rats. The procedure was to inject 15 μc of L-T_4-I^{131} intracardially, and the first blood sample was obtained intracardially 4 hours later. Thereafter, blood samples were collected at 8-hour intervals for 32 hours.

All blood samples were counted in a scintillation well-type detector, using a Berkley scaler Model 2001.

Results. In the first group of rats which was thyroidectomized, L-T_4 at the level of 1.0 μg/100 g bw was administered to maintain them uniformly at approximately their mean normal thyroid secretion rate (TSR). The mean $t_{1/2}$ of L-T_4-I^{131} of this group was 17.8 $\pm$ 1.1 hours. In comparison the mean $t_{1/2}$ of the group of thyroidectomized rats which received no thyroxine replacement therapy was 23.7 $\pm$ 0.7 hours (Table I). This is a difference of 5.9 hours which is statistically significant ($P<.005$). In both groups the $t_{1/2}$ was determined 7 days following thyroidectomy, and the L-T_4-I^{131} was administered subcutaneously allowing 12 hours for absorption into the blood.

In the second experiment, similar female rats with intact thyroid glands were divided into 2 groups of 10 rats each. The group which was kept as controls had a mean $t_{1/2}$ of L-T_4-I^{131} of 19.5 $\pm$ 2.2 hours as compared to a mean of 18.5 $\pm$ 2.1 hours for the experimental group which received 400 μg of methimazole/100 g bw/day for 30 days

* Contribution from Missouri Agri. Exp. Station Journal Series No. 2847. Approved by Director.

† Supported in part by grant from U. S. Atomic Energy Commission Contract AT(11-1)-301-104.

TABLE I. Effect of Thyroidectomy and Methimazole on the t½ of Thyroxine in Female Rats.

Treatment	No. of animals	Body wt, g	t½ hr
Thyroidectomized (L-T_4/1.0 μg/100 g bw)	23	240	17.8 ± 1.1
" (no replacement)	19	240	23.7 ± .7*
Controls, normal	10	232	19.5 ± 2.2
Methimazole 30 days (400 μg/100 g bw/day)	10	237	18.5 ± 2.1

* Difference from group given L-T_4/1.0 μg/100 g bw statistically highly significant ($P<.005$).

(Table I). This is not a significant difference. These groups of rats were injected with the L-T_4-I^{131} intracardially, and collection of blood samples was begun 4 hours later. It is felt to be noteworthy that the t½'s of the 2 control groups did not differ significantly utilizing the different procedures for these determinations.

Discussion. Feldman(4) reported the t½ of L-T_4 in a group of 7 male rats (strain not indicated) to be 18.2 hours and after 5 weeks on an iodine deficient diet, the t½ was 18.6 hours. The t½ of L-triiodothyronine (L-T_3) in a similar group was 11.0 hours in controls and 10.3 hours in iodine deficient rats.

Pittman *et al*(5) reported the t½ of L-T_4 in thyroidectomized Sprague-Dawley rats (sex not indicated), maintained on 1.5 μg L-T_4/100 g bw, to be 16.6 ± 1.5 hours.

The results of the present experiment are in good agreement with these reports. It is interesting to note that the thyroidectomized rats of this study maintained in 1.0 μg of L-T_4 had a t½ of L-T_4-I^{131}, 1.2 hours longer than those reported by Pittman *et al*(5) which were maintained on 1.5 μg of L-T_4. Although this is certainly not a significant difference, it is in conformity with other observations which indicate that increasing hyperthyroidism tends to decrease the t½ of L-T_4(6). This would certainly be plausible since the effect of short term but acute hyperthyroidism due to thyroidectomy increased the t½ of L-T_4-I^{131} to 23.7 hours in this experiment. From this study it appears that in the case of the thyroidectomized rat the general metabolic pattern is such that the rate at which L-T_4-I^{131} leaves the blood is somewhat inhibited regardless of the fact that peripheral tissues may well be semi-starved for thyroid hormones.

In the second phase of this study, it is interesting that the effect of hypothyroidism of inhibiting the rate at which L-T_4 leaves the blood was not obtained in methimazole treated rats. Two possible explanations can be presented. First, it is possible that minute amounts of thyroid hormone are synthesized in the presence of this level of methimazole although thyroidal-I^{131} uptake is acutely depressed and thyroid weight is increased by 98.7% by this 30-day treatment(3). If so, then very small amounts of thyroid hormone are able to support a sufficiently normal metabolic pattern to maintain a comparatively normal t½ of L-T_4-I^{131}. Secondly, and more plausible, it is possible that after this comparatively long period of hypothyroidism the peripheral tissue is so starved for hormone that there is an excess of tissue binding sites available for thyroid hormone. Therefore, regardless of the depressed metabolic pattern, thyroid hormone leaves the blood at a normal control rate. In other words, the tissue lack of thyroid hormone allows the hormone to leave the blood more readily thereby compensating for the depressed metabolic rate due to the hypothyroidism.

It is important to note that administration of 0.0125% methimazole drinking water for 2 weeks did not alter the excretion of radioactivity in the urine or feces of rats injected with 1 μc of L-T_4-I^{131} during two 24-hour periods in comparison to control rats(7). Since the use of a goitrogen is often deemed necessary to prevent the recycling of I^{131} by the thyroid in experiments where one is determining the TSR, it appears that methimazole is much more acceptable than other goitrogens such as thiouracil, propylthiouracil, and methylthiouracil which interfere with the deiodination and extrathyroidal metabolism of thyroid hormone(8,9,10,11,12,13,14).

It is apparent also that the rate of absorp-

tion of L-T_4-I^{131} from subcutaneous injection is relatively rapid in the rat. On the basis that the thyroidectomized rats which received TSR levels of replacement thyroxine were comparable to those controls with intact thyroids, it appears that route of administration of the L-T_4-I^{131} had no effect on the $t\frac{1}{2}$. However, it is, of course, necessary that absorption of the L-T_4-I^{131} be essentially complete prior to utilization of a blood sample for determination of the $t\frac{1}{2}$. By plotting the radioactive counts of the blood samples as a per cent of injected dose on semilog paper, it was found that the rate of disappearance of the L-T_4-I^{131} remained linear after allowing 12 hours for absorption of the L-T_4-I^{131}.

Summary. Data on the biological half-life ($t\frac{1}{2}$) of L-thyroxine-I^{131} (L-T_4-I^{131}) in thyroidectomized, thyroidectomized thyroxine replaced, normal and methimazole treated female rats are presented. Rats thyroidectomized for 7 days had a significantly longer mean $t\frac{1}{2}$ of L-T_4-I^{131} ($P<.005$) of 23.7 $\pm$ 0.7 hours than thyroidectomized rats which received a mean TSR level of replacement thyroxine of 1.0 μg L-T_4/100 g bw and had a mean $t\frac{1}{2}$ of 17.8 $\pm$ 1.1 hours. These thyroxine replaced rats did not differ from intact controls which had a mean $t\frac{1}{2}$ of 19.5 $\pm$ 2.2 hours. Hypothyroidism induced by subcutaneous injection of 400 μg methimazole/100 g bw/day for 30 days had no effect on the $t\frac{1}{2}$ of L-T_4-I^{131} as shown by a mean $t\frac{1}{2}$ of 18.5 $\pm$ 2.1 hours. It was shown also that administration of the tracer dose of L-T_4-I^{131} could be made by either subcutaneous or intracardial injection without effect on the $t\frac{1}{2}$ if proper intervals were maintained between time of injection and collection of the initial blood sample.

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Received November 9, 1964. P.S.E.B.M., 1965, v120.

Determination of Carbonic Anhydrase in the Epiphysis of Endochondral Bone.* (30551)

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There is some evidence that carbonic anhydrase (CA) plays an important role in the calcification process in invertebrates. Among other studies, Wilbur and Jodrey(1) showed that acetazolamide, a CA inhibitor, would decrease the rate of calcium deposition in the mantle shell of the oyster. Goreau(2) likewise showed that similar reactions occurred in coral after CA inhibition with acetazolamide.

Among the vertebrates, Scott *et al*(3) and Benesch *et al*(4) have found that egg shell formation in hens is inhibited by unsubstituted sulfonamides. Little evidence is pre-

* Supported by Nat. Inst. of Health Grant NB-01297.