Effect of Experimentally Altered Thyroid States on the *In Vivo* Labeling of Red Blood Cells in the Rat

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Introduction

The *in vivo* procedure for labeling red blood cells with technetium-99m (Tc-99m) involves the intravenous injection of a reducing agent, such as stannous ion, followed by the intravenous injection of sodium [Tc-99m]pertechnetate (9). It is thought that Tc-99m enters the red blood cells, is reduced by the stannous ion present there, and binds to intracellular constituents (2,7).

Alteration of thyroid function may cause disruption of the red blood cell labeling process. Hypothyroidism and hyperthyroidism have been shown to produce changes in protein metabolism (10), which could affect the transport of pertechnetate since it is 65-80% bound to proteins (5). Hypothyroidism reduces the level of 2, 3-diphosphoglycerate in the blood and therefore its oxygen tension (3) which could change the amount of tin in the stannous ion state in the blood (8). The labeling of red blood cells could thus be affected. This study was performed to determine the effect of experimentally altered thyroid states on the efficiency of the labeling of red blood cells in rats using the *in vivo* technique.

Materials and Methods

Male Sprague-Dawley descendent rats (Laboratory Supply Co., Indianapolis, Indiana) with a weight range of 185-250 g were used. They were housed five to a cage by random assignment and were given free access to food and water. A 1-day period of acclimation was allowed before the experiment began.

A state of hypothyroidism was induced with propylthiouracil (PTU). The dosing solution was made fresh daily to contain 2 mg PTU/0.5 ml/rat. A state of hyperthyroidism was induced with L-thyroxine sodium pentahydrate (T4). The dosing solution was made fresh daily to contain 20 μg T4/0.5 ml/rat. Euthyroidism was maintained with 0.5 ml of 0.9% saline per rat daily. The drug pretreatments were administered by intraperitoneal injection and continued for 17 days (6).

Sodium [Tc-99m]pertechnetate was obtained daily from a commercial Mo-99/Tc-99m generator (Mallinckrodt, Inc., St. Louis, Missouri). The eluate was diluted with oxygen-free 0.9% saline to provide about 15 μCi of activity per milliliter. The volume injected into each rat was 0.5 ml, yielding an injected dose of about 7.5 μCi. An aliquot of the dosing solution was prepared for a standard so that the total activity injected into each rat could be accurately determined.

Stannous chloride solution was prepared daily by dissolving stannous chloride dihydrate in 16.7 ml of oxygen-free 6 *M* HCl and then adding sufficient oxygen-free doubly distilled water to make the total volume 100 ml. Immediately before use 0.1 ml of this solution was diluted to 25 ml with oxygen-free acidic (pH approximately 3) saline and then passed through a 0.22-μm cellulose filter to yield a solution of 42.1 μg Sn^{++} per milliliter. Oxygen purging was achieved by bubbling the solutions with nitrogen gas. The volume to be injected was based on rat weight so as to yield 50 μg Sn^{++} per kilogram of body weight.

The three treatment groups, hypothyroid, euthyroid, and hyperthyroid, each

contained 30 rats. Each treatment group was subdivided for five time periods, 0.5, 1, 2, 4, and 8 hours, each subgroup containing 6 rats. Initiation of the thyroid state induction treatment was timed so that five rats, one from each time period and one or two from each treatment group, completed the 17-day regimen simultaneously. The statistical design was a nonorthogonal, incomplete block design based on a pseudo-factorial construction (1).

On the 18th day, each of these five rats was given an intravenous injection of stannous chloride solution followed 25 min later by an intravenous injection of sodium [Tc-99m]pertechnetate solution, both injections being made into a tail vein. After the designated time period, each rat was anesthetized with ether, its chest was cut open, and blood was drained from its heart using a heparinized evacuated tube. Three milliliters of this blood was centrifuged at 1800 rpm for 5 min, the plasma layer was drawn off, and the erythrocytes were washed twice with 4 ml of 0.9% saline.

Weighed aliquots of whole blood and red blood cells as well as the standard prepared from the dosing solution were counted in a small well-type NaI(Tl) scintillation counter. The counting error did not exceed 1% at the 95% confidence level.

Results and Discussion

The percentages of the total activity injected per gram for whole blood and washed red blood cells were calculated using the activity present in the aliquots of these tissues and the activity present in the standard prepared from the solution injected. These percentages are shown in Table 1. Only 79 animals out of the original 90 were used to provide data for analysis. Of the 11 unused rats, one died of unknown causes during the labeling procedure, one was eliminated from the study due to its being unmanageable, and nine were discarded due to improper injection. The data were tested and found to be homogeneous ($P < 0.025$). A three way analysis of variance was then run to test for time, treatment, and blocking effects, all of which were found not to be significant ($P > 0.05$) (1).

TABLE 1. *Percentage of total Tc-99m activity injected per gram of tissue.*[a]

Time (hr)	Hypothyroid	Euthyroid	Hyperthyroid
	Whole Blood		
0.5	3.8 ± 0.7[b]	4.2 ± 0.8[b]	4.0 ± 0.5
1	4.0 ± 0.6	4.5 ± 0.4[b]	3.7 ± 0.7[b]
2	3.9 ± 0.4	3.5 ± 0.7[b]	3.6 ± 0.7[b]
4	4.0 ± 0.4[b]	3.3 ± 0.5[b]	3.6 ± 0.4
8	3.2 ± 0.5[c]	3.5 ± 0.8[b]	3.2 ± 0.2
	Red blood cells		
0.5	7.4 ± 1.8[b]	8.1 ± 1.5[b]	7.5 ± 1.2
1	8.0 ± 0.8	8.6 ± 1.0[b]	6.9 ± 1.3[b]
2	7.6 ± 0.5	6.8 ± 1.5[b]	6.6 ± 1.4[b]
4	7.4 ± 0.6[b]	6.3 ± 1.2[b]	6.7 ± 0.6
8	5.8 ± 0.7[c]	6.7 ± 1.2[b]	6.0 ± 0.3

[a]Mean ± standard deviation for six animals unless otherwise noted.

[b]Mean for five animals.

[c]Mean for four animals.

Since the blocking effect was not significant and since the statistical design assumed that there were no block-by-time, block-by-treatment, or block-by-time-by-treatment interactions, the data could be pooled across blocks and examined using a two way analysis of variance. In this analysis, the treatment effect remained insignificant but the effect of time became significant for both the whole blood and the red blood cells.

A Newman-Keuls test was run to find where significant differences ($P < 0.05$) occurred. The results are shown in Table 2. Although few significant differences are seen, there is a trend for the later time groups to have lower amounts of Tc-99m in both the whole blood and the red blood cells. This finding was not unexpected since the labeling of red blood cells with Tc-99m is known to degrade over time with a reported half-time of 19.5 hr (4). More interesting than the decrease in Tc-99m activity with time is the fact the decrease follows a similar pattern for all treatment groups, indicating that the altered thyroid states do not affect the rate of labeling or the rate of degradation of red blood cells.

The results of this study indicate that the experimentally altered thyroid states in the rat do not affect the labeling of red blood cells. If these states in the rat are physiologically identical to clinical hypothyroidism and hyperthyroidism in humans, the clinician need not be concerned with interference in the *in vivo* red blood cell labeling process due to altered thyroid state. Statistically identical labeling efficiencies can be expected in the hypothyroid, euthyroid, and hyperthyroid

TABLE 2. *Newman-Keuls multiple comparison of the treatment means.*

Treatment	Percentage of total Tc-99m injected per gram of tissue[a]				
	Whole blood				
Hypothyroid	1[a]	4	2	0.5	8
	4.0	4.0	3.9	3.8	3.2
Euthyroid	1	0.5	8	2	4
	4.5	4.2	3.5	3.5	3.3
Hyperthyroid	0.5	1	4	2	8
	4.0	3.7	3.6	3.6	3.2
	Red blood cells				
Hypothyroid	1	2	4	0.5	8
	8.0	7.6	7.4	7.4	5.8
Euthyroid	1	0.5	2	8	4
	8.6	8.1	6.8	6.7	6.3
Hyperthyroid	0.5	1	4	2	8
	7.5	6.9	6.6	6.6	5.9

[a]The designations represent the time intervals after injection of Tc-99m at which the rats were sacrificed (0.5, 1, 2, 4, and 8 hr). They are arranged in order of decreasing magnitude from left to right. Those underlined are not significantly different at $P > 0.05$ level. The mean value for each time is shown.

patient. If unusual labeling efficiencies occur in hypothyroid or hyperthyroid patients, the clinician should look elsewhere for a causal factor.

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