

Disappearance Rate of Endogenously Radioiodinated Thyroglobulin and Thyroxine after Radioiodine Treatment

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Endogenously radioiodinated thyroglobulin (tg) and thyroxine (T_4), in the circulation following, therapeutic doses of radioiodine were studied in five patients with differentiated thyroid carcinoma. Radioactive serum was fractionated on a Sephadex G-200 and the various radioactive peaks thus obtained were analyzed for their biochemical and immunologic characteristics using precipitation with trichloroacetic acid (TCA) and antithyroglobulin antibody extraction with acidified n-butanol and electrophoresis on agar gel. The disappearance rates of endogenously labelled tg and T_4 were 3.12 ± 0.396 days and 10.14 ± 1.81 days, respectively.

Cancer 49:2281-2284, 1982.

IT IS AN ESTABLISHED FACT that iodoproteins in the circulation, including iodinated tg, are released after radioiodine treatment in patients with differentiated thyroid carcinoma.¹⁻⁴ The various proteins such as tg, thyroxine binding globulin TBG, iodoalbumin, and/or an unknown protein X, whose biologic function is as yet poorly understood, have been studied in these sera; biophysical and biochemical methods, such as electrophoresis, salting out, and immunoreactivity, have been used. However, the physiologic significance and the amount of functioning mass required to produce certain definite amounts of tg remain unclear. Moreover, the biologic half-life of tg in the circulation appears to be at variance.^{5,6} Lo Gerfo *et al.* have shown that the post-operative immunoreactive tg disappears from the circulation, with an average half-life of 14 hours in patients with thyroid carcinoma and no metastases.⁶ In contrast, studies of patients with disease other than thyroid carcinoma have shown serum tg as a multicomponent system with a varying biologic half-life.⁵

In both the above-mentioned studies, the biologic half-life of tg in circulation was estimated using radioimmunoassay (RIA) after thyroidectomy. It was therefore of interest to study the disappearance rate of endogenously labelled tg and T_4 in thyroid carcinoma patients who had undergone thyroidectomy but had

functioning metastatic mass and had received therapeutic doses of radioiodine.

Materials and Methods

Study Subjects

Five patients with thyroid carcinoma who had undergone thyroidectomy but had functioning metastases, as evidenced by ^{131}I scintiscan, were studied. The histologic diagnosis, metastatic lesion, and previous ^{131}I therapy of these patients are shown in Table 1.

Blood specimens were drawn at different time intervals after radioiodine therapy for a period of 10-22 days, depending on the amount of radioactivity in circulation.

Fractionation of Patient's Serum on Sephadex G-200 Column

One-half milliliter of serum was chromatographed on a 100×2.5 cm Sephadex G-200 column (Pharmacia, Uppsala, Sweden) using phosphate-buffered saline (PBS) sodium phosphate 0.0035 mol/liter, NaCl 0.15 mol/liter with NaN_3 , 0.2 g/liter as an eluent. An LKB 7000 Ultrorac fraction collector was used to collect 1-ml fractions and each milliliter was counted for radioactivity with gamma ray spectrometer.

Characterization of Radioactive Peaks

The three radioactive peaks (A, B, and C) obtained by chromatography (Fig. 1) were subjected to precipitation with trichloroacetic acid (TCA) and antithyroglobulin antibody, extraction with acidified n-butanol

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Accepted for publication March 23, 1981.

TABLE 1. Histologic Diagnosis and Therapeutic Data for Thyroid Cancer Patients

Patient	Histologic diagnosis	Metastases	Dose of ^{131}I in mCi	Amount of previous ^{131}I therapy
N	Follicular carcinoma	Lymph node	100	1 (90 mCi ^{131}I)
M	Follicular carcinoma	Skull	100	—
K	Follicular and papillary carcinoma	Lymph node	230	2 (110 + 250 mCi ^{131}I)
S	Follicular carcinoma	Sternum	140	—
P	Follicular carcinoma	Sternum	100	—

and electrophoresis on agar gel for identification of iodine linkage.

Percent Radioiodinated tg in the Sample

The percent of radioiodinated tg (peak A) present in the sample was calculated as:

Percent activity with peak A

$$= \frac{\text{Radioactive counts under peak A}}{\text{Total counts in all three peaks}} \times 100.$$

Biologic Half-Life of Radioiodinated tg

One milliliter of serum of all the samples collected on various days from the same patient after radioiodine therapy were counted on the same day to take care of the physical decay correction. Absolute radioactive counts associated with tg in each sample were then calculated by multiplying the total counts/ml of serum with percentage radioactivity of thyroglobulin and dividing by 100. The counts thus obtained were plotted against the day on which the sample was collected. The

slope of the declining phase of radioactivity was taken for the determination of biologic half-life.

Biologic Half-Life of Radioactive Thyroxine (T_4)

Acidified n-butanol was used to extract thyroxine from the serum of patients treated with radioiodine; 0.25 ml of serum was diluted to 1 ml with normal saline and counted for radioactivity. This was then extracted three times successively with 3 ml of acidified n-butanol. The organic phase was pooled, made to a volume, washed with alkali (5% Na_2CO_3 in 4N NaOH) and 1 ml of alkali washed organic phase was counted for radioactivity (mainly T_4 and a small amount of T_3). Percent radioactivity with thyroxine was then calculated from the original serum counts (it is assumed that the contribution of T_3 to BEI is negligible in comparison with T_4 , as the amount of T_4 in circulation is much higher than T_3). Absolute counts associated with T_4 in the serum were derived, as with the case of tg, and the values obtained were plotted as a function of time for calculation of biologic half-life.

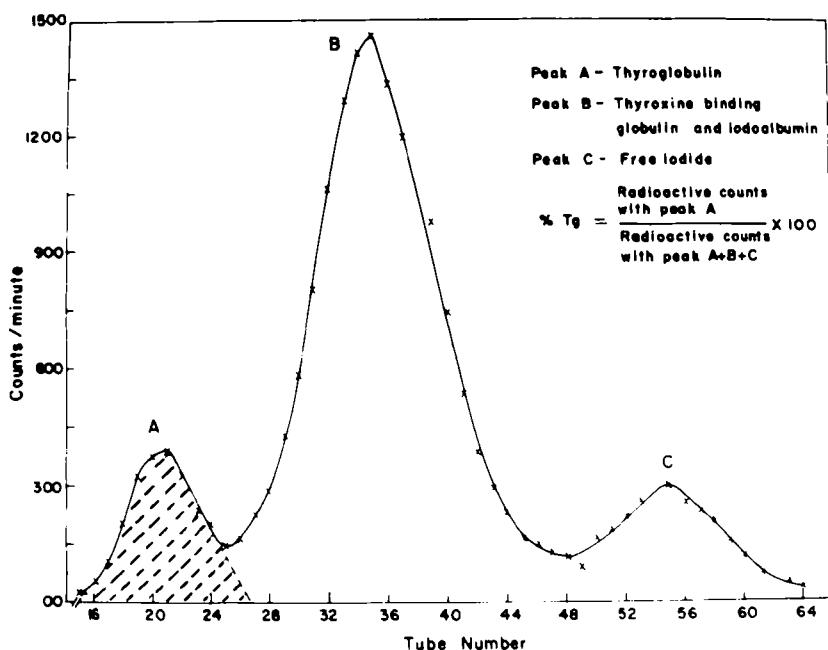


FIG. 1. Chromatographic separation of radioactive serum on Sephadex G-200. The void volume peak (Peak A) is shown by shaded area.

Results

Characterization of Radioactive Peaks Obtained by Molecular Sieve Chromatography

The first radioactive peak obtained on chromatography (peak A) was precipitable with TCA and also by anti-tg antibody, indicating the protein nature and the reactivity of tg. Radioiodine with this protein could not be extracted with butanol (NBEI), indicating that the binding of radioiodine is covalent in nature. Electrophoretic mobility of this protein in agar gel was between the α_1 - α_2 region.

Peak B radioactivity also was precipitable with TCA. About 60–80% of the radioactivity in this peak could be extracted with acidified n-butanol (BEI), indicating the presence of thyroid hormones and that the remaining non-extractable radioactivity (NBEI) could be iodalbumin or an unidentified protein X.

The last radioactive peak (peak C) was mainly composed of free iodide.

Biologic Half-Lives of Radioiodinated tg and Radioactive T_4

Radioiodinated tg in the circulation initially rose in all patients studied, reached a maximum value (the period varied from patient to patient), and then slowly disappeared as a function of time. The disappearance curve of radioiodinated tg in the circulation of one of the patients who received 90 mCi of radioiodine is depicted in Figure 2. The radioiodinated tg reached a maximum value on the eighth day after radioiodine therapy, and then disappeared; it had a biologic half-life of three days. The day on which the maximum amount of tg appeared in circulation varied between 2–8 days in the five patients studied. Although the attainment of maximum activity varied, the disappearance rate of circulating radioiodinated tg was fairly constant (2.8–3.8 days) in the five patients studied, irrespective of amount of tg in the circulation (Table 2).

The variation in the level of radioactive T_4 in the same patient (P) is depicted in Figure 2 (dotted line). On the eighth day, the release of radioactive T_4 also reached a maximum value similar to that of radioiodinated tg. In all five cases studied, radioactive T_4 rose to a maximum along with radioiodinated tg, and then declined. The disappearance rate of radioactive T_4 , however, varied between 7.2–12.2 days with a mean of 10.14 ± 1.81 days (Table 2).

Discussion

Quantitation of serum tg in patients with differentiated thyroid carcinoma is now considered a true bio-

TABLE 2. Biologic Half-Life of Radioiodinated tg and Radioactive T_4 of Thyroid Carcinoma Patients Studied

S No.	Biologic half-life	
	Radioiodinated tg	Radioactive T_4
1	3.8	12.2
2	2.8	10.5
3	3.0	10.5
4	3.1	7.2
5	2.9	10.3
Mean	3.12 ± 0.396	10.14 ± 1.81

chemical marker for the presence of metastases or recurrence of disease after treatment.^{7–11} However, very little is known about its biologic half-life, metabolic fate, and physiologic significance in circulation. The data presented in this paper indicate that the biologic half-life of endogenously labelled tg is between 2.8–3.8 days with a mean of 3.1 ± 0.39 days, which is in agreement with that reported by Ulla-feldt *et al.* using RIA procedure.⁵ They have shown the presence of different

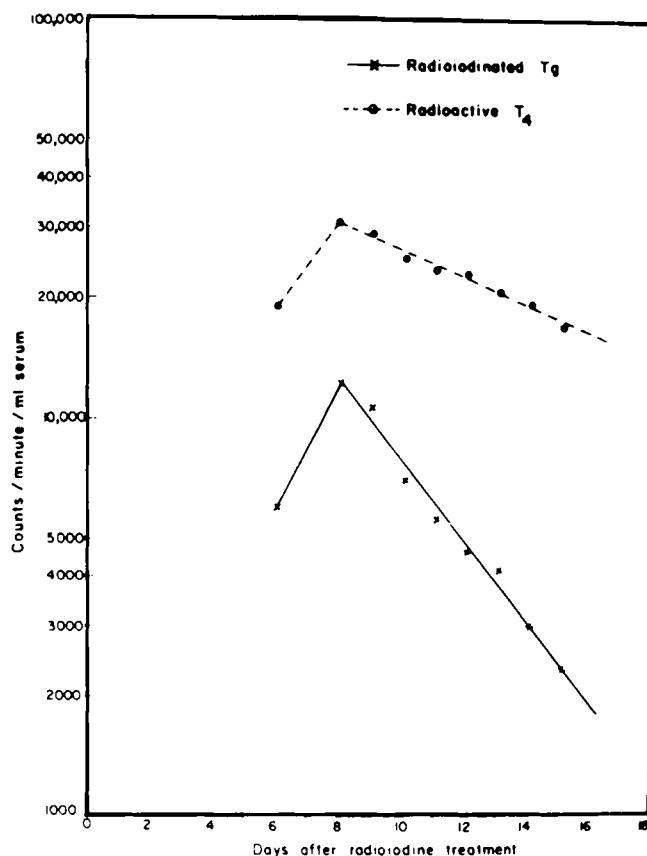


FIG. 2. A typical graph showing the biologic half-life of radioiodinated tg and radioactive T_4 . The experimental procedure is described in the text.

molecular weight tg in the circulation, of which a larger component—19 S tg—disappeared with a biologic half-life of 3.7 ± 0.9 days and smaller molecules disappeared with a biologic half-life of 4.3 ± 2.1 hours. In our study, we followed the first radioactive peak, eluted near the void volume, which is believed to be predominantly the 19 S type. Moreover, it is assumed that during the declining phase of tg level, radioiodination of newly synthesized tg, if any, would be negligible for these reasons: (1) the amount of radioiodine in serum from the initial administered dose would be negligible by 72 hours and thus its contribution in radioiodination of tg is expected to be insignificant; (2) the free radioiodine derived from metabolism of iodoaminoacids and iodoproteins also would not make a significant contribution in radioiodination of newly synthesized tg.

Lo Gerfo *et al.*, in contrast, have reported that the biologic half-life of tg in patients with thyroid carcinoma without metastases is 14 hours.⁶ One of the possibilities could be the lower molecular weight (s) of tg released into circulation after thyroidectomy, which might bring down the biologic half-life of total tg in the circulation as the biologic half-life of lower molecular weight tg is as low as 4.3 ± 2.1 hours.⁵ The other possibility could be the lower sialic acid content of tg in patients with thyroid carcinoma. It has been shown that the sialic acid content of tg from a rat thyroid cancer is low and also that asialo glycoproteins are removed at a much faster rate than that of their native forms.^{12,13} Also, in our experience with animal studies, native tg disappeared with a biologic half-life of 4 hours, whereas the desialylated tg disappeared with a biologic half-life of 5 minutes when injected intravenously into a rat (unpublished data). The sialic acid content does not appear to be the cause of discrepancy, as our patients also had carcinoma of the thyroid with metastases. Thus, the discrepancy in biologic half-life of tg may be because of the presence of tg of various molecular weights in the circulation after thyroidectomy and the heteroassay system of RIA or hitherto unknown factors.

The circulating radioiodinated tg reached a maximum level between 2–8 days in different patients studied. This could depend on the amount of radiation dose delivered to the tumor mass, type of tumor, histology, and/or some unknown factors present. The disappearance of radioiodinated tg does not necessarily mean absence of functioning tumor mass. Serial determination of serum tg by RIA is therefore required to monitor the efficacy of radioiodine treatment in these patients.

Both radioiodinated tg and radioactive T₄ showed a parallel rise—they reached a peak at the same time but disappeared with different biologic half-lives. The biologic half-life of T₄ (10.14 ± 1.81 days) obtained in this study appears to be slightly but significantly higher than that reported in the literature.¹⁴ Moreover, the time

period of increase, attainment of peak, and decline thereafter coincided with that of radioiodinated tg (Fig. 2). If thyroid is the only source of T₄ production, then T₄ production would have declined at a normal biologic half-life of 6–8 days after a maximum release is obtained. The increased biologic half-life of T₄ in these patients could be due to extra thyroidal synthesis, possibly from tg circulation during its metabolism. These data support the hypothesis formulated by Daniel *et al.* that because of the intrinsic thyroxine (T₄ and T₃) content of tg, metabolic effects may occur in the peripheral tissue.¹⁵ Brown and Jackson also have established by injection of radioactively labelled tg in rats and monkeys that T₄ appears in plasma.¹⁶

Thus, it is possible that a certain amount of T₄ produced during tg metabolism would add to the existing pool of T₄ and show an apparent increase in the biologic half-life.

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