MEASUREMENT OF SERUM THYROXINE BY THE TETRALUTE® PROCEDURE: THE EFFECT OF ^{131}I AND ^{99m}Tc ADMINISTRATION

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Since ^{99m}Tc and $Na^{131}I$ are now used quite commonly, we have frequently encountered these radionuclides in sera submitted for thyroxine (T_4) assay. Neither ^{99m}Tc in doses of up to 10 mCi nor ^{131}I in doses of up to 5 mCi appreciably alter serum thyroxine concentration measured by the Tetralute ®method using ^{125}I-labeled thyroxine.

The inorganic pertechnetate and inorganic iodide ions are effectively eliminated from the column by the first buffer wash. A small amount of ^{131}I-labeled thyroxine is produced after therapy of thyrotoxicoses with ^{131}I. This did not elevate total T_4 concentration significantly in the subjects studied. Gamma spectroscopy eliminated this ^{131}I-T_4 contribution to the assay.

Procedures using sodium pertechnetate (^{99m}Tc) or $Na^{131}I$ for diagnosis or treatment are enjoying increased usage. We have frequently encountered these radionuclides in sera submitted for thyroxine T_4 assay. We used a commercial modification (*1*) of the isotopic displacement technique of Murphy and Pattee (*2*) for T_4 measurement. Since the modification, the Tetralute® (^{125}I-Column T_4 Test, Ames Company, Elkhart, Ind.) method for the measurement of serum T_4 monitors ^{125}I-T_4 added to serum in vitro. It is possible that the test subject's previous isotope experience might alter assay results. We have examined this possibility and have found that neither ^{131}I nor ^{99m}Tc in serum impairs the validity of the T_4 measurement.

METHOD

Each of the four patients who were previously untreated received 3–5 mCi of $Na^{131}I$ orally as therapy for thyrotoxicosis. A treatment was not followed by aggravation of the hyperthyroidism or tenderness of the thyroid gland. Two patients each received 10 mCi of sodium-pertechnetate (^{99m}Tc) intravenously for brain scan. Sera were obtained before the radiopharmaceutical was administered and at intervals thereafter. The T_4 concentration of each specimen was determined by the Tetralute® method. Sera were frozen and studied within 3 days. In addition, the performance of the administered radioisotope was monitored throughout each step of the assay procedure.

The validity of the Tetralute® method for T_4 measurement has already been established (*1*). We have made minor changes in our own laboratory that extend somewhat the useful range of the procedure and enhance its precision. The technique itself utilized Sephadex G-25 columns prepared in 0.1 *N* NaOH. In this highly alkaline medium serum, T_4 readily disassociates from thyroxine binding globulin (TBG) and binds to the Sephadex. Tracer ^{125}I-T_4 added with the serum aliquot is similarly bound to the resin. The addition to the column of 0.075 *M* barbitol buffer, pH 8.6, elutes the TBG plus any inorganic ^{125}I that may be present as a contaminant of the ^{125}I-T_4. Stable and tracer T_4 remain bound to the Sephadex particles. These columns are counted for ^{125}I-T_4 activity. Next, a critical amount of human alpha-beta globulin (Nutritional Biochemical Corp., Cleveland, Ohio, in our modification of the procedure) is added. The TBG contained in this mixture competes with the resin for a portion of both species of T_4. A second barbitol buffer rinse elutes that part of the T_4 which has bound to the alpha-beta globulin. The residual radioactivity remaining on the column is then counted. This activity is proportional to the concentration of T_4 in the test serum. The serum concentration of T_4 is calculated from a standard curve developed by adding known amounts of T_4 to

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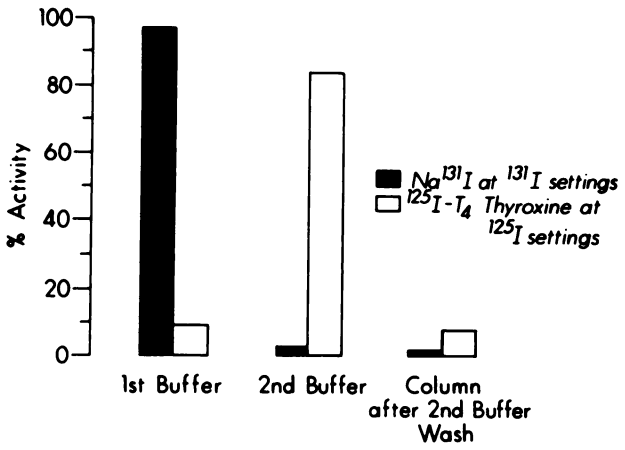

FIG. 1. Behavior of $Na^{131}I$ and ^{125}I-T_4 on Sephadex G-25 columns, when added separately.

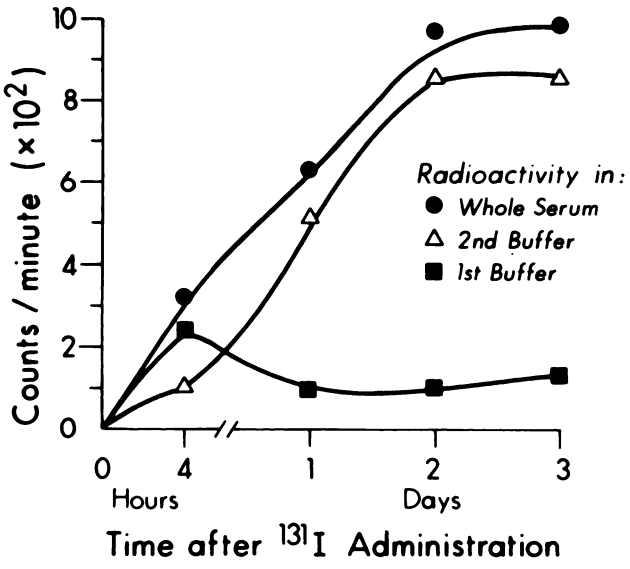

FIG. 2. Radioactivity of serum and its behavior on Sephadex G-25 column following ^{131}I administration to one thyrotoxic patient.

a series of columns and deriving the ratios of ^{125}I-T_4 counts remaining on the columns after the second barbitol buffer rinse to those retained after the first buffer rinse.

The columns themselves and the eluates from the column were counted in a standard 1¾ × 2-in. counter scintillation well with the spectrometer set to detect the photopeak of each of the radioisotopes: for ^{99m}Tc, lower level of 100 keV and a window of 80 keV; for ^{131}I, lower level of 314 keV and a window of 100 keV; for ^{125}I, a lower level of 200 keV and a window of 25 keV. All counts were corrected for background.

The Thyrimeter®, an automated, direct-reading well counter equipped with a discriminator set for ^{125}I (Direct Ratio Reading Gamma Counter, Ames Co. Elkhart, Ind.), is commercially available and is frequently used by some laboratories. The contribution of ^{99m}Tc or ^{131}I to the ^{125}I readings on this instrument was also determined.

RESULTS

Behavior of $Na^{131}I$ and ^{125}I-T_4 alone on Sephadex G-25 columns. When a 2.5 μCi of $Na^{131}I$ alone was added to the column, more than 98% of the radioactivity was eluted by the first buffer wash. The ^{131}I remaining on the column did not significantly elevate the background readings at the ^{125}I spectrometer settings. Virtually all of this residual isotope was eluted by the second buffer wash.

Iodine-125-T_4 alone was also added to the column. Less than 9% of the radioactivity was eluted by the first buffer. This probably represented the inorganic ^{125}I contaminating the ^{125}I-T_4. Eighty-four percent of the ^{125}I-T_4 activity was eluted by the second buffer after alpha-beta globulin had been added. The behavior of $Na^{131}I$ and ^{125}I-T_4 on the column is shown in Fig. 1.

Behavior of patient's sera on the column before and after the oral administration of 3–5 mCi of $Na^{131}I$. Aliquots of sera (0.1–0.2 ml) obtained from three patients 4 hr to 7 days after receiving ^{131}I contained at least 800 times background activity when measured at the ^{131}I settings. Four hours after therapy, 66% of this activity was removed from the test system in the first buffer wash thus behaving in a manner similar to that of inorganic ^{131}I. After 24 hr only 14% of the radioactivity appeared in the first buffer wash. Activity which was eluted after the addition of globulin and the second buffer wash was detectable in all sera drawn after 4 hr and reached peak concentration in sera drawn within 48–72 hr of isotope administration. This activity behaved like the radiolabeled thyroxine. The data for one particular patient may be seen in Fig. 2.

When the same serum specimens and eluates were read at the ^{125}I photo peak, the contribution of this ^{131}I-T_4 was found to be less than 1%. When quantities of ^{125}I-T_4 similar to those used in the assay method, were added to a column, a reading of approximately 20,000 cpm was obtained at the ^{125}I setting while only 500 cpm were recorded at the ^{131}I settings.

Serum T_4 concentrations were measured at intervals in these four patients following administration of the therapeutic doses of $Na^{131}I$. No significant changes occurred during this time (Table 1).

Behavior of patient's sera on the column before and after $^{99m}TcO_4^-$ administration. One-tenth ml ali-

TABLE 1. SERUM THYROXINE IN FOUR THYROTOXIC PATIENTS AT VARIOUS TIMES AFTER 3–5 mCi OF ^{131}I ADMINISTRATION

	Thyroxine Concentration (μgT$_4$I/100 ml)				
	Time				
Subject	Before	4 hr	24 hr	48–96 hr	1 week
1	22	25	—	22	24
2	14	13	15	14	—
3	18	—	18	16	18
4	10.2	9.4	8.4	8.1	9.6

quots of sera from two patients before and 5 and 10 min after they had received 10 mCi $^{99m}TcO_4^-$ intravenously were studied on the Sephadex G-25 columns. Measurements of radioactivity were made at the ^{99m}Tc and ^{125}I spectrometer settings. Specimens obtained immediately before isotope administration contained activity indistinguishable from background at both settings. Immediately after the injection of ^{99m}Tc and in all subsequent specimens studied, activity was at least 50,000 times background in the first buffer wash than at the ^{99m}Tc settings. The same specimens contained only 20 times background activity at the ^{125}I settings. Activity after the globulin had been added in the second buffer wash and on the columns was not significantly different from background in any of the specimens at either the ^{99m}Tc or ^{125}I settings. Serum T_4 concentrations were not significantly altered by the injection of ^{99m}Tc-labeled pertechnetate.

The results were similar whether the Thyrimeter® or a well counter equipped with a spectrometer were used in the analysis.

DISCUSSION

We have found the Tetralute® procedure for the measurement of T_4 to be clinically useful, simple, and reliable. The present data show that even large millicurie amounts of Na^{131}I such as those used in the therapy of thyrotoxicosis do not interfere with the assay. The meager contribution from ^{131}I is fortuitous and is attributed to several conditions. The administered isotope is vastly diluted so that only a fraction appears in the assay system. The physical chemistry of the columns separates inorganic iodide, which is a contaminant in the assay procedure, from the ^{131}I-T_4 that may be produced in the biologic system. Pulse-height analysis facilitates final differentiation of any ^{131}I-T_4 produced by the patient and the larger amount of ^{125}I-T_4 added in vitro in the assay system. Indeed, the added ^{125}I-T_4 is 50–100 times greater than the ^{131}I-T_4 encountered in these sera.

The findings extend the observations reported by Vagenakis, et al (*3*), who found that 60 μCi tracer quantities of ^{131}I did not spuriously elevate thyroxine. Their report addressed itself to the effects of radiation damage from ^{131}I but the data are also applicable to the present context.

Inorganic iodide is almost completely removed by the first buffer wash before any counting procedures are undertaken. Furthermore, any remaining ^{131}I counts are virtually eliminated from consideration by appropriate gamma spectroscopy. Thus, at ^{125}I settings, reagent ^{125}I-T_4 is easily differentiated from any organic or inorganic ^{131}I compounds. Because the direct reading automatic gamma counter (Thyrimeter®) is equipped with a discriminator capable of excluding the 364-keV photopeak of ^{131}I from the 35.5-keV photopeak of ^{125}I, it is a satisfactory instrument for this assay.

The fact that inorganic iodide is eliminated from the columns before any monitoring of counts is performed is an added advantage of the Tetralute® procedure. Inorganic ^{125}I, which may contaminate the reagent ^{125}I-T_4, would be virtually eliminated.

In the course of our studies we found evidence that administered Na^{131}I was converted to an ^{131}I-labeled serum compound that behaved on the columns in a manner similar to the authentic T_4. Conversion of Na^{131}I to ^{131}I-T_4 and its rapid releases into the serum would be expected in thyrotoxic patients. We found evidence of this as early as 4 hr after the therapeutic dose. This ^{131}I-T_4 that was produced did not interfere in the assay of the total T_4 by the ^{125}I Tetralute® method as shown by the virtual failure to detect counts from this source at the ^{125}I settings.

In the four patients we tested, there was no detectable rise or fall in total serum T_4 concentration over a 1-week period following treatment; and there was no clinical evidence of radiation thyroiditis. This was fortuitous because an increase in serum T_4 has been reported in some patients after treatment with ^{131}I as a result of disruption of the thyroid by radiation damage (*4*). We used relatively small amounts of ^{131}I and perhaps that is why we did not have acute thyroid injury. Nevertheless, the important point here is that in the absence of glandular disruption due to radiation injury, the T_4 concentration, as measured by the Tetralute® method, did not increase; the administered ^{131}I was not spuriously detected as T_4. Thus, the Tetralute® procedure is technically adequate for measuring a patient's circulating T_4 even after a patient has received therapeutic amounts of ^{131}I.

Clinical scanning with $^{99m}TcO_4^-$ requires the use of large doses in order to achieve sufficiently high levels of serum radioactivity for proper imaging. Although this isotope enjoys a short half-life (6 hr), significant serum radioactivity is detectable for some time. Fortunately, the overlap of ^{99m}Tc activity (140 keV) into the ^{125}I (35.5-keV) portion of the spectrum is minimal. We have shown that all of the circulating ^{99m}Tc counts are eluted from the Sephadex columns by the first buffer rinse. Thus, there is no need to delay measurement of serum T_4 because of the prior administration of even very large quantities of ^{99m}Tc. It should be noted that ^{99m}Tc-pertechnetate is trapped by the thyroid gland but is not incorporated into the thyroid hormone. Therefore, by no mechanism can ^{99m}Tc alter serum T_4 values as determined by the Tetralute® method.

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