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Adaptation of a T_3-Uptake Test and of Radioimmunoassays for Serum Digoxin, Thyroxine, and Triiodothyronine to an Automated Radioimmunoassay System—"Centria"

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We report the adaptation of four radioassays to the prototype of an automated radioimmunoassay system ("Centria," Union Carbide). The system consists of three integrated modules: (*a*) an automated pipettor, which dispenses samples and reagents; (*b*) the key module, an incubator/separator, in which centrifugal force is used to initiate and terminate multiple radioassay incubations and separations simultaneously; and (*c*) a gamma-counter/computer, which counts three tubes simultaneously and converts counts into concentration units. Radioimmunoassays for thyroxine, triiodothyronine, and digoxin were developed with use of well-characterized antibodies and of prepackaged Sephadex-containing columns to separate bound and free radioactive ligand. A triiodothyronine-uptake test in which the same kind of columns were used was also adapted to the instrument. Results for clinical samples compared favorably with those obtained by manual procedures. We report data on correlation between different methods and preliminary data on precision of the prototype system.

Additional Keyphrases: *centrifugal mixing and separation* • *separation on Sephadex columns* • *nonequilibrium assay* • *analytical systems*

Radioimmunoassay (*1*), because of its extreme sensitivity and specificity, is rapidly becoming a standard technique in endocrinology and biochemistry laboratories. However, the many tedious and critical operations inherent in present radioimmunoassay procedures have contributed to delay of its acceptance as a routine clinical laboratory technique. Initial attempts to mechanize the technique (*2*) did not find wide acceptance, and most efforts toward partial automation were aimed at facilitating data interpretation rather than assay manipulation (*3*).

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The "Centria" system (Union Carbide Corp.) was conceived in response to this need for automation and has been designed to eliminate all critical manual operations in performing a radioimmunoassay. The modular design of the system, which consists of three integrated units, provides both the flexibility required when several test procedures are performed on many small sample batches, and high throughputs, which are particularly desirable when large numbers of specimens are analyzed for a single constituent. The technical features and mode of operation of the Centria system represent a departure from conventional ways of performing radioimmunoassays in addition to giving impetus to new assay design. Like the centrifugal clinical chemistry analyzer (*4*), the system provides identical reaction conditions for standards, controls, and samples, thus creating a new tool for kinetic, nonequilibrium immunoassays (*5*).

The present work demonstrates how the prototype system can be applied to perform those radioassays most frequently requested in the clinical chemistry laboratory. Development of four automated procedures is described and assay results are compared with those obtained by manual methods performed outside our own laboratory.

Materials and Methods

Instrumentation

A laboratory prototype of the Centria system was used for the experiments. Thirty analysis positions are provided in the system, which consists of three modules: a reagent and sample pipettor, an incubator/separator, and a counter/computer.

The Centria pipettor and transfer disc. The Centria pipettor (Figure 1) is designed around a turntable, which is first loaded with a ring containing 30 sample cups. A disposable plastic transfer disc consisting of 30 radially aligned pairs of cavities (Figure

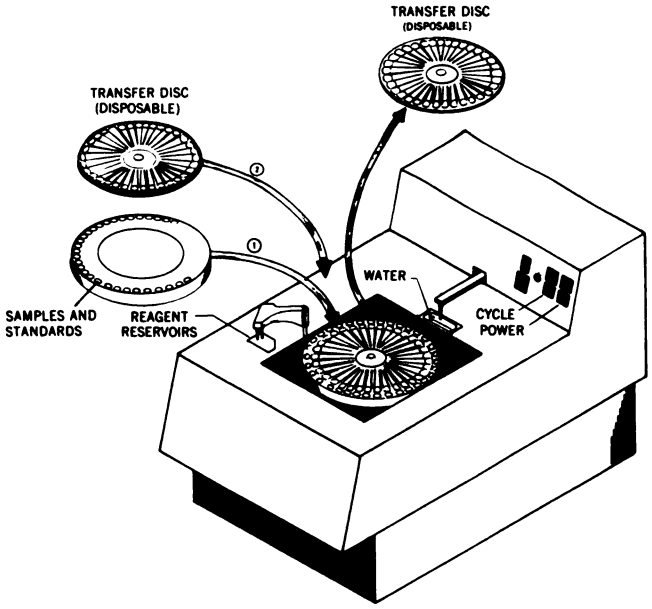

Fig. 1. The Centria pipettor

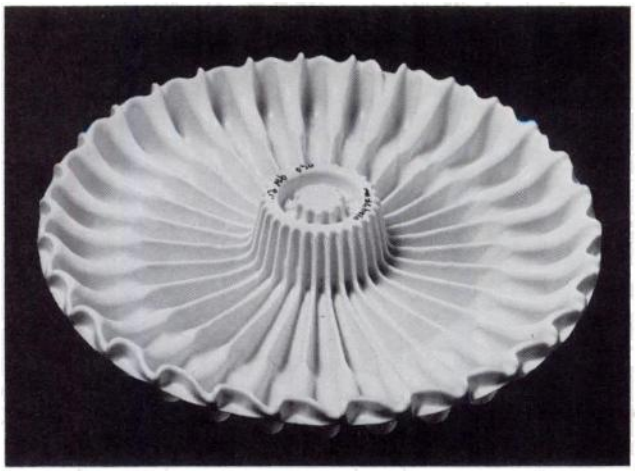

Fig. 2. The Centria transfer disc

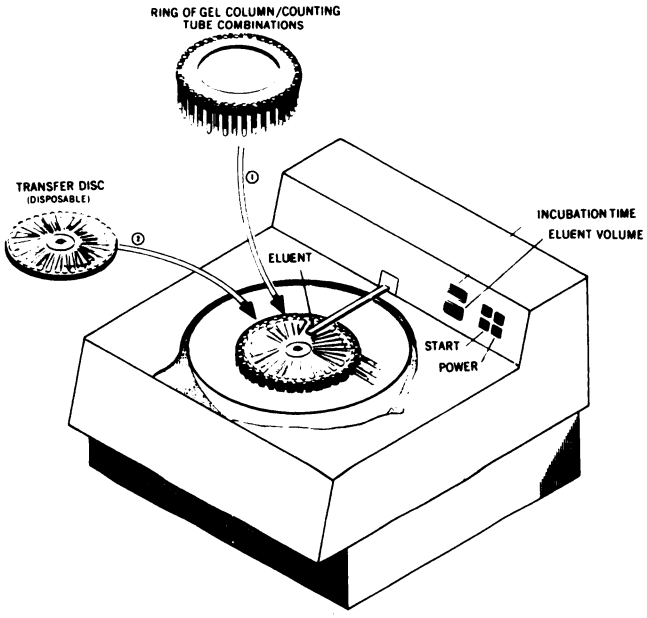

Fig. 3. The Centria incubator/separator

2) is then placed on the turntable. Standards or samples are automatically withdrawn from the cups and loaded into the outer cavities of the disc. The sample volume is either 15, 35, or 50 μl, depending on the assay. In each case the sample is diluted to 100 μl, to complete transfer of the measured volume and to minimize carryover problems. Before the next sample is aspirated, the outside of the sample tip is rinsed in a water reservoir. While sample or standard is being loaded, antiserum (200 μl) and radioactive competing antigen (50 μl) are automatically withdrawn from reservoirs and dispensed into the radially aligned cavities—antiserum into the inner cavity and radioactive ligand into the outer cavity, by a second mechanical system. Because the system uses air-displacement pumps, purging and priming of the measuring systems is not needed and hence little reagent is wasted. Neither antiserum nor radioactive antigen solution is loaded into positions not receiving sample, standard, or control serum.

The Centria incubator/separator. This module is the heart of the system, where incubation and separation of the reactants are performed (Figure 3). It consists of a variable-speed turntable, which is first loaded with a plastic ring holding 30 small ready-to-use separating columns packed with swollen Sephadex G-25 (Pharmacia Labs. Inc., Piscataway, N.J. 08854). They are positioned in test tubes, which in turn are suspended by their upper rims in ball seats in the plastic ring. The filled transfer disc is placed on the turntable and automatically keyed such that each pair of cavities is aligned with the opening of a separating column.

Figure 4 shows a single sample position. When rotation of the turntable is started, antiserum flows from the inner into the outer cavity, where all reactants are instantaneously mixed and held for a preset interval. The steep angle and overall shape of the outer cavity prevent any overflow at the first speed. After the incubation time has elapsed, the instrument automatically accelerates the turntable rotation to a second, higher speed, causing the incubated mixture to be transferred onto the columns, where it embeds in the separating medium. After embedding has taken place, a pump is started, which dispenses a predetermined amount of liquid onto the spinning transfer disc, where it is divided into equal aliquots by the geometry of the disc. While the eluent is driving the incubated mixtures through the separating medium, the free radioactive tracer is either adsorbed or partitioned out on the column. The antibody-bound tracer in turn moves with the front of the eluent and is collected in the test tubes that support the spinning columns. The elution process takes

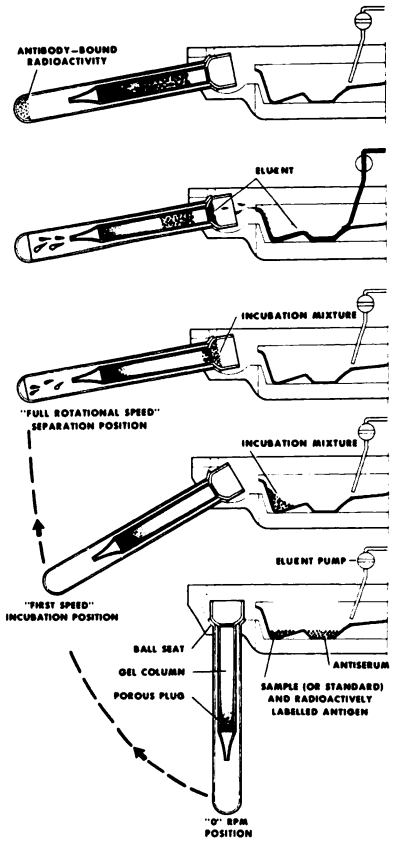

Fig. 4. Side view of a single sample position in the Centria incubator/separator

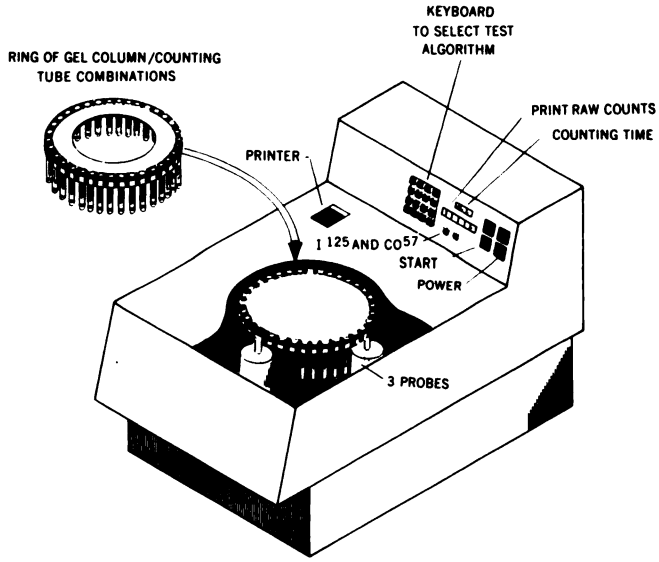

Fig. 5. The Centria counter/computer

about 60 s. After the turntable has come to rest, the transfer disc is discarded and the column ring holding the column/test tube combinations is lifted and transferred to the turntable of the counter/computer.

The counter/computer. This third module of the system consists of a three-channel gamma counter/computer combination, which counts the radioactivity in the test tubes and calculates concentrations based on standards run simultaneously with the clinical samples (Figure 5). The ring of gel column/test tube combinations is placed on the counter turntable, which at the beginning of a run is in its elevated position. Keying the column ring to the turntable assures the proper assignment of counting positions of all standards and samples. Test code, counting time, standard values, and method of data reduction are then fed into a microprocessor. When the start button is pushed, the turntable indexes and descends to its lower position, and three test tubes at a time are guided mechanically into three counting wells, which are fixed 120° apart. The three counting systems are 1.75 × 2 inch sodium iodide crystal/photomultiplier tube combinations. When the first counting interval has elapsed, the turntable lifts up, advances, and lowers the next set of three tubes into the probes. This change-over takes 8 s. At the end of the counting cycle, the counts are either printed out or processed on-line, according to the initially chosen data-reduction method, which converts them into concentration units. The counter is designed to count iodine-125 and cobalt-57.

Reagents

Antisera. Anti-digoxin was elicited in rabbits with a conjugate of digoxin/human serum albumin, prepared according to the procedure of Smith et al. (*6*). Ultraviolet analysis of the conjugate indicated a digoxin/human serum albumin molar ratio of approximately 11. The anti-digoxin (Ra 55) used in these experiments was harvested six months after a primary injection of 0.2 mg of conjugate, emulsified in Freund's complete adjuvant; we used the low antigen dose and multidermal injection route of Vaitukaitis et al. (*7*). During the six-month immunization schedule, the rabbits received three approximately equally spaced booster injections of 0.1 to 0.5 mg of conjugate emulsified in adjuvant. The harvested anti-digoxin showed a titer (the final dilution of raw antiserum in the incubate that will bind 50% of the radiolabeled hapten) of 110 000. Antibody specificity studies (expressed in terms of percentage cross-reactivity and defined in Table 1) show the antiserum to be highly specific for digoxin. The cross-reactivity, with digitoxin and spironolactone as inhibitors, was 4.3 and <0.7%, respectively. In addition, three commercially obtained control sera, known to be negative for digoxin, showed insignificant displacement of radiolabeled digoxin in the incubate mixture. The average intrinsic affinity constant of this antiserum, determined by the equilibrium dialysis procedure of Smith et al. (*6*), was 1.7×10^9 liter/mol.

Anti-triiodo-L-thyronine (T_3) was elicited in rabbits with a triiodo-L-thyronine/bovine serum albumin conjugate, synthesized by the procedure of Gharib et al. (*8*). Ultraviolet analysis of the conjugate showed a T_3/bovine serum albumin molar ratio of 4–5. The anti-T_3 (Ra 94-I) used in these experiments

Table 1. Cross-Reactivity of Antiserum to Triiodothyronine

Hapten	% Cross-reaction[a,b]	% Cross-reaction[a,c]
Triiodo-L-thyronine	100.0	100.0
Triiodo-D-thyronine	100.0	100.0
L-Thyroxine	0.2	0.2
3,5-Diiodo-L-thyronine	0.9	0.8
Triiodothyropropionic acid	9.8	6.9
Diiodo-L-tyrosine	<<0.1	<<0.1

[a] % cross-reaction is defined as the reciprocal of that molar ratio (multiplied by 100), of the hapten inhibitor to the unlabeled hapten, required to halve antibody binding of the labeled hapten.

[b] Values for % cross-reaction were determined by using Sephadex G-25 columns (Centria) to separate free and antibody-bound hapten.

[c] Values for % cross-reaction were determined by using dextran-coated charcoal in a batch method to separate free and antibody-bound hapten.

Table 2. Cross-Reactivity of Antiserum to Thyroxine

Hapten	% Cross-reaction[a]
L-Thyroxine	100.0
Triiodo-L-thyronine	1.0
Triiodo-D-thyronine	1.6
Triiodothyroacetic acid	1.0
Triiodothyropropionic acid	3.0
Diiodo-L-tyrosine	<<0.2

[a] See footnotes [a] and [b] of Table 1.

was harvested four months after a primary injection of 75 μg of conjugate, emulsified with Freund's complete adjuvant; again, we used the low antigen dose and multidermal injection procedure of Vaitukaitis et al. (7). Two boosting injections of 50 μg and 250 μg, emulsified with adjuvant, were given during the four-month immunization period. The anti-T_3 showed a titer of 1:1750.

The cross reactivity of five inhibitors is shown in Table 1. Similar values for the percentage cross-reaction are seen whether dextran-coated charcoal or the Centria gel-permeation columns were used in the separation step. The average intrinsic affinity constant for the anti-T_3 was calculated to be 2.4×10^9 liter/mol.

Anti-L-thyroxine (T_4) was produced in rabbits with an L-thyroxine/bovine serum albumin conjugate. The method used for conjugation was the same as described for the T_3-conjugate. Analysis of the conjugate showed a T_4/bovine serum albumin molar ratio of 4. The anti-T_4 used in these experiments was harvested six months after the primary injection. During the six-month immunization period, three boosting injections were given subcutaneously and intravenously. The anti-T_4 exhibited a titer of 1:525. Antibody specificity studies indicate an antiserum of low cross-reactivity. The percentage cross-reaction for five inhibitors is seen in Table 2.

Radioactive ligands used were: [^{125}I]thyroxine, [^{125}I]triiodothyronine (Amersham/Searle, Arlington Heights, Ill. 60004), and digoxin-[^{125}I]tyrosine methyl ester, (Burroughs-Wellcome Co., Research Triangle Park, N.C. 27709).

Standards. Thyroxine, triiodothyronine (Sigma Chemical Co., St. Louis, Mo. 63178); digoxin (Burroughs-Wellcome).

Normal human serum (Hyland Laboratories, Costa Mesa, Calif. 92626).

Activated charcoal (Darco-G-60; Matheson, Coleman and Bell, Rutherford, N.J. 07470).

Buffer components: Disodium phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium barbital, "Tween-80," sodium azide (Fisher Scientific Co., Fair Lawn, N.J. 07410); tris(hydroxymethyl)aminomethane ("Trizma" base), bovine serum albumin (Fraction V), human serum albumin (Fraction V) (Sigma); gelatin (Eastman Organic Chemicals, Rochester, N.Y. 14650), thimerosol ("Merthiolate") (Elanco Prod. Co., Indianapolis, Ind. 46206); and 8-anilino-1-naphthalene sulfonic acid (ANS; K&K Labs, Plainview, N.Y. 07065).

Separation columns. Materials used in these columns were: disposable Sarpette (Walter Sarstedt Inc., Princeton, N.J. 08540), porous polyethylene (Porex Materials Co., Atlanta, Ga. 30346), and Sephadex G-25 (Pharmacia).

Methods

Separation procedure. In all four procedures, Sephadex G-25 (fine) gel is used to separate free and protein-bound ligand, as monitored radiochemically. This type of separation material was chosen primarily because the theory underlying gel fractionation separations is well understood (*9*) and a standardized commercial material is available. In addition, Sephadex G-25 does not disrupt antibody/antigen complexes with high association constants even when it displays additional adsorptive characteristics as are seen in the cases of thyroxine (*10–13*) and triiodothyronine (*14, 15*). A typical elution pattern for the radioactive ligands of digoxin and triiodothyronine is shown in Figure 6. These curves are generated by adding 350 μl of the respective incubated radioimmunoassay mixtures to the columns, eluting successively with 0.2-ml increments of buffer, and counting the radioactivity in the fractions collected from the columns after spinning them at the second speed on the incubator/separator module. Triiodothyronine emerges relatively late at pH 5.2 under the conditions of the T_3-uptake test, but, like thyroxine, is completely retained at pH 8.9.

Radioimmunoassays for thyroxine, triiodothyronine, and digoxin. Table 3 summarizes the details of the procedure. The first two positions on the transfer disc are used to determine nonspecific binding. Buff-

Table 3. Details of Loading the Transfer Disc

	Thyroxine RIA	Triiodothyronine RIA	Digoxin RIA	T_3-uptake test
Transfer disc loading	200 μl of antiserum	200 μl of antiserum	200 μl of antiserum	200 μl of T_3-^{125}I
Inner cavity	(RTx4) diluted 1:300 in Buffer A	(Ra 94-I) diluted 1:1000 in Buffer B	(Ra 55) diluted 1:65,000 in Buffer C	(0.5 μ Ci/2.5 μg/liter) in Buffer D
Outer cavity	(1) 15 μl of sample or serum based standard plus 85 μl of water	(1) 50 μl of sample or serum based standard plus 50 μl of water	(1) 50 μl of sample or serum based standard plus 50 μl of water	35 μl of sample plus 65 μl of water
	(2) T_4-^{125}I (2 mCi/2 μg/liter) plus 0.6% 8-anilino-1-naphthalene sulfonic acid (ANS) in 50 μl of Buffer A	(2) T_3-^{125}I (1 mCi/μg/liter) in 50 μl of Buffer B	(2) Digoxin-tyrosine methyl ester-^{125}I ~ 250 μCi/~0.5 μg/liter) plus 0.1% Tween in 50 μl of Buffer C	
Column buffer	Buffer A	Buffer B	Buffer C	Buffer D
Incubation time, min	15	30	15	5
Eluent	Buffer A	Buffer B	Buffer C	Water

Definitions: Buffer A: 0.075 mol/liter barbital, containing 20 ml normal rabbit serum per liter, pH 8.6. Buffer B: 0.05 mol/liter Tris-HCl, containing 1.0 g of gelatin per liter, pH 8.9. Buffer C: 0.01 mol/liter phosphate, containing 1.0 g of gelatin per liter, pH 7.4. Buffer D: 0.05 mol/liter phosphate, pH 5.2.

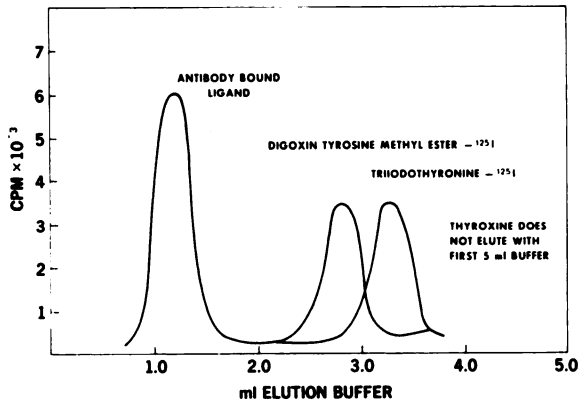

Fig. 6. Separation of free thyroxine, triiodothyronine, and digoxin ligands from their protein complexes

er without antibody is pipetted into the inner cavities; radiolabeled ligand and serum without the analyte of interest fill the outer cavities. Positions 3 to 12 are allocated to duplicates of four standards and antibody binding in the absence of standard (B_0), while the remainder of the positions are available for clinical samples and controls. After processing the filled disc on the incubator/separator and then counting the column eluates, one obtains a print-out such as that shown in Figure 7. In this example, the logit-log algorithm (*16–18*) is used to linearize the standard curve and to calculate concentrations of clinical samples. Slope and intercept of the linear logit-log plot are printed out along with the residual variance of the regression line. The residual variance was selected for use in characterizing how closely the standards fit the linear regression line. The print-out indicates to the operator that positions 1 and 2 were treated as nonspecific binding and positions 3 and 4 as B_0 or antibody binding in the absence of analyte. Positions 5, 7, 9, and 11 show the actual concentration of standards as read into the computer, while positions 6, 8, 10, and 12 show, at the left of the slash, the mean concentrations of those same standards computed from the "best fit" slope and intercept. On the right side of the slash an individual standard's deviation from the mean concentration is printed out. Positions 13 through 30 are reserved for clinical samples, controls, and total counts. The latter always exceed the B_0 counts and are therefore interpreted as a concentration which is out of range (*OR*).

The T_3-uptake test. This test provides a semiquantitative measure for the saturation of thyroxine-binding globulin with thyroxine (*19*). Radioactive triiodothyronine (T_3) is added to serum and free, unreacted T_3 is then removed from the solution through adsorption (*20*), ion exchange (*21*), or gel filtration (*22, 23*).

In the Centria procedure, Sephadex G-25 is used to take up the unbound T_3. Differently from the radioimmunoassay procedures, the radioactive ligand is dispensed into the inner cavity of the transfer disc and thus is not added to the serum sample until the disc is placed on the incubator/separator and spun up to the first speed of rotation. This way, all samples

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Digoxin Run 3

Date    2-19-75
Run     01A
Logit-Log
RV=0.00993
Slope = -1.099
Intercept = 0.596

        NG/ML
1   S   NSB
2
3   S   0.0
4
5   S   4.00
6       4.21/0.00
7   S   2.00
8       2.05/0.10
9   S   1.00
10      1.05/0.05
11  S   0.50
12      0.51/0.05
13      1.47
14      1.21
15      3.48
16      3.16
17      0.91
18      1.10
19      6.18
20      5.10
21      0.83
22      1.00
23      1.00
24      0.91
25      1.96
26      1.96
27      0.68
28      0.91
29      OR
30      OR
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Fig. 7. Print-out of assay results obtained with the Centria system

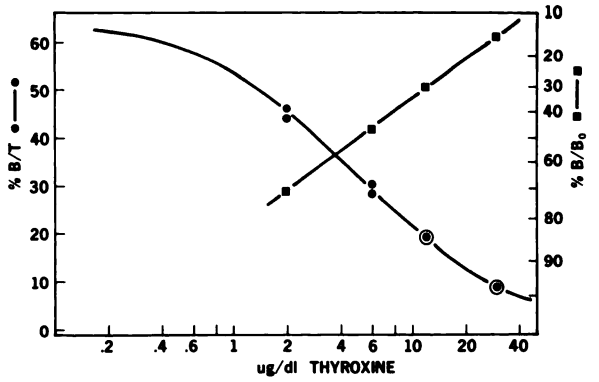

Fig. 8. Standard curve for thyroxine radioimmunoassay

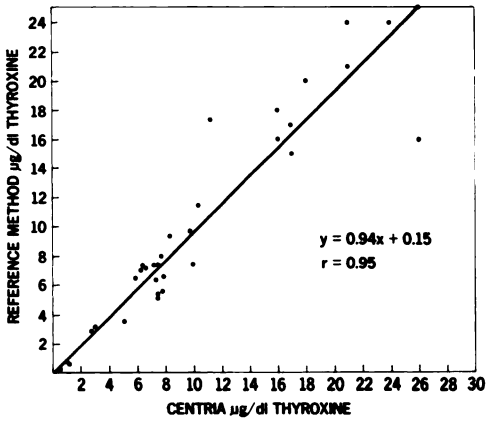

Fig. 9. Comparison of results of manual and Centria radioimmunoassay procedures for thyroxine

react under identical conditions simultaneously. The T_3 uptake ratio is computed according to

$$\% \text{ uptake} = (\text{counts eluate/counts total}) \times 100\%$$

Results and Discussion

Thyroxine Radioimmunoassay

Since the first radioimmunoassay for thyroxine was developed (*24*), many groups have published similar procedures (*25–30*). In our case, a standard curve was established that accommodates thyroxine concentrations between 1 and 30 µg/dl (Figure 8). Excellent linearization of the curve is achieved by the logit-log transformation, which made this algorithm perfectly acceptable for data reduction.

Samples previously analyzed at the Steroid Research Institute of Montefiore Hospital, Bronx, N.Y. by radioimmunoassay were run on the prototype Centria system (Figure 9). Assuming a normal range for thyroxine of 5.1 to 11.7 µg/dl, all serum samples showed the correct assigned pathologic or normal status when analyzed by the two differing procedures.

Table 4 gives an indication of the reproducibility obtainable with the prototype system. The first four lines show the reproducibility of the recalculated standard values of five consecutive runs. Thyroxine concentrations of control sera derived from simultaneously run standards are shown on the next four lines. Running duplicates enables one to identify occasional gross errors—as indicated for control 2 in run 3.

Standard data from previous runs can be stored in the microprocessor for use in calculating concentrations in serum in subsequent runs. This feature permits the loading of discs after the initial run with as many as 15 samples in duplicate, thus considerably increasing the throughput of the system. When intrarun precision was determined, data from a previous run was stored in the computer and retrieved for the calculation of precision in a second run in which all 30 sample positions were filled with a specimen of pooled serum. Intrarun precision (CV) on the prototype system has ranged from 3.5 to 5%; run-to-run precision was 7%.

Triiodothyronine Radioimmunoassay

To determine triiodothyronine by radioimmunoassay (*31–35*), we used the system's unique capacity to conveniently perform kinetic, nonequilibrium assays. Figure 10 shows the standard curves obtained after

Table 4. Results of Five Consecutive Runs of a Thyroxine Radioimmunoassay on Centria

Standard 1 (2 μg/dl)	1.8 ± 0.1	1.7 ± 0.4	2.0 ± 0.1	1.9 ± 0.5	1.9 ± 0.2
Standard 2 (6 μg/dl)	6.4 ± 0.5	7.0 ± 0.0	5.9 ± 0.6	6.2 ± 0.4	6.9 ± 0.3
Standard 3 (12 μg/dl)	12.4 ± 0.5	12.3 ± 2.6	11.4 ± 0.0	13.0 ± 0.0	13.4 ± 0.5
Standard 4 (30 μg/dl)	27.7 ± 1.9	27.8 ± 2.0	30.2 ± 0.0	28.3 ± 1.0	28.0 ± 3.1
Control 1	8.5	8.6	8.1	8.1	8.3
Control 1	9.1	8.0	8.1	8.1	7.8
Control 2	17.0	16.0	15.1	18.3	17.3
Control 2	15.9	17.1	9.9	17.1	15.9

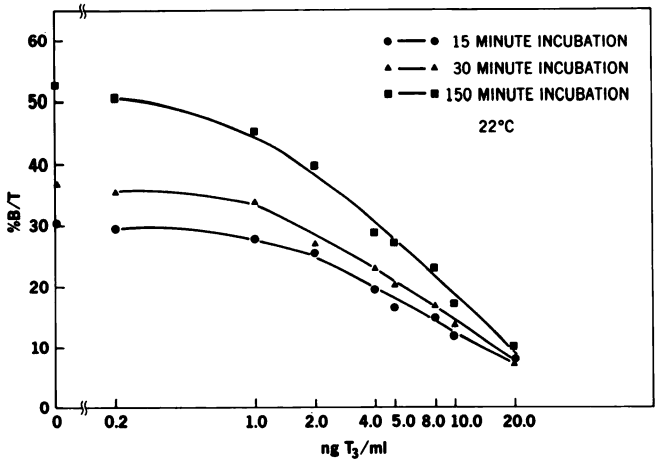

Fig. 10. Standard curve for triiodothyronine radioimmunoassay obtained after three incubation times

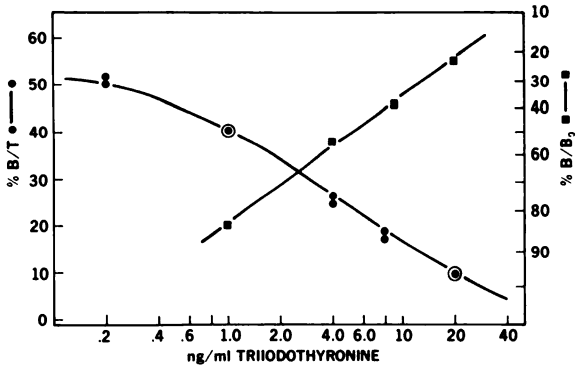

Fig. 11. Standard curve for triiodothyronine radioimmunoassay with 30-min incubation

15-, 30-, and 150-min incubations. Because abnormally high T_3 concentrations appear to be of foremost clinical interest, sensitivity of the assay was reduced in favor of speed of analysis. If higher sensitivity is desired, incubation times can easily be increased by removing the transfer disc from the incubator/separator after mixing and subjecting the incubated mixtures to separation after 2 h have elapsed.

A standard curve covering concentrations between 0.2 and 20 ng of T_3 per milliliter was established after 30-min incubation (Figure 11) and concentrations in serum were determined in a series of mostly pathologic samples. These have been analyzed by an equilibrium procedure performed at 4 °C, with use of a second antibody to separate free from bound radiolabel.

Thirty-four samples were compared by establishing the regression equation $y = 0.95x - 0.12$, with a correlation coefficient of 0.97 (Figure 12). These results indicated a bias in favor of higher values for our procedure but show an excellent correlation in view of the totally different assay approaches. Precision (CV) of the procedure between runs, determined on control samples at two different concentrations, was 9%; within-run precision was 7%.

The kinetic procedure uses the tris(hydroxymethyl)aminomethane buffer/Merthiolate reagent system originally suggested by Hüfner and Hesch (*35*). We confirmed these authors' observation that antibody binding is essentially independent of Merthiolate concentration. In addition, the Merthiolate is more stable than 8-anilino-1-naphthalene-sulfonic acid, which has to be prepared freshly each day.

T_3 Uptake Test

Use of Sephadex G-25 in the T_3 uptake test was first proposed by Lissitzky et al. (*22*). Of all the separation media suggested for this test, Sephadex G-25 subjects the incubated mixture to the mildest separation process, which is essentially based on dialysis and does not affect the equilibrium.

Results obtained with our procedure on 40 clinical samples were compared to results given by the manual "Trilute" test (Ames. Co., Elkhart, Ind. 46514). A linear regression equation of $y = 0.87x + 9.33$ and a

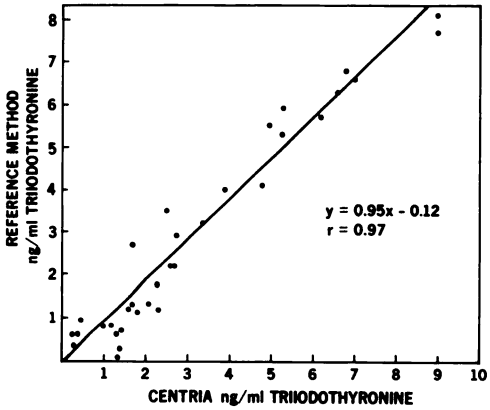

Fig. 12. Comparison of results of manual and Centria radioimmunoassay procedures for triiodothyronine

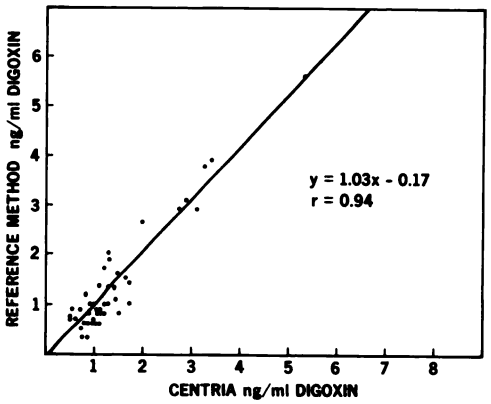

Fig. 14. Comparison of results of manual and Centria radioimmunoassay procedures for digoxin

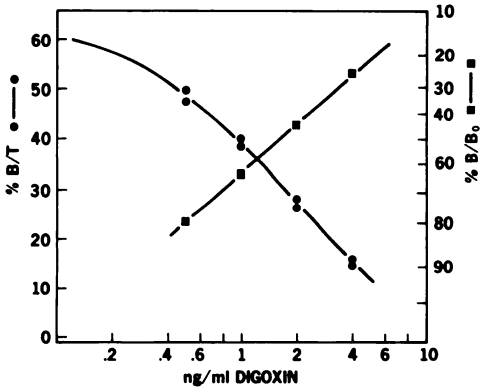

Fig. 13. Standard curve for digoxin radioimmunoassay

correlation coefficient of $r = 0.87$ were observed. Most of the results are clustered in and around the normal range, which usually leads to poor correlation data. The means of all data were, however, very close: 42.1% for the Trilute test and 43.9% for the Centria System.

Digoxin Radioimmunoassay

Soon after Butler and Chen (*36*) raised the first highly specific antibodies against digoxin, a radioimmunoassay was developed by Smith et al. (*37*). In several procedures, the originally used tritiated digoxin has since been replaced with iodinated ligands, which are derived from the tyrosine methyl ester of either digoxin or digoxigenin (*38, 39*). High specific activities can be achieved with iodinated labels, and quenching problems generated by icteric or hemolyzed samples are circumvented (*40*).

The Centria procedure was developed with an iodinated digoxin/tyrosine methylester as supplied by Burroughs-Wellcome. The standard curve covers the range of concentrations between 0.2 and 8 ng of digoxin per milliliter and is adequately linearized by the logit-log transformation (Figure 13). Intra-run precision was determined to be 3.5 to 4%, while inter-run precision for the prototype was 9%.

Fifty-eight clinical samples, previously analyzed at Methodist Hospital, Indianapolis, by a procedure in which a tritiated digoxin ligand and dextran-coated charcoal are used in the separation step, were reassayed on the Centria system. Regression analysis ($y = 1.03x - 0.17$; $r = 0.94$) revealed good agreement (Figure 14). In the toxic range—i.e., above concentrations of 2 ng/ml digoxin—the two methods disagreed for only one sample.

The four assays will now be extensively field-evaluated. Additional information on precision, recovery, and correlation with alternative procedures is being collected for these four assays, along with similar data for other Centria immunoassays.

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