Semi-Automated T_3 Uptake Test That Uses Magnetic Albumin Microparticles

D. O. Kubiatowicz, D. S. Ithakissios, J. H. Wicks, and P. E. Heerwald

3M Company, St. Paul, Minnesota

We describe a T_3 uptake test that uses magnetic albumin microparticles to separate free from bound [^{125}I] T_3. The test uses 0.1 ml serum in a total assay volume of 1.1 ml, and is performed in barbital buffer of pH 7.6, ionic strength 0.2. The accuracy of the test is not affected by the following ranges: volume, 1–3 ml; incubation time, 15–120 min; and incubation temperature, 4–35°C. We describe a magnetic separator device that allows simultaneous handling of 100 assay tubes with minimum manipulation. The intra-assay and interassay coefficients of variance were 2.87% and 5.20%, respectively.

J Nucl Med 19: 854–857, 1978

Albumin microparticles bind T_3 reversibly with a weak noncovalent bond and allow establishment of equilibrium in an assay mixture (*1,2*). In this paper we describe a room-temperature preparation of albumin microspheres containing barium ferrite and their use in a time- and temperature-independent T_3 uptake test. In addition we describe a magnetic separator device that allows semi-automation of the test with excellent reproducibility.

MATERIALS AND METHODS

Barbital buffer A contained 1.03 g of sodium barbital, 1.6 g of barbital, and 0.5 μg of triiodothyronine per liter. Barbital buffers B were similar but also contained 1.0 g of human serum albumin per liter, and 2.63, 5.56, 11.4, or 23.1 g of NaCl per liter to provide respective ionic strengths of 0.05, 0.1, 0.2, and 0.4. All buffers were of pH 7.6.

For the investigation of [^{125}I] T_3 binding to magnetic albumin microparticles (MAM) (data in Table 1), radiolabeled triiodothyronine, [^{125}I] T_3, having a specific activity of about 50 mCi/mg, was diluted with barbital buffer A to an [^{125}I] T_3 concentration of 8 μg of [^{125}I] T_3 (400 μCi) per liter. It was also diluted with barbital buffer B to an [^{125}I] T_3 concentration of 800 ng of [^{125}I] T_3 (40 μCi) per liter for the T_3 uptake test and for the data in Table 2.

Magnetic Albumin Microparticles (MAM). These are prepared by first stirring a mixture of 500 ml castor oil, 500 ml n-butanol, and 15 ml of glutaraldehyde (contained in a 2-liter beaker) at 450–500 rpm. A homogenous suspension of 3 g barium ferrite (less than 1 μ in diam) in 9 ml of bovine serum albumin is then added dropwise into the edge of the beaker at a rate of 1 drop/sec. The mixture is stirred for 2 hr. The MAM thus formed are separated by filtration and then washed by refluxing in a Soxhlet extractor, first with 200 ml of n-heptane for 8 hr, then with 200 ml of H_2O for another 8 hr. The MAM are dried under vacuum and classified by sieving to obtain a size of 10–80 μ. For convenient use in the T_3 uptake test, 30 mg of MAM were compressed into tablets using lactose as a binder. The tablets were exposed to a magnetic field of about 5,000 gauss produced by an electromagnet to permanently magnetize the barium ferrite in the MAM.

Magnetic separator device. This consists of two racks made of plexiglass, an aluminum housing, a permanent-magnet assembly, and a motor to rotate the racks about a horizontal axis (Fig. 2). Each rack is capable of holding 50 12- × 75-mm test tubes and can easily be inserted or removed from the aluminum housing. The housing includes a cover that can be closed over the test tubes to hold them physically in the rack and to seal them. The interior surface of the cover is a rubber sheet, which can be overlayed with either a disposable parafilm sheet to seal the liquids into the test tubes during rotation, or with a disposable absorbant paper mat to absorb liquids from the test tubes.

The permanent-magnet assembly is mounted in the base of the aluminum housing. This is easily raised into contact

TABLE 1. BINDING OF [^{125}I] T_3 TO 30 MG OF MAM AS AFFECTED BY THE T_3 CONCENTRATION

T_3 (μg/liter)	Percentage of radioactivity in MAM
Tracer	54.2
2	52.4
4	49.8
6	51.1
8	52.2
10	53.3

Received Aug. 8, 1977; revision accepted Nov. 22, 1977.

For reprints contact: D. S. Ithakissios, Medical Products Div., 3M Co., 3M Ctr.—230-3, St. Paul, MN 55101.

TABLE 2. EFFECTS OF IONIC STRENGTH AND WEIGHT OF MAM ON SENSITIVITY OF T_3 UPTAKE TEST

Amount of MAM (mg)	Ionic strength 0.05	0.1	0.2	0.4
7.5	0.23(0.887)*	0.11(0.790)	0.36(0.986)	0.25(0.821)
15	0.54(0.906)	0.53(0.965)	0.56(0.925)	0.55(0.962)
30	0.51(0.972)	0.51(0.974)	0.63(0.962)	0.52(0.958)
60	0.53(0.982)	0.51(0.953)	0.42(0.911)	0.48(0.899)

* Slope and (correlation coefficient) describing the linear relationship between the binding of I-125 T_3 to MAM (at various ionic strengths) and concentration of T_4 added to pooled human serum.

with the housing (cover closed) to place the test tubes within the magnetic field, or shifted to a position away from the housing (cover open) to remove them from the magnetic field. The housing is electrically rotated during the assay. For the T_3 uptake test the magnetic separator is used in the following manner.

T_3 uptake test. Carry out the test at room temperature in 12- × 75-mm polypropylene tubes. Pipet 100 μl pooled human serum or patient serum and 1.0 ml of [^{125}I] T_3 solution into each designated test tube on the rack. Then add one MAM tablet to each tube, except the total activity tubes, and insert the rack into the magnetic separator. Cover the tubes with a parafilm sheet and close the cover to seal them. Before applying the magnetic field, rotate the housing about its horizontal axis for 30 min. Then apply the magnetic field, discard the parafilm, and place a mat of absorbent paper over the top of the tubes. Close the cover again and turn the housing upside down for 15 sec. Finally remove and discard the absorbant mat and count the radioactivity in each test tube containing the MAM until 10,000 counts are accumulated.

Calculations.

$$T_3 \text{ uptake index} = \frac{\text{net cpm in serum}}{\text{net cpm in human pooled serum}}.$$

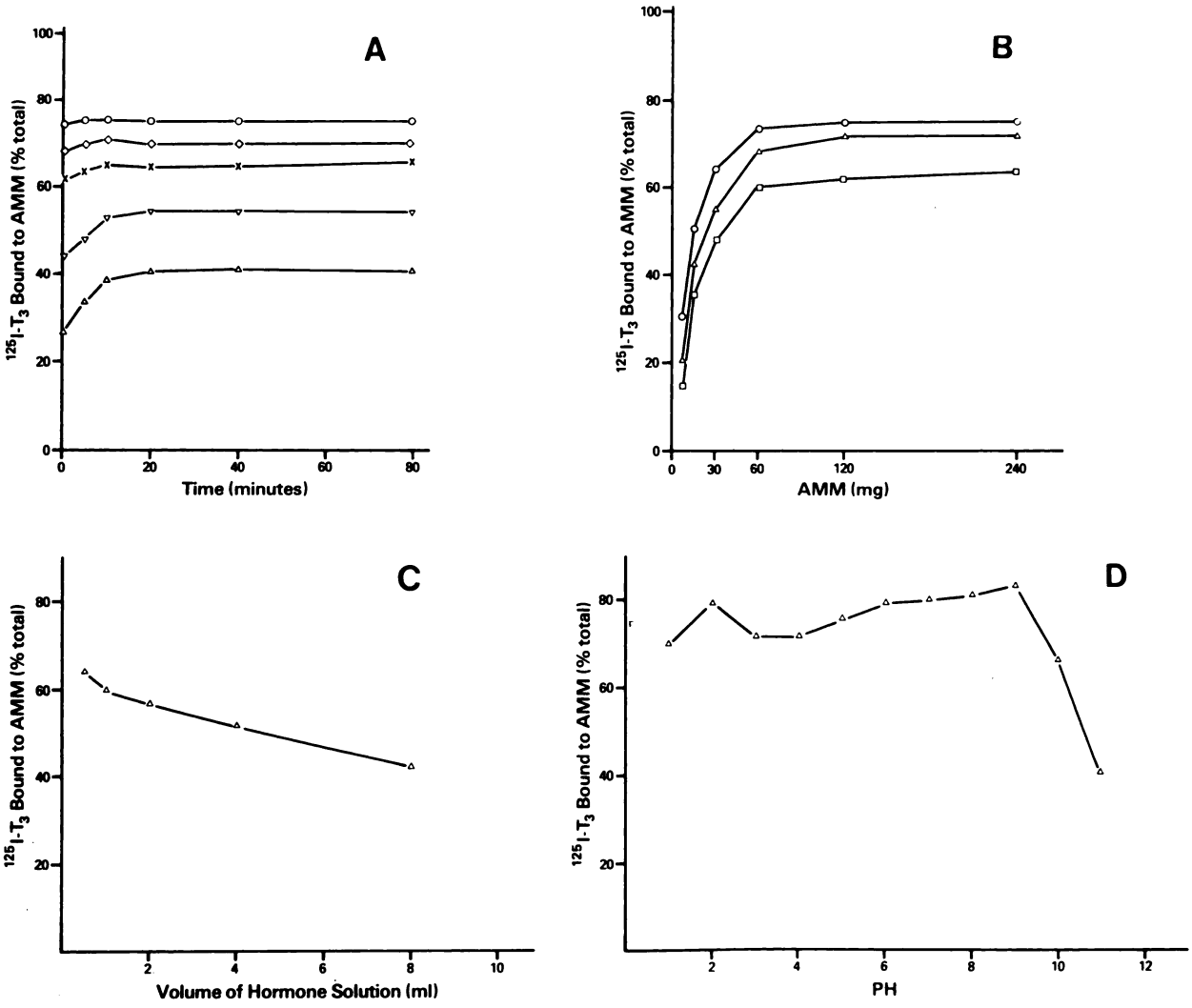

FIG. 1. Binding of [^{125}I] T_3 to MAM. (A) against incubation time, with mg MAM as follows: (△) 7.5, ( ) 15, (×) 30, ( ) 60 and (○) 120. (B) against wt. of MAM, at temperatures: (□) 35°C, (△) 22°C, (○) 4°C. (C) against reaction volume. (D) against pH.

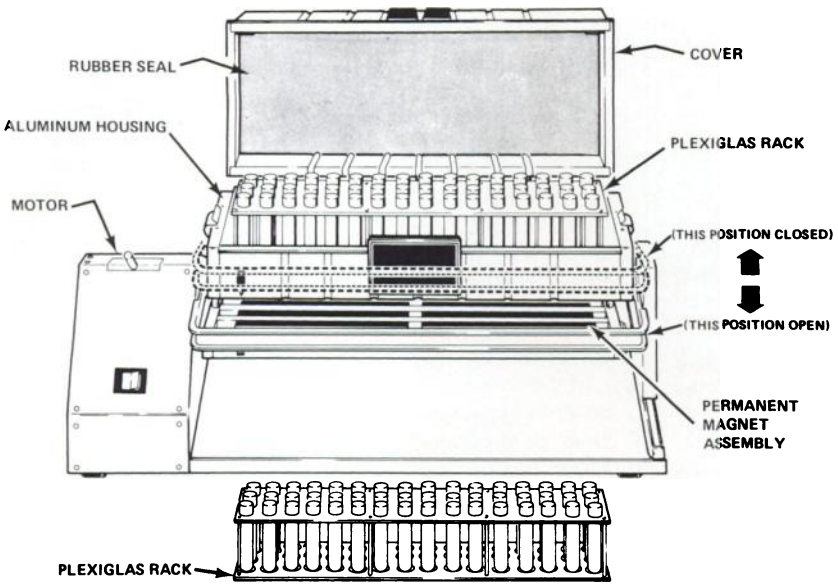

FIG. 2. Diagramatic representation of Magnetic Separator.

RESULTS AND DISCUSSION

We prepared the MAM by modifying previously reported methods (*3,4*) in which albumin microspheres were made water-insoluble by heat denaturation at 100–150°C. In our procedure MAM are insolubilized by chemical cross-linking techniques at room temperature (*5*). This process consists of injecting a suspension of barium ferrite in an aqueous solution of protein into a stirred bath of oil containing n-butanol (dehydrating agent) and glutaraldehyde (cross-linking reagent). The cross-linking process is monitored by sampling; the samples are first washed with n-heptane and then with water, and cross linking is stopped when no protein is released into the water (spectrophotometric determination).

Correlation studies using MAM and heat-denatured microspheres of human serum albumin (*4*) showed that the two types of particles exhibited similar sorption* of [^{125}I] T_3 when the weight of the MAM was increased to allow for the presence of nonadsorbing barium ferrite. A paired t-test comparison showed no difference at the 99% confidence level.

The magnetic separator allows simultaneous handling of 100 tubes with minimum manipulation. Two correlation studies of this device with centrifugation, using 15 tubes per assay, indicated that the two methods gave similar reproducibility. (Coefficients of variation were 2.9 and 2.3%, respectively.)

Binding of [^{125}I] T_3 to MAM. For a given suspending volume, MAM have a saturation degree close to zero for up to 10 μg of triiodothyronine per liter. This is shown in Table 1 by the constant fraction of [^{125}I] T_3 sorbed by the MAM. Figure 1A indicates that the time to reach equilibrium depends on the weight of MAM. This is practically instantaneous above 50 mg. Figure 1B also shows a temperature dependence: more [^{125}I] T_3 binds to MAM at 4°C than at 37°C. Figures 1C and 1D show a decrease in the binding of [^{125}I] T_3 to MAM with increasing reaction volume of barbital buffer, or solution pH >9. We found the latter effect to be caused by partial hydrolysis of the MAM in the basic medium.

In other studies, we found that iodinated proteins such as I-125 human serum albumin and I-125 IgG do not bind to MAM. We therefore believe that a clean separation takes place between free [^{125}I] T_3 and that bound to serum proteins when MAM is used in the T_3 uptake test.

Aspects of the T_3 uptake test. The assay we have described is a modification and simplification of the procedure reported by Rolleri et al. (*2*). We reduced the volume of serum used per test to 0.1 ml and reduced the total assay volume to 1.1 ml.

For a given weight of MAM, the radioactivity found in MAM is inversely proportional to the number of serum TBG binding sites available. However, as the weight of MAM varies so does the amount of [^{125}I] T_3 sorbed due to the equilibrium shift. In order to obtain T_3 uptake values independent of the weight of MAM, we report the data as a T_3 uptake index.

We optimized the sensitivity of the T_3 uptake test by

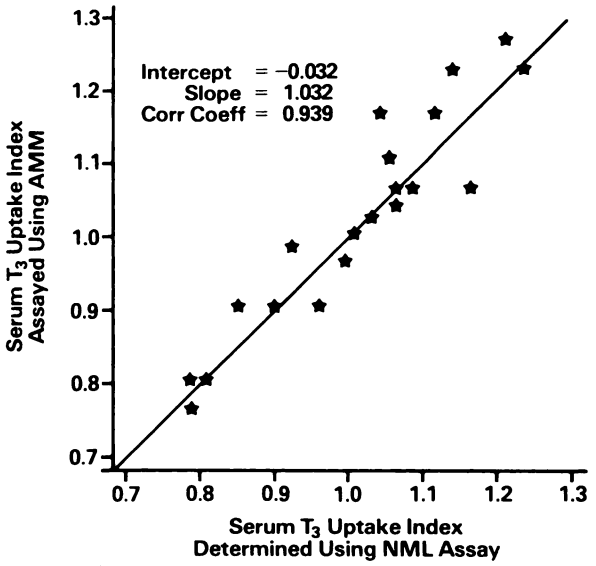

FIG. 3. Correlation of T_3 uptake indices obtained using this method and those obtained with assay from nuclear medical laboratories.

TABLE 3. PRECISION OF T_3 UPTAKE TEST

Sera	Intra-assay T_3 uptake index	CV*	Interassay T_3 uptake index	CV
Hypothyroid	0.804 ± 0.024†	3.1	0.798 ± 0.062‡	7.8
Euthyroid	0.993 ± 0.038	3.8	0.996 ± 0.045	4.5
Hyperthyroid	1.117 ± 0.019	1.7	1.120 ± 0.037	3.3

* CV = (s.d./mean) 100.

† Mean ± s.d., n = 6. The s.d. was calculated from the equation: $\sqrt{[\Sigma(d^2)]/2n}$, where d = difference between duplicates and n = the number of pairs.

‡ Mean ± s.d., n = 9. The s.d. was calculated from mean of duplicate determinations done on each assay.

TABLE 4. DETERMINATION OF T_3 INDICES IN CONTROL SERUM SAMPLES

Control sera	Lot No.	T_3 uptake index mean ± s.d.	Samples tested
Curtis (depressed)	11201E4	0.792 ± 0.032	12
Curtis (normal)	11001E4	0.965 ± 0.028	12
Curtis (elevated)	10001E4	1.163 ± 0.015	12
Dade (Level I)	RCS-14	0.811 ± 0.022	14
Dade (Level II)	RCS-14	0.972 ± 0.033	14
Dade (Level III)	RCS-14	1.201 ± 0.020	14
Lederle I	2945-432	0.925 ± 0.019	8
Lederle II	2946-406	1.120 ± 0.026	8

TABLE 5. EFFECT OF TIME AND TEMPERATURE ON UPTAKE VALUES MEASURED USING MAM*

Incubation time (min)	T_3 uptake index	Incubation temperature (°C)	T_3 uptake index
15§	0.989 ± 0.020†	4‡	0.997 ± 0.018
30	0.995 ± 0.025	22	0.982 ± 0.021
60	0.993 ± 0.029	35	0.995 ± 0.031
120	0.997 ± 0.027		

* Pooled normal human serum was used.

† Mean ± s.d., n = 4.

‡ Incubation time was 30 min.

§ Measurements were done at room temperature (22°C).

evaluating the slopes of plots in which the percentage of [^{125}I] T_3 bound to various weights of MAM from solutions of various ionic strength was studied as a function of thyroxin added to a sample of pooled human serum. The study was performed by incubating for 30 min 1 ml barbital buffer B (varying the ionic strength from 0.05 to 0.4) containing [^{125}I] T_3, 0.1 ml pooled human serum, 0.1 ml thyroxine solution (0–24 μg/100 ml), and increasing amounts of MAM (7.5, 15, 30, and 60 mg). The human serum was obtained by pooling 50 equal volumes of serum taken from healthy donors. Table 2 shows these slopes as we determined them by regression analysis. We judged that the optimum assay conditions were those producing plots with the greatest slope—i.e., those in which the ability of MAM to detect a change in thyroxin was highest. We concluded that the most sensitive T_3 uptake test would be obtained with the use of 30 mg of MAM and a buffer of ionic strength 0.2.

Normal range of the assay. We calculated this from 108 serum samples containing 4.5–11.4 μg T_4 per 100 milliliters as determined by RIA. The T_3 indices determined were normally distributed, having a mean of 0.990 and a range of 0.887–1.093 (99% confidence for 75% of the population).

Precision of the assay. We determined the intra-assay and interassay precision utilizing hypothyroid, normal, and hyperthyroid pooled serum samples. For the intra-assay precision we ran six assays in duplicate over a period of 2 wk, and for the interassay precision we ran nine assays in duplicate over a period of 2 mo. The respective coefficients of variation are shown in Table 3. These averaged 2.87% and 5.2%, respectively, over the range of sera tested.

Accuracy of the assay. We evaluated this by using the assay to run T_3 uptake indices on commercial samples of control serum (Table 4) and comparing the results with the expected normal or abnormal regions found by other methods as reported by the control-serum manufactures. All sera tested were within the normal or obnormal regions expected. Additional studies showed that the T_3 uptake values measured using MAM were independent of time and temperature (Table 5).

In other studies we determined T_3 uptake values for patient sera representative of various typical thyroid states both by the present method and by a commercial kit†. Figure 3 shows the correlation between the assays indicating that both provided similar clinical information for the sera tested (corr. = 0.939). A paired *t*-test comparison showed no difference at the 95% confidence level.

CONCLUSION

We have found this T_3 uptake test to be precise, sensitive, and fast. Less than 70 min was required to perform T_3 uptake tests for 100 serum samples with excellent reproducibility (this time did not include counting). Furthermore, we found the assay to be independent of time and temperature.

FOOTNOTES

* Sorption refers to a process that may involve adsorption and/or absorption.

† Nuclear-Medical Lab, Inc., Dallas, Tex.

REFERENCES

1. ROLLERI E, HEGESIPPE M: Use of serum albumin microspheres as a reversible adsorbent for studying protein-small molecule interactions. *Biochim Biophys Acta* 295: 429–437, 1973

2. ROLLERI E, BUZZIGOLI G, PLASSIO G: Serum triiodothyronine uptake using albumin microspheres in the assessment of thyroid function. *J Nucl Med* 13: 893–898, 1972

3. PASQUALINI R, PLASSIO G, SOSI S: The preparation of albumin microspheres. *J Biol Nucl Med* 13: 80–84, 1969

4. RHODES BA, BOLLES TF: Albumin microspheres-current methods of preparation and use. In *Radiopharmaceuticals*, Subramanian G, Rhodes B, Cooper J, et al, eds. New York, Society of Nuclear Medicine Inc., 1975, pp 282–283

5. ITHAKISSIOS DS, KUBIATOWICZ DO: Thyroxin radioassays utilizing albumin magnetic microparticles. *J Nucl Med* 18: 622–623, 1977 (Abst)