Measurement of Triiodothyronine in Urine

KATSUMI YOSHIDA, TOSHIRO SAKURADA, NOBUKO KAISE,
MAKIKO YAMAMOTO, KAZUO KAISE, SHINTARO SAITO and
KAORU YOSHINAGA

*The Second Department of Internal Medicine, Tohoku
University School of Medicine, Sendai 980*

YOSHIDA, K., SAKURADA, T., KAISE, N., YAMAMOTO, M., KAISE, K., SAITO, S. and YOSHINAGA, K. *Measurement of Triiodothyronine in Urine*. Tohoku J. exp. Med., 1980, 132 (4), 389-395 — A radioimmunoassay procedure to measure triiodothyronine (T_3) in unextracted urine is described. One hundred μ l of T_3 standards or samples were incubated with 100 μ l of T_3 antiserum (1:40,000), 100 μ l of tracer $^{125}\text{I}-T_3$ and 700 μ l of 0.05 M borate buffer, pH 8.6. Separation of free from bound antigen was achieved by dextran coated charcoal. The cross-reaction of L- T_3 with L- T_4 in this assay system was less than 0.2%. Dilution of high T_3 urine was parallel to the standard curve. Recovery was 101 ± 8 %. Coefficients of variation were 3-8% within each assay and 13% between assays. Euthyroid subjects excreted 0.81 ± 0.39 μ g (mean $\pm$ s.d.) in 24 hr. Similar values were obtained in pregnant women, euthyroid patients with chronic thyroiditis, and a patient with thyroxine-binding globulin deficiency. Excretion of urinary T_3 was high (7.48 ± 3.32 μ g/24 h) in patients with hyperthyroidism, and low (0.14 ± 0.15 μ g/24 hr) in patients with hypothyroidism. Urinary T_3 excretion was almost undetectable in terminal renal failure. Positive correlations were found between urinary T_3 and serum total T_3 ($r=0.89$) and especially serum free T_3 ($r=0.97$). A positive correlation was also found between urinary T_3 and creatinine clearance ($r=0.63$). Mean urinary clearance of T_3 (C_{T_3} =Urinary T_3 excretion/serum free T_3) was 166 ml/min and was significantly higher than their mean creatinine clearance (110 ml/min). The measurement of T_3 in urine is reliable and easy to perform, and may allow a new approach to the understanding of thyroid hormone metabolism. — triiodothyronine in urine; radioimmunoassay; free thyroid hormone

The free thyroid hormone is available to the tissue to induce the effects on the cell metabolism (Ingbar and Freinkel 1960; Robbins and Rall 1960). Therefore, the concentration of the free thyroid hormone acts as an important determinant of the metabolic state. Since only a small percentage (0.2%-0.3%) of circulating triiodothyronine (T_3) is present in the free form, the measurement of free T_3 is too complicated to be applied as a routine test (Sterling and Hegedus 1962; Schussler and Plager 1967; Lee et al. 1964). It is considered that the urinary concentration of T_3 might change in accordance with the level of free T_3 in serum. In the present paper, a method of radioimmunoassay (RIA) for urinary T_3 is described.

MATERIALS AND METHODS

Radioimmunoassay for T_3 in urine

Urinary T_3 was measured by RIA on unextracted urine according to the RIA method for serum T_3 described previously (Sakurada et al. 1973). Specific antiserum against T_3 was raised by immunizing the rabbits with T_3 -methylester hydrochloride-bovine serum albumin (BSA) conjugate in complete Freund's adjuvant.

As shown in Fig. 1, 100 μ l of undiluted urine samples or T_3 standards were incubated with 100 μ l of T_3 antiserum (1:40,000), 100 μ l of tracer ^{125}I - T_3 and 700 μ l of buffer overnight (16–20 hr) at 4°C. The buffer used in the assay system was 0.05 M borate buffer, pH 8.6, containing 0.5% BSA. Standard tubes were received equivalent volume (100 μ l) of pooled hormone-free urine treated with 7 per cent activated charcoal. Separation of free from bound antigen was achieved by dextran-coated charcoal.

Serum T_3 was measured by RIA previously described. Serum free fractions of T_3 were determined by a gel filtration technique (Sakurada et al. 1969), modified from that of Lee et al (1964).

Fig. 1. Method of radioimmunoassay for T_3 in urine.

Materials

Urines were obtained from 45 euthyroid patients with no thyroid disorders and no proteinuria, 18 hyperthyroid patients, 14 hypothyroid patients, 7 euthyroid patients with chronic thyroiditis, 3 patients with anorexia nervosa, one patient with thyroxine binding-globulin (TBG) deficiency, 6 patients with severe chronic renal failure (creatinine clearance, Ccr, <10 ml/min), and 11 subjects in the third trimester of pregnancy. Urine was collected from each subject over a period of 24 hr. The total volume was measured and an aliquot was stored frozen at -20°C.

Validation

Fig. 2 shows the typical curvilinear standard curve and dilution curve for T_3 . Dilution curve was parallel to the standard curve. Cross reaction of L- T_3 with L- T_4 in this assay system was less than 0.2%. Various amounts of L- T_3 were added to urine and recovery was $101 \pm 8\%$ (mean $\pm$ s.d.). Coefficients of variation were 3–8% within each assay and 13% between assays. Correction of urinary pH was unnecessary, since the urinary pH ranging between 4 and 11 did not affect the T_3 determination by RIA.

Fig. 2. Standard curve and dilution curve for radioimmunoassay of T_3 in urine. $\blacktriangle$, T_3 standard curve; $\circ$, euthyroid; $\triangle$, hyperthyroid; $\bullet$, hypothyroid.

Calculations

Serum free T_3 concentration was calculated as the product of total T_3 and % free T_3 by gel filtration. Clearances of free T_3 (CT_3) were calculated as follows:

$$CT_3 = \frac{\text{Urine } T_3 \text{ (ng/24 h)}}{\text{Serum free } T_3 \text{ (ng/ml)} \times 1,440} \text{ (ml/min)}$$

RESULTS

Urinary T_3 concentrations

Excretion of urinary T_3 in 45 euthyroid patients was $0.81 \pm 0.39 \mu\text{g/day}$ (mean $\pm$ s.d.), ranging from 0.3 to $1.96 \mu\text{g/day}$. The urinary T_3 in a patient with TBG deficiency was normal ($0.9 \mu\text{g/day}$). The mean urinary T_3 in 18 hyperthyroid patients was $7.48 \pm 3.32 \mu\text{g/day}$ (range, 2.88 – $14.6 \mu\text{g/day}$), a value significantly higher than that observed in euthyroid subjects (unpaired t -test, $p < 0.001$).

The mean urinary T_3 in 14 hypothyroid patients was $0.14 \pm 0.15 \mu\text{g/day}$ (range, 0 – $0.54 \mu\text{g/day}$), a value significantly lower than the corresponding normal value. ($p < 0.001$).

The mean urinary T_3 in 7 euthyroid patients with chronic thyroiditis was $0.54 \pm 0.21 \mu\text{g/day}$, a value slightly lower than the corresponding normal value, but the difference was not statistically significant.

Mean urinary T_3 in the 11 pregnant women was $1.06 \pm 0.39 \mu\text{g/day}$, a value slightly higher than that observed in euthyroid patients, but the difference was not significant.

All three patients with anorexia nervosa had low urinary T_3 levels, and all patients with chronic renal failure had very low urinary T_3 levels (Fig. 3).

Fig. 5. Correlation between serum free T_3 and urinary T_3 excretion. $\circ$, euthyroid; Δ , hyperthyroid; $\bullet$, hypothyroid.

Correlation between urinary T_3 and Ccr.

A positive correlation was also found between urinary T_3 and Ccr ($r=0.63$, $p<0.01$). Urinary T_3 was significantly reduced in chronic renal failure (Fig. 6).

Renal clearance of free serum T_3

The mean renal clearance rate of free T_3 in 15 euthyroid patients was 174 ml/min, and the range was wide from 42 to 366 ml/min (Fig. 7). The mean T_3 clearance rates in 10 hyperthyroid patients and in 2 hypothyroid patients were 166

Fig. 6. Correlation between urinary T_3 and creatinine clearance.

Fig. 7. Correlation between urinary T_3 clearance and serum T_3 . o, euthyroid; Δ , hyperthyroid; ●, hypothyroid.

ml/min and 106 ml/min, respectively. As a whole, the mean T_3 clearance rate was 166 ml/min and was significantly higher than their mean Ccr (110 ml/min) ($p < 0.05$).

DISCUSSION

Since Chan and coworkers (1972) have reported the measurement of urinary T_3 a number of studies of urinary T_3 have been published (Burke et al. 1972; Hüfner and Hesch 1973; Gaitan et al. 1975; Rogowski et al. 1978). In the first study of urinary T_3 by Chan et al., urine was acidified to pH 4.0 and urinary T_3 was extracted with ethyl acetate prior to the RIA. Many subsequent papers pointed out that when T_3 was assayed on unextracted urine, the urinary T_3 values were about one-third of those reported by Chan et al., and suggested that the extraction procedure might induce the hydrolysis of T_3 conjugates.

We also measured urinary T_3 by RIA on unextracted urine and the obtained result in euthyroid controls was of the same order as those of several of the studies mentioned above, about one-third of those by Chan et al. We have not established whether T_3 conjugates or metabolites are measured in our RIA system. But, using enzymatic and acid hydrolysis, Burke et al. (1972) and Gaitan et al. (1975) reported that the conjugate was not measured in their system, because urinary T_3 value increased after hydrolysis. Our normal value is close to those of Burke et al. and Gaitan et al. Therefore, the conjugate might not be measured in our system either.

As previously reported (Sakurada et al. 1973), our antisera against T_3 were specific, but they cross-react slightly with T_3 derivatives such as triiodothyroacetic

acid, triiodothyropropionic acid and so on. Therefore, there is still a possibility that these metabolites were also measured in the present RIA system, though the serum values are reported to be very low (Nakamura et al. 1978).

Anyway, excellent correlations were found between urinary T_3 and serum T_3 , and especially serum free T_3 . Furthermore, urinary T_3 was not influenced by changes in circulating TBG concentration, since the normal values were found in pregnancy with high serum TBG as well as in TBG deficiency. These results indicate that urinary T_3 values reflect serum free T_3 concentrations.

The mean T_3 clearance rate was significantly higher than creatinine clearance. This might be the result of tubular excretion of T_3 , perhaps T_3 being produced in this tissue from T_4 by deiodination.

The development of a simple and reliable assay for T_3 in urine will permit further studies on T_3 metabolism.

References

- 1) Burke, C.W., Shakespear, R.A. & Fraser, T.R. (1972) Measurement of thyroxine and triiodothyronine in human urine. *Lancet*, **2**, 1177-1179.
- 2) Chan, V., Landon, J., Besser, G.M. & Ekins, R.P. (1972) Urinary triiodothyronine excretion as index of thyroid function. *Lancet*, **2**, 253-256.
- 3) Gaitan, E.J., Wahner, W.H., Gorman, A.C. & Jiang, N. (1975) Measurement of triiodothyronine in unextracted urine. *J. Lab. clin. Med.*, **86**, 538-546.
- 4) Hüfner, M. & Hesch, R.D. (1973) Triiodothyronine determinations in urine. *Lancet*, **1**, 101-102.
- 5) Ingbar, S.H. & Freinkel, N. (1960) Regulation of the peripheral metabolism of the thyroid hormones. *Recet. Progr. Hormone Res.*, **16**, 353-403.
- 6) Lee, N.D., Henry, R.J. & Golub, O.J. (1964) Determination of the free thyroxine content of serum. *J. clin. Endocr.*, **24**, 486-495.
- 7) Nakamura, Y., Chopra, I.J. & Solomon, D.H. (1978) An assessment of the concentration of acetic acid and propionic acid derivatives of 3, 5, 3'-triiodothyronine in human serum. *J. clin. Endocr.*, **46**, 91-97.
- 8) Robbins, J. & Rall, J.E. (1960) Proteins associated with the thyroid hormones. *Physiol. Rev.*, **40**, 415-498.
- 9) Rogowski, P., Siersbok-Nielsen, K. & Molholm Hansen, J. (1978) Urinary excretion of thyroxine and triiodothyronine in different thyroid function states in man. *Acta Endocr.*, **87**, 525-534.
- 10) Sakurada, T., Saito, S., Inagaki, K., Takayama, S. & Torikai, T. (1969) Quantitative determination of total and free triiodothyronine and thyroxine. *Tohoku J. exp. Med.*, **99**, 179-188.
- 11) Sakurada, T., Saito, S., Yamaguchi, T., Yamamoto, M., Demura, R., Demura, H., Fukuchi, S., Yoshida, K. & Torikai, T. (1973) Radioimmunoassay of triiodothyronine. *Tohoku J. exp. Med.*, **110**, 329-340.
- 12) Schussler, G.C. & Plager, J.E. (1967) Effect of preliminary purification of ^{131}I -thyroxine on the determination of free thyroxine in serum. *J. clin. Endocr.*, **27**, 242-250.
- 13) Sterling, K. & Hegedus, A. (1962) Measurement of free thyroxine concentration in human serum. *J. clin. Invest.*, **41**, 1031-1040.