Clinical application and mechanization of a simple and inexpensive method for estimating T₃ uptake in serum

W. W. WALTHER

From the Department of Pathology, Whipps Cross Hospital, London

SYNOPSIS This very inexpensive method of estimating T₃ uptake, already described (Walther, 1971), uses only 0.1 ml of patient's serum and discriminates between thyrotoxicosis and myxoedema much better than the resin sponge method. It can readily be mechanized and the 'free thyroxine index' derived has normal limits of 3.0 to 8.0.

The protein-bound iodine (PBI) alone is commonly used as a diagnostic aid in suspected thyroid disease. Combining the result of a T₃ uptake test with the PBI gives a 'free thyroxine index' (Clark and Horn, 1965) which provides a more accurate means of

Received for publication 15 July 1971.

diagnosis. The free thyroxine index (FTI) is in fact an arbitrary figure which indicates the level of 'free' thyroxine which probably determines the actual thyroid status of the patient. It is easily calculated using the present Sephadex method, by multiplying the Sephadex uptake ratio which is uptake with test

Fig. 1 The results in 230 patients where the PBI is plotted against the FTI (see text). The normal limits for each method are delineated.

○ indicates myxoedema
 . indicates euthyroid
 △ indicates hyperthyroid
 X indicates anomalous results (see text)

Fig. 2 Results of calculating the free thyroxine index recorded in separate columns according to different thyroid status.

serum/uptake with standard serum by the PBI content of the serum in $\mu\text{g/ml}$. Figure 1 shows the effect of applying this correction to the protein-bound iodine. It will be seen that a number of sera with low PBI concentrations are within the normal FTI range, and the same is true in a number with raised concentrations. The special advantage of calculating the FTI is well shown in Fig. 2 which tabulates the results obtained in 310 patients according to thyroid function. The column marked 'anomalous results' includes cases in which there was temporary enhancement of thyroid function (febrile cases) as well as those in which thyroid function had been reduced by therapy, for instance, antidiabetic drugs. A further advantage of this method over the resin method is that no correction factor (Osorio, 1967) is required to calculate the free thyroxine index. This was shown by making progressive

Fig. 3 Centrifuge tube fitted with plastic collar to retain it in sampler plate.

Fig. 4 Sampler and plate with scintillation detector in position.

dilutions of the serum of a pregnant woman whose PBI level was $10\text{ }\mu\text{g/ml}$ and Sephadex uptake ratio was 0.4. The latter was found to vary inversely with the concentration (see Table). The concentration of FTI results about the mean level of 5.5 is obvious. The method has been partly mechanized. A Technicon AutoAnalyzer sampler plate was modified to hold the $4\text{ in} \times \frac{5}{8}\text{ in.}$ plastic centrifuge tubes in

Concentration of Serum (%)	Sephadex Uptake Ratio	Free Thyroxine Index (Value Based on Serum Concentration)
100	0.4	4.0
80	0.48	3.8
60	0.65	3.9
40	1.0	4.0
20	1.83	3.7

Table Free thyroxine index

Fig. 5 Section of lead shield to show position of centrifuge tube and inclined plane.

Fig. 7 Tracing obtained from recording ratemeter. This reads from right to left.

which the reaction is done. Alternate holes in the sampler plate were bored out to size and short guides consisting of $\frac{3}{4}$ in. long butt ends of disposable 10 ml syringe barrels stuck underneath the sampler plate. Plastic collars were fitted to the centrifuge tubes to stop them falling too far through the sampler plate (Fig. 3).

A thin crystal end-window scintillation detector (particularly suited for counting a 'soft' isotope like ^{125}I) is fixed at a suitable level (Fig. 4) and fitted

Fig. 6 Special timing disc for Technicon sampler.

Fig. 8 Rubber stopper prevents the suction tube from accidentally disturbing the Sephadex beads.

with a lead shield $\frac{1}{16}$ in. thick with a $\frac{5}{8}$ in. diameter hole in the centre. This lead shield also provides an inclined plane so that the plastic centrifuge tubes can ride up it and drop into the central hole to contact the upper surface of the crystal container (Fig. 5). The scintillation detector is connected with a recording ratemeter. A special timing disc was made so that each centrifuge was being counted for 80 seconds and the transfer to the next tube took 10 seconds (Fig. 6). A typical record of results is shown

in Fig. 7, the first two peaks indicating a normal and a high standard. Mixing the reagents while binding is occurring can be facilitated by placing the sampler plate with its burden of plastic tubes on a Matburn type mixer propped up so that the long axis of the tubes is about 20° from horizontal. The mixer revolves at about 30 rpm and this can be continued for one hour thus eliminating the laborious mixing at 10-minute intervals of the original method. During the washing process, the supernatant fluid is removed by a Pasteur pipette attached to a filter pump. A distance piece consisting of a rubber bung with a hole in it is attached to the Pasteur pipette to prevent

it disturbing the Sephadex precipitate (Fig. 8). If the various pieces of equipment are to hand, their modification costs almost nothing apart from a little time. The method is now in routine use and gives excellent results.

References

- Clark, F., and Horn, D. B. (1965). Relationship between serum protein-bound iodine and resin uptake of ^{131}I -triiodothyronine. *J. clin. Endocr.*, **26**, 352-353.
 Osorio, C. (1967). Calculation of the free thyroxine index from T_3 uptake tests. *J. clin. Path.*, **20**, 351-352.
 Walther, W. W. (1971). Simple inexpensive micro method for the estimation of T_3 uptake. *J. clin. Path.*, **24**, 177-178.

Reports and Bulletins prepared by the Association of Clinical Biochemists

The following reports and bulletins are published by the Association of Clinical Biochemists. They may be obtained from The Administrative Office, Association of Clinical Biochemists, 7 Warwick Court, Holborn, London, WC1R 5DP. The prices include postage, but airmail will be charged extra. Overseas readers should remit by British Postal or Money Order. If this is not possible the equivalent of 50p is the minimum amount that can be accepted.

SCIENTIFIC REPORTS

3 Automatic Dispensing Pipettes. An assessment of 35 commercial instruments 1967 P. M. G. BROUGHTON, A. H. GOWENLOCK, G. M. WIDDOWSON, and K. A. AHLQUIST 85p (\$2)

4 An Evaluation of five Commercial Flame Photometers suitable for the Simultaneous Determination of Sodium and Potassium March 1970 P. M. G. BROUGHTON and J. B. DAWSON 85p (\$2)

SCIENTIFIC REVIEWS

1 The Assessment of Thyroid Function March 1971 F. V. FLYNN and J. R. HOBBS 62½p (\$1.50)

TECHNICAL BULLETINS

9 Determination of Urea by AutoAnalyzer November 1966 RUTH M. HASLAM 42½p (\$1)

11 Determination of Serum Albumin by AutoAnalyzer using Bromocresol Green October 1967 B. E. NORTHAM and G. M. WIDDOWSON 42½p (\$1)

13 An Assessment of the Technicon Type II Sampler Unit March 1968 B. C. GRAY and G. K. MCGOWAN 42½p (\$1)

14 Atomic Absorption Spectroscopy. An outline of its principles and a guide to the selection of instruments May 1968 J. B. DAWSON and P. M. G. BROUGHTON 42½p (\$1)

15 A Guide to Automatic Pipettes (2nd edition) June 1968 P. M. G. BROUGHTON 42½p (\$1)

16 A Guide to Automation in Clinical Chemistry May 1969 P. M. G. BROUGHTON 62½p (\$1.50)

17 Flame Photometers (2nd edition) 1969 P. WILDING 62½p (\$1.50)

18 Control Solutions for Clinical Biochemistry (4th edition) March 1970 P. M. G. BROUGHTON 62½p (\$1.50)

19 Spectrophotometers. A comparative list of low-priced instruments readily available in Britain May 1970 C. E. WILDE and P. SEWELL 62½p (\$1.50)

20 Quantities and Units in Clinical Biochemistry June 1970 P. M. G. BROUGHTON 62½p (\$1.50) More than 30 copies in units of 10 at 20p

21 Filter Fluorimeters: A comparative list of 18 instruments September 1970 H. BRAUNSBURG and S. BROWN 62½p (\$1.50)

22 Bilirubin standards and the Determination of Bilirubin by Manual and Technicon AutoAnalyzer Methods January 1971 BARBARA BILLING, RUTH HASLAM and N. WALD 62½p (\$1.50)

23 Interchangeable Cells for Spectrophotometers and Fluorimeters September 1971 E. S. BROWN and A. H. GOWENLOCK 62½p (\$1.50)