Comparison of three simple methods for the assessment of 'free' thyroid hormone

T. M. D. GIMLETTE¹

From the Radio-Isotope Laboratory, St. Thomas's Hospital, London

SYNOPSIS A dialysis method for estimating 'free' thyroxine is compared with two indirect methods using the uptake of triiodothyronine by the red cells and by a Sephadex column. The three methods give results which correlate well and the Sephadex method is the simplest.

The theoretical basis of such tests and their role in the assessment of thyroid status is briefly discussed.

A number of direct and indirect methods for evaluating the free fraction of the total plasma thyroxine have been described. The most direct method, using radioiodine-labelled thyroxine, is equilibrium dialysis (Sterling and Hegedus, 1962); simpler techniques for estimating 'dialysable thyroxine' as a rate of transfer across a semi-permeable membrane (Christensen, 1959; Gimlette, 1964) and by Sephadex chromatography (Lee, Henry, and Golub, 1964) have also been described. More indirect methods using labelled triiodothyronine have included the estimation of the uptake of triiodothyronine by the red cells (Hamolsky, Stein, and Freedberg, 1957), by ion exchange resins (Mitchell, 1958) and resin sponge (Mitchell, Harden, and O'Rourke, 1960), Sephadex columns (Shapiro and Rabinowitz, 1962; Gimlette, 1967), and charcoal (Herbert, Gottlieb, Lau, Gilbert, and Silver, 1965). This paper reports a comparison of three simple methods, namely, the dialysable thyroxine technique, the red cell uptake of triiodothyronine, and Sephadex column chromatography.

METHODS

1 Sixteen-hour dialysable thyroxine was estimated by a method previously described (Gimlette, 1964). This is the estimation of the percentage of a tracer quantity of ¹³¹I thyroxine added to the plasma under investigation which is dialysed, during 16 hours at 37°C., out of a small cellophane bag immersed in a sample of the same plasma in a test tube. The normal range was 0.46–0.80%.

2 The red-cell uptake of triiodothyronine was estimated by the method of Hamolsky *et al.* (1957) with some

modifications: 2 ml. samples of blood were used, and incubation and washing were carried out in glass test tubes. The results were calculated in terms of a triiodothyronine-binding coefficient (Adams, Specht, and Woodward, 1960).

$$\text{Red cell binding coefficient} = \frac{1-H}{H} \times \frac{U}{1-U}$$

$$\text{Where } H = \frac{\text{Haematocrit}}{100} \quad U = \frac{\% \text{ Red cell uptake}}{100} \quad (\text{uncorrected})$$

The normal range found in extensive previous studies was 0.070 to 0.120 with the technique used.

3 Sephadex chromatography was carried out by a method previously described (Gimlette, 1967). The results were expressed in terms of a triiodothyronine-binding coefficient and the normal range was 0.050–0.100.

MATERIAL

One hundred and twenty patients were studied by the three methods described. All the patients had been referred for the establishment of their thyroid status and this was done by routine methods including radioiodine studies. Fifty-one patients were euthyroid and without significant organic disease, 12 were euthyroid but suffering from serious organic disease of various kinds, 38 were hyperthyroid, and 19 were hypothyroid.

In 39 of these patients serum thyroxine was also estimated by a modification of the method of Ekins, previously described (Gimlette, 1965). The normal range was 5.5–14.0 µg./100 ml.

In the majority of these patients thyroxine-binding globulin and prealbumin maximum binding capacities were estimated (Oppenheimer, Squef, Surks, and Hauer, 1963). The normal ranges were 15–25 µg./100 ml. and 110–300 µg./100 ml.

¹Present address: Department of Nuclear Medicine, The Liverpool Clinic, 1 Myrtle St., Liverpool 7.

RESULTS

The results of the study are indicated in Tables I and II and in Figures 1, 2, and 3. Clearly there is a correlation between the three methods of assessing 'free' thyroxine, but this is not precisely linear.

TABLE I¹

THYROID STATUS ESTIMATED BY THREE METHODS

Method	Euthyroid	Euthyroid and Ill	Hyperthyroid	Hypothyroid	Total
Sephadex	51 (2)	12 (5)	38 (5)	19 (4)	120 (16)
Red cell uptake	51 (2)	12 (7)	38 (3)	19 (4)	120 (16)
Dialysable T ₄	51 (3)	12 (9)	38 (3)	19 (7)	120 (22)

¹The figures indicate the number of patients in each group and the figures in brackets those incorrectly assigned.

The results with the red cell method and the Sephadex method both indicated the correct diagnosis in 87%. The dialysable thyroxine method indicated the correct diagnosis in 82%. The difference between the results is probably not significant

and may be due to the normal range in the latter, established in an earlier study (Gimlette, 1964), being a little low. With all three methods the diagnostic accuracy was better in hyperthyroid than in hypothyroid patients and least good in ill patients; over one-third of the incorrect results were high values in this relatively small group, in whom abnormal levels of binding proteins, particularly of thyroxine-binding prealbumin, are frequently present.

The group of patients in whom plasma thyroxine has been estimated is small, but promising results from combining this test with an estimation of 'free' thyroxine by using the product of plasma thyroxine and Sephadex-binding coefficient may be seen (Table II). Misleading results when only one test was used were not infrequent, particularly in the euthyroid, ill group; but when the above product was used a correct separation of all the patients was achieved.

In the euthyroid, ill patients plasma thyroxine and the maximum binding capacity of thyroxine-

TABLE II

RESULTS OF ESTIMATIONS OF PLASMA AND FREE THYROXINE

Patients	Thyroxine (g./100 ml.)	Dialysable Thyroxine	Binding Coefficient		Thyroxine × Sephadex Binding Coefficient	Maximum Binding Capacity	
			Red Cells	Sephadex		T.B.G.	T.B.P.A.
1 Euthyroid	9.0	0.53	0.068	0.063	0.57		
2 Euthyroid	11.7	0.55	0.075	0.066	0.77	26	140
3 Euthyroid	10.3	0.69	0.086	0.070	0.72	16	160
4 Euthyroid	12.7	0.67	0.078	0.066	0.84		
5 Euthyroid	7.4	0.63	0.089	0.066	0.49	20	210
6 Euthyroid	11.0	0.70	0.090	0.065	0.71	22	—
7 Euthyroid	7.7	0.71	0.086	0.076	0.59	17	—
8 Euthyroid	10.5	0.50	0.070	0.056	0.59	23	245
9 Euthyroid	6.8	0.51	0.071	0.062	0.42	26	110
10 Euthyroid	11.2	0.53	0.077	0.062	0.69	22	175
11 Euthyroid, ill	7.0	0.89	0.137	0.108	0.76	17	100
12 Euthyroid, ill	6.3	0.81	0.138	0.118	0.74	21	90
13 Euthyroid, ill	4.0	1.49	0.158	0.124	0.50	16	60
14 Euthyroid, ill	5.7	0.99	0.121	0.108	0.62	20	40
15 Euthyroid, ill	5.3	0.86	0.075	0.064	0.34	14	110
16 Euthyroid, ill	8.7	0.68	0.080	0.070	0.81	16	180
17 Hyperthyroid	21.0	0.79	0.128	0.098	2.06	25	180
18 Hyperthyroid	20.5	0.69	0.108	0.079	1.62	26	160
19 Hyperthyroid	13.5	0.95	0.130	0.099	1.34		
20 Hyperthyroid	15.8	0.91	0.130	0.108	1.71	14	220
21 Hyperthyroid	19.9	1.01	0.151	0.150	2.99	22	70
22 Hyperthyroid	14.2	0.84	0.134	0.120	1.71	23	230
23 Hyperthyroid	24.5	0.86	0.136	0.126	3.09	18	260
24 Hyperthyroid	13.7	0.93	0.130	1.121	1.61	17	250
25 Hyperthyroid	15.0	0.95	0.130	0.096	1.44		
26 Hyperthyroid	15.5	1.01	0.131	0.150	2.33	18	—
27 Hyperthyroid	18.0	0.84	0.134	0.138	2.48	22	—
28 Hyperthyroid	21.6	0.92	0.133	0.116	2.51		
29 Hyperthyroid	20.2	0.78	0.113	0.096	1.94	17	180
30 Hypothyroid	2.0	0.49	0.069	0.044	0.09	31	—
31 Hypothyroid	2.2	0.48	0.072	0.048	0.11	19	220
32 Hypothyroid	6.0	0.41	0.064	0.039	0.23	32	180
33 Hypothyroid	2.5	0.54	0.066	0.040	0.10	27	170
34 Hypothyroid	6.2	0.49	0.075	0.052	0.32		
35 Hypothyroid	1.7	0.55	0.066	0.051	0.08	37	240
36 Hypothyroid	0.0	0.35	0.053	0.034	0.00	28	210
37 Hypothyroid	1.0	0.43	0.061	0.044	0.04	20	260
38 Hypothyroid	1.8	0.52	0.063	0.043	0.24	24	240
39 Hypothyroid	5.7	0.54	0.066	0.048	0.27	25	150

FIG. 1. Comparison of Sephadex binding coefficient and dialysable thyroxine. (The normal ranges are shown by the vertical and horizontal lines.)

FIG. 2. Comparison of Sephadex binding coefficient and red cell binding coefficient.

FIG. 3. Comparison of red cell binding coefficient and dialysable thyroxine.

binding prealbumin was usually decidedly low, but the capacity of thyroxine-binding globulin was only slightly reduced compared with the normal. This suggests that reduced thyroxine-binding pre-albumin may play an important part in producing the raised values for 'free' thyroxine found by all three tests in this small group of ill patients.

DISCUSSION

The object of the tests described above, and of others based on the same principle, is to obtain a value which is proportional to the fraction of the total plasma thyroxine which is 'free', or not bound to protein. The value obtained can be multiplied by the total serum thyroxine or chemical protein-bound iodine (P.B.I.) to give a product which is proportional to the absolute level of free thyroxine, a quantity too small to be measured by direct means. It is generally believed that only 'free' hormone, or hormone unbound to large protein molecules, is able to enter cells and thereafter have its effect on metabolism (Robbins and Rall, 1960; Sterling and Brenner, 1966), and therefore the level of free thyroxine in the plasma probably constitutes the best available index of thyroid status, particularly in the absence of adequate knowledge of the behaviour and mode of action of the hormone within the cell under normal and abnormal conditions.

The direct methods using equilibrium dialysis give a value for the free fraction of the serum

thyroxine under the particular experimental conditions of the technique, although this is not necessarily identical with the value under physiological conditions. The simpler techniques of estimating the rate of dialysis across a semipermeable membrane (Christensen, 1959; Gimlette, 1964) can be used in the same way and give results which are probably proportional to those obtained by equilibrium dialysis.

The indirect methods on the other hand compare the binding power of serum or plasma for triiodothyronine with that of the red cells, resin, Sephadex, or whatever indicator substance is used. The binding power of plasma is proportional to the concentration of free binding sites available (Myant and Osorio, 1959) and this is inversely proportional to the fraction of added radioactive triiodothyronine transferred to the indicator substance, the binding power of which is constant under the experimental conditions (Robbins and Rall, 1960). These techniques give a value which may be expressed as a binding coefficient, and which is related to the free hormone without being a direct estimate (Sterling and Brenner, 1966). Triiodothyronine is used since it has a greater affinity for the indicator substances than thyroxine has, and when quantities of the order of 0.1 $\mu\text{g./100 ml.}$ are added to serum, an easily measurable proportion of this radioactive triiodothyronine is bound by the indicator substances, while the distribution of thyroxine on the plasma-binding sites is not significantly altered. The binding properties of triiodothyronine with plasma proteins are not exactly the same as those of thyroxine. A decrease in binding power of prealbumin, which does not itself bind triiodothyronine, will tend to increase binding of triiodothyronine by the indicator substance, since such a decrease makes fewer plasma binding sites available for thyroxine and so increases the competition for the limited capacity protein sites which thyroxine and triiodothyronine share (Robbins and Rall, 1960). The increase in the 'free' fraction of thyroxine, estimated by equilibrium dialysis, noted in many ill patients and attributed to decreased prealbumin binding (Oppenheimer *et al.*, 1963), is also noted when triiodothyronine binding by red cells or Sephadex is used as the method of assessment.

In the selection of a suitable test for assessment of 'free' thyroid hormone, the ease and convenience of those available must be considered, since there appears to be no gross disparity in the relative values obtained by the various methods in different conditions. A number of direct and indirect methods, some of them rather complex, for the assessment of 'free' hormone have been described. For simple routine purposes, the estimation of triiodothyronine-

binding coefficient by the Sephadex method described (Gimlette, 1967) is suggested as a convenient method which has much to recommend it for the purpose.

REFERENCES

- Adams, R., Specht, N., and Woodward, I. (1960). *J. clin. Endocr.*, **20**, 1366.
- Christensen, L. K. (1959). *Scand. J. clin. Lab. Invest.*, **11**, 326.
- Gimlette, T. M. D. (1964). *J. clin. Path.*, **17**, 58.
- (1965). *Ibid.*, **18**, 293.
- (1967). *Ibid.* In the press.
- Hamolsky, M. W., Stein, M., and Freedberg, A. S. (1957). *J. clin. Endocr.*, **17**, 33.
- Herbert, V., Gottlieb, C. W., Lau, K.-S., Gilbert, P., and Silver, S. (1965). *J. Lab. clin. Med.*, **66**, 814.
- Lee, N. D., Henry, R. J., and Golub, O. J. (1964). *J. clin. Endocr.*, **24**, 486.
- Mitchell, M. L. (1958). *Ibid.*, **18**, 1437.
- , Harden, A. B., and O'Rourke, M. E. (1960). *Ibid.*, **20**, 1474.
- Myant, N. B., and Osorio, C. (1959). *J. Physiol. (Lond.)*, **146**, 344.
- Oppenheimer, J. H., Squef, R., Surks, M. I., and Hauer, H., (1963). *J. clin. Invest.*, **42**, 1769.
- Robbins, J., and Rall, J. E. (1960). *Physiol. Rev.*, **40**, 415.
- Shapiro, B., and Rabinowitz, J. L. (1962). *J. nucl. Med.*, **3**, 417.
- Sterling, K., and Hegedus, A. (1962). *J. clin. Invest.*, **41**, 1031.
- , and Brenner, M. A. (1966). *Ibid.*, **45**, 153.