

The measurement of serum thyroxine in children

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SYNOPSIS A competitive protein-binding method was used to determine serum thyroxine concentration throughout childhood, adolescence, and in young adults. The values showed a gradual decrease as the child became older and reached adult levels by the time of adolescence. The range (mean \pm 2 SD $\mu\text{g}/100$ ml) for cord serum was 8.8-14.8; for 4 weeks to 11 years, 6.5-13.9; for 11 to 17 years, 5.4-12.2, and for young adults 6.0-11.2.

The measurement of serum thyroxine provides a valuable index of thyroid function, and with the introduction of the competitive protein-binding method by Ekins (1960) and the subsequent development of the assay, its determination has been simplified. Many workers have shown its use in diagnosis in adults (Murphy, Pattee, and Gold, 1966; Arango, Mayberry, Hockert, and Elveback, 1968; Ekins, Williams, and Ellis, 1969; Howorth and Maclagan, 1969).

The technique can easily be adapted to estimate the thyroxine content of small samples and therefore seems particularly suited to the study of thyroid function in children. In this paper, a protein-binding method based on that of Maclagan and Howorth (1969) is described and the results of thyroxine determinations in 145 'normal' subjects with ages ranging from the neonatal period to young adulthood are reported.

Subjects

Blood samples were obtained from (1) 114 children and adolescents who were undergoing investigation in the Hospital for Sick Children and in whom there was no clinical evidence of thyroid disease nor any other factor causing alteration in thyroxine-binding protein levels; (2) 11 healthy young adults aged 20 to 27 years; (3) 20 cord bloods obtained from full-term deliveries; and (4) six children being treated with L-thyroxine.

Materials and Methods

Serum thyroxine was determined by the protein-

binding method of Maclagan and Howorth (1969) with minor modifications. Dried human plasma was reconstituted and stored in small fractions at -20°C to provide a convenient source of thyroxine-binding globulin (TBG) for the assay. A 1.5% solution of TBG and ^{125}I thyroxine solution containing approximately 75 000 counts per 10^3 seconds per ml were prepared in barbitone buffer as required. Serum (or plasma), 0.2 ml, was extracted with 1 ml extraction fluid and duplicate aliquots of extract and of thyroxine standards (0.20 ng) were processed. Following resin uptake with Amberlite IRA 400 (Cl^-) resin, 1 ml of supernatant was removed and the radioactivity determined. A standard curve was drawn of percentage radioactivity bound to protein against the amount of thyroxine, and from this the thyroxine content of the unknown samples was calculated.

Protein-bound iodine (PBI) was estimated as described by Edwards and McCracken (1967).

Student's *t* test was used to test differences between mean values of subgroups. The correlation coefficient was calculated from the regression line of the PBI values plotted against thyroxine iodine values.

Results

Thyroxine determinations were performed in duplicate unless otherwise indicated.

EVALUATION OF THE COMPETITIVE PROTEIN-BINDING METHOD

Details of recovery and precision studies are given in Table I. Number of batches implies the number of separate occasions on which the particular assay was performed.

The recovery from the extraction procedure was

	Number of Batches	Percentage Recovery	
		Mean	SD (±)
Recovery			
Extraction of ¹²⁵ I thyroxine from serum	3	99	3
Addition of 5 ng thyroxine to extract	19	102	5
Addition of 5 ng thyroxine to serum	5	98	14
Addition of 7.5 ng thyroxine to serum	4	101	11
Thyroxine (µg/100 ml)			
		Mean	SEM (±)
Precision			
Inter-batch variation	8	3.4	0.10
	12	13.8	0.31
	25 ²	6.0	0.12
	25 ²	9.8	0.11
Within-batch variation ³		5.9 (7)	0.08
		8.8 (8)	0.24
		14.0 (4)	0.22
			3.1

Table I Evaluation of competitive protein-binding method

¹CV = SD × 100 × mean⁻¹

²Control sera (Dade, Florida) were used over a three-month period

³Numbers of determinations are shown in brackets.

assessed by adding ¹²⁵I thyroxine in trace amounts to serum and determining the radioactive content of aliquots of the extract. Five ng thyroxine was added to the extract with an endogenous thyroxine content of 7.4 ng, and recovery was assessed by expressing the observed increment of the thyroxine value as a percentage of that added. Similarly, 5 ng or 7.5 ng thyroxine was added to serum with endogenous thyroxine content of 5 ng.

The coefficients of variation were calculated for replicate determinations (a) within a single batch, and (b) between successive batches.

SERUM THYROXINE VALUES IN NORMAL CHILDREN AND YOUNG ADULTS

Individual values for serum thyroxine are shown in

Figure 1. No sex difference was observed. Mean values (±SD) for different groups are shown in Table II.

	No. of Patients	Thyroxine (µg/ 100 ml)	
		Mean	SD (±)
Cord serum	20	11.8	1.5
4 weeks to 11 years	79	10.2	1.9
11 years to 17 years	23	8.8	1.7
Adults	11	8.6	1.3

Table II Serum thyroxine in childhood and adolescence

Correlation of thyroxine iodine values with PBI determinations

Thyroxine iodine (thyroxine value × 0.653) and PBI

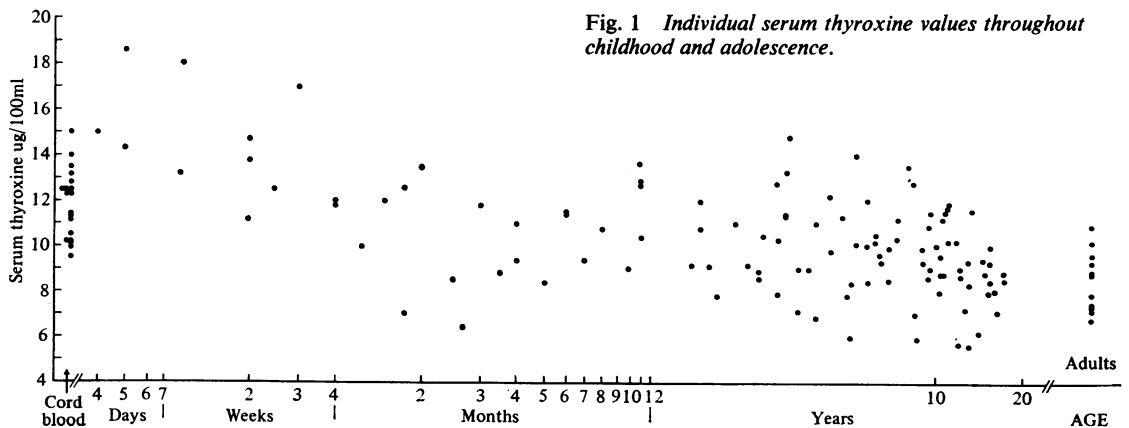


Fig. 1 Individual serum thyroxine values throughout childhood and adolescence.

values were determined in a total of 50 patients including six receiving treatment with L-thyroxine. The correlation coefficient was 0.56 ($p < 0.001$).

Discussion

The method is simple, specific, and reliable, and uses only a microsample of serum. The only compounds which interfere with it, other than analogues of thyroxine and tri-iodothyronine, are phenylbutazone and diphenylhydantoin (Ekins *et al.*, 1969). Unlike the method for PBI, serum thyroxine determination by competitive protein binding is not affected by excess iodide, iodine-containing compounds, and mercurial diuretics.

There have been many studies on normal ranges of serum thyroxine for adult populations. The range obtained from this study on a group of 11 young adults of 6.0 to 11.2 $\mu\text{g}/100$ ml (mean \pm 2 SD) agrees closely with those reported by Murray, Joasoo, and Parkin (1971), by Nakajima, Kuramochi, Horiguchi, and Kubo (1966), and by Nusynowitz and Waliszewski (1969).

Few accounts have been devoted to values found in children. Arango *et al.* (1968) included in their series patients aged between 10 and 20 years. No statistically significant trend with age was noted. Murray *et al.* (1971), in a more extensive study of Australian children aged between 6 months and 15 years, recorded a gradual fall in serum thyroxine with increasing age and the present study confirms this observation. The mean values obtained here are all slightly lower than the values for the corresponding age groupings of Murray *et al.* (1971) (Table III), the difference being significant only in the 6 mth to 6 year group ($p < 0.05$).

We have extended the age range to include samples taken from cord blood obtained at delivery from 20 full-term infants, 11 children aged between 4 weeks and 6 months, and nine adolescents aged between 15 and 17 years. Results for cord serum (mean value 11.8 ± 1.5 (SD) $\mu\text{g}/100$ ml) agree with those reported by Robin, Reketoff, Fang, and Selenkow (1969) and

Fisher, Odell, Hobel, and Garza (1969). Sierzbaek-Nielsen and Mølholm Hansen (1967) and Malkasian and Mayberry (1970) record wider ranges in their studies. The values for a few children studied during the first week of life (Fig. 1) were significantly higher than either the range for cord serum or for the 4-week to 6-month age group. This indication of hyperactivity of the thyroid gland in the neonatal period has been well documented with respect to levels of PBI (Fisher and Oddie, 1964) and has also been noted using the competitive protein-binding method for serum thyroxine (Sierzbaek-Nielsen and Mølholm Hansen, 1967). Mean values obtained in the present study for several age groupings (Table II) showed significant differences between the values for cord serum and children older than 4 weeks ($p < 0.005$), and between pre-pubertal children and adolescents ($p < 0.005$).

Serum thyroxine values expressed as thyroxine iodine were usually found to be higher than the corresponding PBI values. This was observed also by Murray *et al.* (1971). Children on thyroxine therapy often gave high values.

Non-thyroidal factors may reduce the circulating level of thyroxine (tri-iodothyronine diphenylhydantoin) or cause alterations in TBG levels as in inherited TBG abnormalities, nephrotic syndrome, and malnutrition. In such situations, the total serum thyroxine value may give a misleading indication of the individual's thyroid status, and a measure of the concentration of thyroxine-binding proteins is necessary. This may be achieved either by direct (Sterling and Brenner, 1966) or indirect measurement (Maclagan and Howorth, 1969) of the free thyroxine fraction which is inversely proportional to the concentration of binding proteins.

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Age Group	This Study			Murray <i>et al.</i> (1971)		
	No. of Patients	Thyroxine ($\mu\text{g}/100$ ml)		No. of Patients	Thyroxine ($\mu\text{g}/100$ ml)	
		Mean	SD (\pm)		Mean	SD (\pm)
0.5 to 6 years	42	10.3	2.0	70	11.4	1.9
6 to 12 years	30	10.0	1.6	80	10.7	2.0
12 to 15 years	15	8.4	1.6	30	9.5	1.8
Adults	11	8.6	1.3	—	9.1	1.3

Table III Comparison of results with those obtained by Murray *et al.* (1971)

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