The Iodotyrosine Content of Normal Human Serum

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This report is related to the controversy on whether MIT* and DIT occur in significant concentrations in the serum of normal human subjects and animals.

The orthodox view is that the iodothyronines, namely thyroxine and traces of tri-iodothyronine, account for virtually all of the organic iodine in normal serum (Laidlaw, 1949; Bird & Farran, 1960; Pitt-Rivers & Rall, 1961; Wellby & Hetzel, 1962; Pileggi, Segal & Golub, 1964). By contrast there are claims that free MIT and DIT contribute significantly to the organic iodine in serum (Block, Werner & Mandl, 1958; Werner & Block, 1959; Beale & Whitehead, 1960; Werner & Radichevich, 1963; Ouellette, Balcius & Zuppinger, 1966; Weinert, Masui, Radichevich & Werner, 1967), with estimates of their contribution ranging from approx. 20% (Weinert *et al.* 1967) to 50% (Werner & Block, 1959). There is an obvious objection to these claims in that the active peripheral deiodination of both MIT and DIT (Stanbury, Kassenaar & Meijer, 1956) makes the presence of the free compounds in normal serum extremely unlikely. However, Weinert *et al.* (1967) countered this objection with their suggestion that MIT and DIT exist in a 'masked' form (i.e. protected from deiodination) in the circulation.

Results supporting the orthodox view have been obtained mostly with methods employing either simple butan-1-ol or ion-exchange resins (cationic and anionic) for the extraction of the iodinated compounds from serum followed by their identification by standard paper-chromatographic procedures. The extraction procedure described in most of the papers claiming MIT and DIT in the serum is with boiling acidified butan-1-ol, followed by identification and quantitative determination by either paper chromatography or isotopic-dilution analysis.

'Isotopic dilution' has not been used in any study reporting an absence of MIT and DIT, hence our reinvestigation of the controversy using ion exchange for extraction and isotopic dilution for quantitative determination.

The ^{131}I -labelled MIT and DIT were synthesized by iodinating L-tyrosine with ^{131}I in the presence of

* Abbreviations: MIT, monoiodotyrosine; DIT, diiodotyrosine.

KI, KIO_3 and H_2SO_4 . Each was purified by three consecutive chromatographic separations on Toyo no. 51 paper developed with butan-1-ol saturated with 2M-acetic acid. The specific radioactivities of MIT and DIT were determined in terms of c.p.s. per ^{127}I content.

A batch of isotopic-dilution assays usually contained the following specimens. (1) Serum from a normal subject. (2) The same serum enriched by $5\mu\text{g.}/100\text{ml.}$ with DIT (or MIT). This was used as a control on the procedure. The batch was discarded on the few occasions when the recovery was not in the range 80–120%. (3) Serum from a patient with untreated thyrotoxicosis.

Individual specimens of serum were enriched with appropriate amounts of either labelled MIT or labelled DIT. The MIT and DIT were isolated in a form sufficiently pure for isotopic-dilution studies by using Dowex AG 1 (X2; 200–400 mesh) ion-exchange resin. The procedure is a modification of that reported by Wellby (1962) and the principles are as follows.

(1) For DIT. (a) All iodinated compounds in the serum are absorbed on to a resin column equilibrated with sodium acetate buffer, pH 5.2. (b) Serum proteins are removed by washing with sodium acetate buffer, pH 5.2. (c) Iodinated proteins and MIT are eluted in sodium acetate buffer, pH 3.2. (d) DIT is eluted at pH 2.4.

(2) For MIT. (a) All iodinated compounds in the serum are absorbed on resin equilibrated with sodium acetate buffer, pH 5.2. (b) Some iodinated protein is removed by washing the column with iodine-free water. (c) The remaining iodinated protein is removed by washing with sodium acetate buffer, pH 4.0 (some MIT is lost in this wash). (d) The remaining MIT is eluted (free from DIT) at pH 3.2.

Under these sets of conditions it was demonstrated that only negligible amounts of thyroxine, tri-iodothyronine and inorganic iodide are eluted at either pH 3.2 or 2.4.

Specific-radioactivity determinations were made in batches that included DIT (or MIT) both before and after isotopic dilution. Any effects on specific-radioactivity measurements due to variations in counter efficiency were thereby minimized. Chemical iodine determinations of the labelled DIT and MIT

Table 1. *Iodotyrosine concentrations in serum measured by isotopic dilution*

Experimental details are given in the text. Differences between groups of subjects are highly significant ($P < 0.01$) for DIT and MIT.

		DIT				MIT	
		Concn. ($\mu\text{g./100ml.}$)				Concn. ($\mu\text{g./100ml.}$)	
Subjects	No. of samples	Mean	Range	No. of samples	Mean	Range	
Normal	22	-0.12	-0.5 to +0.7	15	0.04	-0.6 to +0.9	
Thyrototoxic	25	1.24	+0.6 to +4.2	10	1.90	+0.9 to +5.2	

before and after dilution were achieved by adding samples of each to glycine solution and performing standard alkaline ash incineration for 1 hr. and catalytic estimation of the inorganic iodide (Acland, 1957).

The degree of dilution of the isotopic MIT and DIT by the MIT and DIT in the serum was derived and the concentrations of MIT and DIT in the serum were calculated with the standard formula.

The results (Table 1) indicate zero or negligible concentrations of both MIT and DIT in normal serum, but elevated concentrations of both in the serum from patients with thyrotoxicosis.

Although there is the possibility of small amounts of MIT and DIT occurring in the serum of the occasional normal subject, the study has confirmed that in general there are insignificant amounts of the 'free' iodotyrosines in normal human serum. This finding is in accord with orthodox views on the composition of the circulating thyroid hormones (Laidlaw, 1949; Bird & Farran, 1960; Pitt-Rivers & Rall, 1961; Wellby & Hetzel, 1962; Pileggi *et al.* 1964); but all of these studies, as acknowledged in connexion with the earlier report from this laboratory (Wellby & Hetzel, 1962), employed quantitative methods where the limit of detection of DIT and MIT was possibly not low enough to exclude small yet significant amounts. However, by employing isotopic dilution for the quantitative determination, the present study has overcome any objections, based on insufficient sensitivity, to the claim of absence of free iodotyrosines from normal serum.

Werner's group (Werner & Block, 1959) originally believed that free iodotyrosines do exist in normal human serum. They have since changed this belief because of technical and physiological objections to such a concept (Pileggi *et al.* 1964; Stanbury *et al.* 1956) and because of their growing number of observations (Werner & Radichevich, 1963; Weinert *et al.* 1967; Radichevich & Werner, 1968), which they interpret as indicating that approx. 25% of the extractable serum iodine consists of iodotyrosines (or precursors of iodotyrosines) associated with serum albumin in such a fashion as to protect them against deiodination. This view has some support

from other workers (Wiener, 1965; Rhodes, 1968), although the exact findings have not yet been confirmed.

If the present findings and those of Werner are compatible, the following postulates must hold. (1) That ion-exchange procedures fail to dissociate the iodotyrosines (or precursors) from serum albumin, in spite of the fact that these resins do dissociate thyroxine from its specific binding proteins. (2) That in the thyrotoxic subjects two forms of iodotyrosines exist, one in the free state and one associated with serum albumin. Presumably the former would be synthesized in the thyroid gland, and the latter in extrathyroidal sites as an integral part of the serum albumin molecule.

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- Acland, J. D. (1957). *Biochem. J.* **66**, 177.
 Beale, D. & Whitehead, J. K. (1960). *Clin. chim. Acta*, **5**, 150.
 Bird, R. & Farran, H. E. A. (1960). *J. clin. Endocrin.* **20**, 81.
 Block, R. J., Werner, S. C. & Mandl, R. H. (1958). *Arch. Biochem. Biophys.* **73**, 9.
 Laidlaw, J. C. (1949). *Nature, Lond.*, **164**, 927.
 Ouellette, R. P., Balcus, J. F. & Zuppinger, K. (1966). *J. Appl. Radiat. Isotopes*, **17**, 649.
 Pileggi, V. J., Segal, H. A. & Golub, O. J. (1964). *J. clin. Endocrin.* **24**, 273.
 Pitt-Rivers, R. & Rall, J. E. (1961). *Endocrinology*, **68**, 309.
 Radichevich, I. & Werner, S. C. (1968). *Proc. Soc. exp. Biol., N.Y.*, **124**, 1009.
 Rhodes, B. A. (1968). *Acta endocr., Kbh.*, Suppl. no. 127.
 Stanbury, J. B., Kassenaar, A. A. H. & Meijer, J. W. A. (1956). *J. clin. Endocrin.* **16**, 735.
 Weinert, H., Masui, H., Radichevich, I. & Werner, S. C. (1967). *J. clin. Endocrin.* **46**, 1264.
 Wellby, M. L. (1962). *Aust. J. exp. Biol. med. Sci.* **40**, 405.
 Wellby, M. L. & Hetzel, B. S. (1962). *Nature, Lond.*, **193**, 752.
 Werner, S. C. & Block, R. J. (1959). *Nature, Lond.*, **183**, 406.
 Werner, S. C. & Radichevich, I. (1963). *Nature, Lond.*, **197**, 877.
 Wiener, J. D. (1965). *Acta endocr., Kbh.*, **48**, 199.