

were phosphorus, 1.57; nitrogen, 6.0; non-protein N, 3.3. The sulfur content of non-radioactive samples was 0.25%.

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### Preparation of L-Methionine-S<sup>35</sup> and L-Cystine-S<sup>35</sup> from Radioactive Yeast<sup>1</sup>

BY JOHN L. WOOD AND GORDON C. MILLS

RECEIVED AUGUST 30, 1951

Radioactive L-cystine and L-methionine have been isolated from yeast labeled with radioactive sulfur.<sup>2</sup> The yeast was prepared by a method which ensured a high specific activity on the yeast sulfur.<sup>3</sup> This made a small scale operation possible and yielded L-methionine and L-cystine of high specific activity.

The yeast protein was separated from the carbohydrate by the procedure of Albanese, *et al.*,<sup>4</sup> and the protein was hydrolyzed with a hydrochloric acid-formic acid mixture. Dowex 50 was used to separate the sulfur amino acids.<sup>5</sup> Each amino acid was isolated from the proper ion-exchange fraction in a pure state after the addition of a small amount of the appropriate non-radioactive carrier.

A 4-g. sample of yeast ( $1.5 \times 10^9$  counts/min.) yielded 161 mg. of L-methionine with a specific radioactivity of  $1.6 \times 10^6$  counts per minute per mg. of methionine and 158 mg. of L-cystine with a specific activity of  $3.7 \times 10^5$  counts per minute per mg. of cystine.

(1) This investigation was supported by research grants from the National Cancer Institute, of the National Institutes of Health, Public Health Service, and from the American Cancer Society.

(2) For complete experimental details order Document 3499 from American Documentation Institute, 1719 N St., N. W., Washington 6, D. C., remitting \$1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film), or \$1.05 for photo copies (6 × 8 inches) readable without optical aid.

(3) J. L. Wood and Jesse D. Perkinson, *THIS JOURNAL*, **74**, 2444 (1952).

(4) A. A. Albanese, D. L. Wagner, J. E. Frankston and V. Irby, *Anal. Chem.*, **16**, 609 (1944).

(5) W. H. Stein and S. Moore, *Symposia on Quant. Biol.*, **14**, 179 (1949).

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### The Synthesis of Thyroxine-1-C<sup>14</sup><sup>1</sup>

BY S. C. WANG, J. P. HUMMEL AND T. WINNICK

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Thyroxine labeled with radiocarbon on the

(1) For detailed descriptions order Document 3497 from American Documentation Institute, 1719 N Street, N. W., Washington 6, D. C., remitting \$1.00 for microfilm (images 1 inch high on standard 35-mm. motion picture film) or \$1.65 for photocopies (6 × 8 inches) readable without optical aid.

carboxyl group has been synthesized by us on a semi-micro scale by a procedure based on the classical method of Harington and Barger.<sup>2</sup>

One hundred mg. of glycine-1-C<sup>14</sup><sup>3</sup> representing 5.45 mc. was treated with benzoyl chloride to yield hippuric acid-1-C<sup>14</sup>. The latter was condensed with 3,5-diiodo-4-(4'-methoxyphenoxy)-benzaldehyde. The resulting azlactone was converted to  $\alpha$ -benzoylamino-3,5-diiodo-4-(4'-methoxyphenoxy)-cinnamic acid-1-C<sup>14</sup>, and the latter in turn to 3,5-diiodothyronine-1-C<sup>14</sup>. The iodination to thyroxine was conducted in ethylamine solution.<sup>4</sup> The yield of thyroxine-1-C<sup>14</sup> was 533 mg. or 53% based on the glycine-1-C<sup>14</sup>. The product had a specific radioactivity of 530,000 counts per minute per mg., with a thin mica window counter.

The infrared spectra of thyroxine and diiodothyronine are given in Fig. 1. Our preparations were indistinguish-

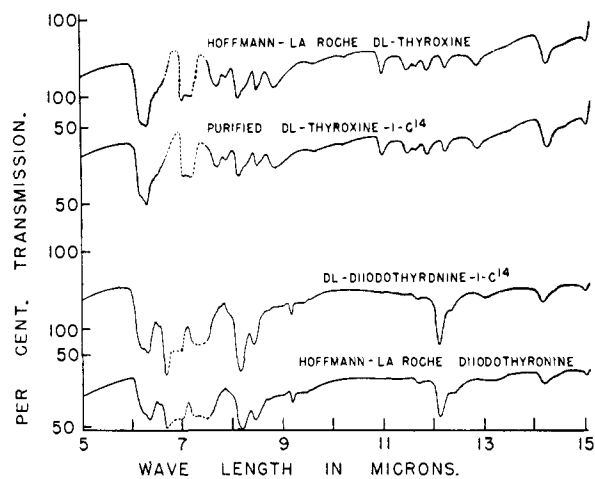


Fig. 1.—The Perkin-Elmer double beam spectrometer with NaCl optics was used; 20 mg. of sample per ml. of Nujol; dotted portions of records represent relatively opaque regions of the Nujol.

able from commercial samples. Likewise thyroxine-1-C<sup>14</sup> had the same biological potency as commercial thyroxine, based on assays with thyroidectomized rats.<sup>5</sup> The position of the labeling was confirmed by the Van Slyke ninhydrin method. The thyroxine was decarboxylated at pH 2.5, and the evolved C<sup>14</sup>O<sub>2</sub> accounted quantitatively for the radioactivity.

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(2) C. R. Harington and G. Barger, *Biochem. J.*, **21**, 169 (1927).

(3) Prepared by Tracerlab, Inc., Boston, Mass., from 10 mc. of BaC<sup>14</sup>O<sub>3</sub> furnished by the U. S. Atomic Energy Commission.

(4) J. C. Clayton and B. A. Hems, *J. Chem. Soc.*, 840 (1950).

(5) Performed by Dr. S. B. Barker and Mr. H. B. Dirks of the Department of Physiology.