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^{35} and L-Cystine-S^{35} from Radioactive Yeast[1]

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.[2] The yeast was prepared by a method which ensured a high specific activity on the yeast sulfur.[3] 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.*,[4] and the protein was hydrolyzed with a hydrochloric acid–formic acid mixture. Dowex 50 was used to separate the sulfur amino acids.[5] 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 × 10^9 counts/min.) yielded 161 mg. of L-methionine with a specific radioactivity of 1.6 × 10^6 counts per minute per mg. of methionine and 158 mg. of L-cystine with a specific activity of 3.7 × 10^5 counts per minute per mg. of cystine.

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[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).

DEPARTMENT OF CHEMISTRY
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The Synthesis of Thyroxine-1-C^{14} [1]

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

RECEIVED JANUARY 7, 1952

Thyroxine labeled with radiocarbon on the carboxyl group has been synthesized by us on a semi-micro scale by a procedure based on the classical method of Harington and Barger.[2]

One hundred mg. of glycine-1-C^{14} [3] representing 5.45 mc. was treated with benzoyl chloride to yield hippuric acid-1-C^{14}. The latter was condensed with 3,5-diiodo-4-(4′-methoxyphenoxy)-benzaldehyde. The resulting azlactone was converted to α-benzoylamino-3,5-diiodo-4-(4′-methoxyphenoxy)-cinnamic acid-1-C^{14}, and the latter in turn to 3,5-diiodothyronine-1-C^{14}. The iodination to thyroxine was conducted in ethylamine solution.[4] The yield of thyroxine-1-C^{14} was 533 mg. or 53% based on the glycine-1-C^{14}. 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 indistinguishable from commercial samples. Likewise thyroxine-1-C^{14} had the same biological potency as commercial thyroxine, based on assays with thyroidectomized rats.[5] The position of the labeling was confirmed by the Van Slyke ninhydrin method. The thyroxine was decarboxylated at *p*H 2.5, and the evolved $C^{14}O_2$ accounted quantitatively for the radioactivity.

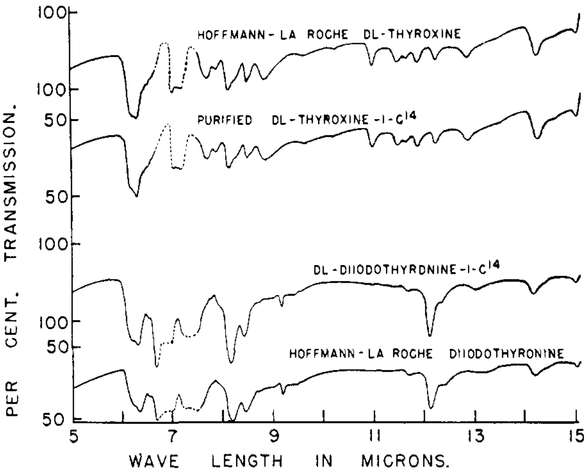

Fig. 1.—The Perkin-Elmer double beam spectrometer with NaCl optics was used; 20 mg. of sample per ml. of Nujol; dotted portions of records represent relatively opaque regions of the Nujol.

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[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.

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

[3] Prepared by Tracerlab, Inc., Boston, Mass., from 10 mc. of $BaC^{14}O_3$ 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.