A yield of 1.95 g. of uracil (62% based on urea) was obtained with a specific activity of 0.33 μc./mg.

The substance exhibited an ultraviolet absorption spectrum and an extinction coefficient identical with those reported in the literature.[11,12] A descending chromatogram of the material in a mixture of *t*-butanol–glacial acetic acid–water (65:25:10 v./v.), using Whatman No. 1 paper, showed a single radioactive component having an R_f value of 0.60. The product was subjected to an 8-plate countercurrent distribution in a system of 1 *M* potassium phosphate buffer at *p*H 6.8 and a mixture of equal volumes of *n*-butanol and *t*-butanol. The optical density at 260 mμ for the aqueous and organic layer of each plate was measured, and the sum of the values for the two phases of the various plates is plotted in Fig. 1. The relative radioactivity of each plate was determined by the addition of a constant volume of methanol and water to each plate to make the two layers mutually soluble. Aliquots of these solutions were then plated in plastic cups, dried and assayed for radioactivity in a gas-flow proportional counter. It was found that in addition to the background a correction for naturally occurring K^{40} of the buffer was necessary. The resulting values are plotted in Fig. 1. The close agreement, within the accuracy of the technique, with the calculated curve for authentic uracil having a distribution of 1.85 in such a system, indicated that the substance was of high purity.

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DEPARTMENT OF PHARMACOLOGY
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WASHINGTON 5, D. C. RECEIVED JULY 18, 1951

Absence of Rapid Exchange of Sulfur Atoms between Sulfate and Persulfate Ions

BY P. C. RIESEBOS AND A. H. W. ATEN, JR.

In aqueous solutions a rapid exchange between Hg^{++} and Hg^{2++}-ions has been reported.[1] This observation suggests that an investigation of exchange reactions between ion pairs of the same type might be a matter of some interest. As the sulfate–persulfate combination is fairly easy to handle, we have performed some experiments with this system. It may be pointed out that in this exchange process an oxygen–oxygen bond is affected, whereas in the sulfur exchanges studied earlier, like the $SO_4^{=}$–HS^-, SO_4–$SO_3^{=}$, $S_2O_3^{=}$–HS^- and $S_2O_3^{=}$–$SO_3^{=}$ reactions,[2] bonds between a sulfur and an oxygen atom or between two sulfur atoms were attached.

Solutions containing radioactive potassium sulfate, labeled with S^{35} (about 0.001 or 0.002 molar) and inactive potassium persulfate (about 0.0005 or 0.001 molar) were kept at room temperature for a week. The sulfate fraction was precipitated as barium sulfate after which the persulfate was decomposed by boiling with hydrochloric acid. Experiments were performed at *p*H values of about 1, about 7 and about 10. In all cases the average value of the specific activity of the sulfur in the persulfate amounted to less than 2% of the specific activity of the sulfate sulfur. (Large differences between figures obtained in duplicate experiments suggest, however, that most or all of the small activity found in the persulfate fraction may well be due to incomplete separation of the two fractions.) Under these circumstances the half-time of exchange amounts to about half a year at least.

Another series of exchange experiments was performed at *p*H about 10 in which the solutions were boiled for 5 minutes. This resulted in the decomposition of about 1/3 of the persulfate, after which the average of the radioactivity in this fraction still did not amount to more than 1.5% of the total activity in the system. (Here again the wide variation of the results suggests that this limit may be far too high.)

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INSTITUTE FOR NUCLEAR RESEARCH
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Synthesis of Histamine-2-C^{14}-Imidazole[1]

BY RICHARD W. SCHAYER[2]

Certain bacteria possess an enzyme which converts L-histidine into histamine and carbon dioxide.[3] Rodwell[4] has isolated unspecified strains of *Lactobacilli* possessing a very high histidine decarboxylase activity. Using an acetone powder preparation of these bacteria,[5] radioactive L-histidine has been decarboxylated and the radioactive histamine isolated as the dipicrate.

Experimental

Thiol-L-histidine-2-C^{14}-imidazole.—Radioactive sodium cyanide (approximately 3 mc.) was prepared from C^{14}-barium carbonate without dilution of the isotope by the method of Belleau and Heard.[6] The sodium cyanide was converted to sodium thiocyanate by the method of Castiglioni[7] as adapted by Borsook, *et al.*[8] After dilution with carrier equal to 1.5 times the estimated weight of the isotopic material, the sodium thiocyanate was treated with α,δ-diamino-γ-ketovaleric acid[9] (γ-ketoörnithine) producing 155 mg. of crystalline thiol-L-histidine which failed to melt up to 300°, as reported by Ashley and Harington.[9] Additional radioactive thiolhistidine was crystallized from the mother liquor after addition of carrier.

L-Histidine-2-C^{14}-imidazole.—One hundred and fifty mg. of thiolhistidine was oxidized with ferric sulfate to histidine[8].

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(2) The author is indebted to Rosa L. Smiley for assistance.

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which was isolated from the reaction mixture as the diflavianate yielding 370 mg. of L-histidine diflavianate, m.p. 245–247°. From the diflavianate was obtained 59.6 mg. of L-histidine, m.p. 294°. Ashley and Harington report 295°. For a 2% aqueous solution of a non-radioactive sample prepared by the same method $[\alpha]^{20}D$ −35.5°. Ashley and Harington found $[\alpha]^{20}D$ −36.0°.

Histamine-2-C^{14}-imidazole.—Histidine decarboxylase was prepared by incubating 50 mg. of acetone powder of the *Lactobacilli* with 5 ml. of McIlvaine buffer at *p*H 4.8 for 6 hours. After removing the cells by centrifugation, the enzyme preparation was added to 18.4 mg. of radioactive L-histidine in a Warburg flask and incubated at 30° for 65 minutes at which time carbon dioxide evolution was complete. The solution was transferred to a small separatory funnel, made strongly alkaline, and extracted four times with *n*-amyl alcohol. At this point 95% of the radioactivity was in the alcohol fraction. After one additional extraction the alcohol fractions were dried and evaporated to dryness *in vacuo*. The residue was dissolved in 3 ml. of water, a hot solution of 60 mg. of picric acid in 4 ml. of water added, the mixture heated to boiling and filtered; 44.3 mg. of histamine dipicrate, m.p. 238–242°, was obtained, a 65% yield from L-histidine. Pyman[10] reported m.p. 238–242°. Additional isotopic histamine dipicrate was crystallized from the mother liquor after the addition of carrier.

The activity using an internal counter was 9.3 × 10^6 c.p.m. per mg. of histamine base.

Anal. (for a non-radioactive sample synthesized in the same manner) Calcd. for $C_5H_9N_3(C_6H_3O_7N_3)_2$: C, 35.8; H, 2.66; N, 22.2. Found[11]: C, 35.7; H, 2.79; N, 22.3.

A paper chromatogram of the histamine (as the dihydrochloride) in butanol–ammonia showed a single sharp radioactive peak at R_F 0.80; under identical conditions the radioactive L-histidine produced a single sharp peak at R_F 0.15. Thus the histamine is free of demonstrable contamination by histidine. Chromatograms of the histamine in other solvents showed single peaks suggesting absence of significant amounts of other radioactive impurities.

Before use in animal experiments the radioactive histamine dipicrate was recrystallized from water, a sample dissolved in 0.15 *N* hydrochloric acid, the picric acid extracted with ether, and the solution of histamine dihydrochloride neutralized with sodium bicarbonate just before use. In a test for pharmacological activity[12] a very dilute solution of the radioactive histamine dihydrochloride produced the same contraction of guinea pig uterus as did the same amount of commercial histamine dihydrochloride.

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(11) Analysis by Micro-Tech Laboratories.

(12) Kindly performed by Dr. Georges Ungar of this Institute.

RHEUMATIC FEVER RESEARCH INSTITUTE
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Synthesis of *dl*-Adrenalin-β-C^{14} and *dl*-Adrenochrome-β-C^{14}

BY RICHARD W. SCHAYER[1]

The syntheses of radioactive adrenalin and adrenochrome were accomplished by known procedures modified for small-scale use and for conserving isotopic materials.

Experimental

Chloroacetic Acid-carboxyl-C^{14}.—Barium carbonate-C^{14} (3.0 millicuries)[2] was diluted to 4.92 g. and converted by the Grignard reaction to 1.74 g. (85% yield) of carboxyl-labeled sodium acetate.[3] Chloroacetic acid was synthesized by the method of Ostwald.[4] After recrystallization from ligroin, 2.01 g., m.p. 58°, a yield of 56% from sodium acetate after allowing for 400 mg. carrier, was obtained.

Chloroacetylcatechol.—Chloroacetic acid, 1.95 g., was heated on a steam-bath with 1.95 g. of catechol and 2.0 ml. of freshly distilled phosphorus oxychloride in an atmosphere of sulfur dioxide.[5] When the reaction was complete (about 45 minutes) the mixture was dissolved in 30 ml. of hot water, filtered and the residue washed. Crude chloroacetylcatechol, 1.50 g., m.p. 169–170°, was obtained. After recrystallization from hot water containing traces of hydrochloric acid and sodium bisulfite, 1.13 g. (29% yield from chloroacetic acid) was obtained having the reported melting point of 173°.

***dl*-Adrenalone Hydrochloride** (4-Methylaminoacetylcatechol Hydrochloride).—Chloroacetyl catechol, 1.00 g., was mixed with 5.0 ml. of 25% methylamine and allowed to stand at room temperature for 20 hours with frequent shaking.[6] Alcohol, 9 ml., was added and after standing 90 minutes in the cold, the brown precipitate was filtered, washed with 50% alcohol, absolute alcohol and finally ether. The crude adrenalone was dissolved in a minimum of dilute hydrochloric acid, diluted to about 20 ml. with water, and reprecipitated by addition of ammonia producing 0.52 g. of adrenalone (54% yield). Adrenalone, 0.52 g., was dissolved in a minimum of 3 *N* hydrochloric acid, filtered, absolute alcohol and finally ether added. Adrenalone hydrochloride, 0.50 g., crystallized, an 81% yield from adrenalone.

***dl*-Adrenalin-β-C^{14}** (Methylaminomethyl-(3,4-dihydroxyphenyl)-carbinol).—Adrenalone hydrochloride, 0.24 g., was dissolved in 10 ml. of water, 0.20 g. of catalyst (5% palladium-on-aluminum oxide) added and the mixture hydrogenated at ordinary pressure and temperature for two hours.[7] After filtering off the catalyst and adding excess ammonia 150 mg. of *dl*-adrenalin-β-C^{14} (74% yield from adrenalone hydrochloride) was obtained. The over-all yield from barium carbonate to adrenalin was 4.4%.

Anal. (for a non-radioactive sample synthesized by the same method) Calcd. for $C_9H_{13}O_3N$: C, 59.00; H, 7.27; N, 7.65. Found[8]: C, 59.11; H, 7.36; N, 7.52.

The activity measured with an internal counter was 2.72 × 10^5 c.p.m. per mg. The compound had the same effect on the blood pressure of a dog as did commercial synthetic epinephrine. A paper chromatogram of the adrenalin in butanol–acetic acid produced a single peak at R_F 0.45.

***dl*-Adrenochrome-β-C^{14}.[9]**—*dl*-Adrenalin-β-C^{14}, 40 mg., plus non-isotopic adrenalin, 60 mg., were dissolved in 3.0 ml. of absolute methanol containing 0.06 ml. of 99% formic acid. After warming to 35°, 0.7 g. of silver oxide was added, the mixture shaken and maintained at 35° for exactly one minute, filtered through a rapid filter and washed with 1 ml. of methanol. Crystals started forming immediately. After storing at −15° for 30 minutes the adrenochrome was filtered and washed successively with 1:1 methanol–ether, 1:3 methanol–ether, and ether. A first crop of 22 mg. red-brown crystals was obtained. By careful addition of ether to the mother liquor an additional 18 mg. of adrenochrome crystallized giving a total yield of 40%.

Anal. (for a non-radioactive sample synthesized by the same method, after correction for 2.12% ash) Calcd. for $C_9H_9O_3N$: C, 60.3; H, 5.06; N, 7.82. Found[8]: C, 59.2; H, 5.30; N, 7.69.

The activity measured with an internal counter was 1.06 × 10^6 c.p.m. per mg. Biological tests indicated that there was no observable contamination by adrenalin.

CONTRIBUTION FROM THE
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