The results obtained from the glycogen contents of the livers of the rats absorbing for three hours are given in Table II.

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The Synthesis of Choline and Acetylcholine Labeled in the Ethylene Chain with Isotopic Carbon

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Further insight into the mechanism by which acetylcholine exerts its profound influence on activity of the nervous system might be expected to result from studies on the fate of the compound labeled in a stable position with isotopic carbon. Since participation of the N-methyl groups of choline in metabolic transmethylation reactions has been demonstrated,[1] an isotopic atom in the ethylene chain is required for a stable carbon label.

Conversion of ethylene-C-14 to acetylcholine requires transformation of the olefin to its halohydrin or oxide as intermediate. Addition of the elements of hypohalous acid to ethylene affords a mixture of the halohydrin and substantial quantities of dihalide, although it has been observed that in the synthesis of ethylene chlorohydrin the formation of dichloride can be minimized by introduction of chlorine and ethylene at carefully controlled rates to a well stirred aqueous reaction mixture.[2] Preparation of ethylene bromohydrin was achieved in a similar manner,[3] and the technique was later modified to avoid the use of a large excess of ethylene.[4]

Since adaptation of these methods to the synthesis of ethylene bromohydrin with high specific activity appeared troublesome, we investigated the reaction of ethylene with N-bromoacetamide in aqueous solution. Although this reagent has been employed in the transformation of a variety of olefins to their bromohydrins and derivatives,[5,6] its reaction with ethylene itself does not appear to have been reported. We have found that in the presence of a trace of sulfuric acid[7] the reaction proceeds smoothly and affords the bromohydrin in yields of 90%. With an excess of trimethylamine in anhydrous ether, conversion of the bromohydrin to choline bromide proceeded in 80–85% yield[8]; acetylation with acetic anhydride under nitrogen atmosphere gave acetylcholine bromide in 85% yield.

Experimental

Ethylene-C-14.—Reduction of acetylene-C-14, obtained in the usual manner from active barium carbonate,[9] was achieved by reaction with chromous chloride solution[10]; traces of acetylene were removed from the product by shaking with alkaline mercuric cyanide solution.[11]

Ethylene Bromohydrin-1,2-C-14.—A solution of 2.76 g. (20 mmoles) N-bromoacetamide[12] in 50 ml. of water containing 2.0 ml. of 0.10 *N* sulfuric acid solution was transferred to a 500-ml. r.b. flask equipped with a break-seal. Twenty millimoles of ethylene was distilled into the reaction mixture under high vacuum; the flask was sealed and then shaken at room temperature for 18 hours. Unreacted ethylene was recovered from the mixture by high vacuum distillation, and in preliminary experiments good agreement was found between the recovered gas and the unreacted N-bromoacetamide determined by titration of an aliquot of the reaction mixture with standard thiosulfate solution.

The reaction mixture, saturated with sodium bromide, was extracted repeatedly with ether; distillation of the combined ether extracts, dried over sodium sulfate, afforded the bromohydrin as an almost colorless liquid which was used for preparation of choline bromide without further purification.

Acetylcholine Bromide (Trimethyl-2-acetoxyethyl-1,2-C-14-ammonium Bromide).—Conversion of 1.40 g. (11.2 mmoles) of ethylene bromohydrin to choline bromide was effected by reaction with excess anhydrous trimethylamine in 10 ml. of dry ether. After five hours at 55° and 15 hours at room temperature, distillation of the ether and excess amine afforded 1.70 g. (83%) of choline bromide, m.p. 275–280° dec.; crystallization from ethanol–ether gave 1.63 g. of the quaternary salt, m.p. 287–289° dec.

Five millimoles of choline bromide heated for four hours with acetic anhydride under nitrogen atmosphere gave, after separation of the excess anhydride, 0.97 g. (86%) of slightly colored acetylcholine bromide, m.p. 143–144° with sintering at 140°; crystallization from ethanol–ether afforded the pure acetate, m.p. 143–144°.

Anal. Calcd. for $C_7H_{16}O_2NBr$: C, 37.18; H, 7.13. Found: C, 37.3; H, 7.3.

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NEW COMPOUNDS

Synthesis of 2,2-Dichloropropane-1,3-diol

2,2-Dichlorodiethyl malonate (28.0 g., 0.122 mole), prepared by passing chlorine through diethyl malonate at 120°,[1] was dissolved in anhydrous ethyl ether (150 ml.) and placed in a one-liter three-necked flask fitted with an air-tight stirrer and a condenser and dropping funnel in which calcium chloride tubes were inserted. The reaction flask was cooled in an ice-bath while lithium aluminum hydride (4.9 g., 0.13 moles) in anhydrous ethyl ether was added slowly. After an additional hour of stirring, water (3 ml.) was added to destroy the excess of lithium aluminum hydride. The mixture was added to cold 10% sulfuric acid solution (500 ml.). The ether and water layers were separated and the ether layer washed with water until neutral. On evaporation of the ether *in vacuo*, an oily compound remained which crystallized on cooling in an ice-bath. The crystals of 2,2-dichlorohydrin after recrystallization several times from ethyl ether melted at 88.5–89.0°. A yield of 7.6 g. (43%) was obtained.

Anal. Calcd. for $C_3H_6O_2Cl_2$: Cl, 48.90. Found: Cl, 48.69, 48.74.

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