The acetobromo derivative was dissolved in acetone[6] (50 ml.) and stirred overnight with 6 g. of silver carbonate. The solution was filtered and evaporated to a sirup under reduced pressure at 35°. The sirup crystallized immediately after solution in cool ethanol; yield 2.7 g., m.p. 111–115°. The dry material was covered with ethanol and allowed to stand overnight. When it was filtered and dried the constants were: m.p. 113–115°, $[\alpha]^{27}D$ + 2.9° (*c* 2.9, chloroform), $[\alpha]^{27}D$ − 7.0° (initial, extrapolated) → +34.1° (5 hr., *c* 3.1, pyridine) with *k* (first order) 0.01 (minutes and decimal logarithms). X-Ray powder diffraction data: 7.90[7]–25,[8] 7.16–20, 6.21–30, 5.54–20, 5.12–30, 4.72–70, 3.99–100, 3.54–15, 3.34–15, 3.10–5, 2.97–5, 2.71–10, 2.80–5, 1.93–5, 1.66–5. Further crystallization from acetone–ether–petroleum ether did not change these constants.

Anal. Calcd. for $C_{12}H_{15}O_{11}(CH_3CO)_7$: C, 49.05; H, 5.70; CH_3CO, 11.0 ml. 0.1 *N* NaOH per 100 mg. Found: C, 49.25; H, 5.66; CH_3CO, 11.0 ml.

Conversion of β-Gentiobiose Heptaacetate to the Alpha-beta Compound.—A sample of β-gentiobiose heptaacetate was heated in an oil-bath at 135° for 45 minutes. The resulting crystalline material was recrystallized from acetone–ether–petroleum ether; m.p. 175–177°, $[\alpha]^{25}D$ + 36.1° (*c*, 3.2, chloroform), $[\alpha]^{28}D$ + 34.7° (*c* 3.25, pyridine, no detectable mutarotation). Further recrystallizations did not change these constants. The above values are in substantial agreement with those of Bergmann and Freudenberg.[2] X-Ray powder diffraction data[7,8]: 5.50–80, 4.90–90, 4.47–100, 3.92–70, 3.57–50, 3.36–60, 3.12–5, 2.92–5, 2.80–20, 2.60–10, 2.03–10, 1.91–10, 1.74–10.

(6) E. Fischer and K. Hess, *Ber.*, **45**, 912 (1912).

(7) Interplanar spacing, Å., Cu K_α radiation.

(8) Relative intensity as percentage strongest line; estimated visually.

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The Rate of Absorption of and the Formation of Glycogen by DL-Homoserine as Compared with DL-Alanine[1]

BY W. J. WINGO AND R. A. SMITH

Homoserine, or α-amino-γ-hydroxybutyric acid, is believed to be one of the primary products of the action of cystathionase upon cystathionine. This hypothesis appears more likely to be so in view of the recent findings of Carroll, Stacy and du Vigneaud,[2] that homoserine, but not α-aminobutyric acid, is converted to α-ketobutyric acid by liver extracts which form cysteine and α-ketobutyric acid from cystathionine. This observation, together with the finding of McCoy, Meyer and Rose[3] that homoserine will not support the growth of rats on a threonine-deficient diet, and Armstrong and Binkley's[4] demonstration that the transulfuration reaction is not reversible, comprise the total knowledge of the metabolism of this compound in animals.

We have determined the rate of absorption of DL-homoserine from the intestine of the rat; similar measurements on DL-alanine were done for comparison. The glycogen contents of the livers of some of the rats used were also determined.

Method.—The DL-homoserine used was prepared from γ-butyrolactone by the method of Livak, *et al.*[5a,b] It was analyzed by the Kjeldahl method and by the carboxyl nitrogen method of Van Slyke, *et al.*,[6] and was found to be pure. The DL-alanine used was analyzed by the carboxyl nitrogen method, and was also pure.

In the first experiments, the amino acids were given by stomach tube to male white Sprague–Dawley rats which had been fasted for 24 hours. These rats had been kept in the laboratory for some weeks after their receipt from Sprague–Dawley, Inc., and were on the average larger than is desirable for studies of this kind. The rats weighed 130 to 330 g.; most of them were near 275 g. in weight. The doses of homoserine ranged from 193 to 273 mg./100 g. rat; the doses of alanine ranged from 185 to 288 mg./100 g. rat. The control rats were fed nothing. Three hours after the compounds were given, the rats were killed. Glycogen was determined in a portion of the liver by the method of Good, Cramer and Somogyi.[7]

For determination of the rate of absorption from the gut, gastro-intestinal tract and its contents were ground in a Waring blendor with 1% picric acid. The resulting slurry was diluted to 100 ml. with 1% picric acid in a volumetric flask and filtered through muslin, and carboxyl nitrogen was determined in aliquots of the picric acid solution.[8] Control experiments, in which homoserine and alanine were added to such gastrointestinal preparations showed that alanine was quantitatively recovered, but that recoveries of 94% were obtained for homoserine. A correction for this low recovery is incorporated in the values for homoserine absorptions reported here.

In the second set of experiments, the rats used were younger, smaller, and more carefully selected with respect to weight. Two absorption periods—two hours and one hour—were studied. Twelve rats which had been fasted for 48 hours were used for each experiment. Four rats served as controls; four were given DL-alanine, and four were given DL-homoserine. The analytical procedures were the same as those used in the first experiment, except that glycogen determinations were not done.

Results.—The results obtained for the rates of absorption are given in Table I.

TABLE I

RATES OF ABSORPTION OF DL-ALANINE AND DL-HOMOSERINE FROM THE INTESTINE OF THE RAT

Amino acid fed	Length of absorption period, hr.	Number of rats in group	Weight range of rats, g.	Dose range of amino acid, mg./100 g.	Rate of absorption, mg./100 g./hr.
DL-Alanine[a]	3	12	170–330	185–288	64 ± 4
DL-Homoserine[a]	3	11	230–310	193–273	60 ± 3
DL-Alanine[b]	2	4	115–135	198–211	94 ± 3
DL-Homoserine[b]	2	4	127–145	203–206	73 ± 2
DL-Alanine[c]	1	4	104–112	147–153	84 ± 4
DL-Homoserine[c]	1	4	100–112	150–153	68 ± 6

[a] The average total carboxyl N content of the gastrointestinal tracts of 11 control rats was 5.14 mg. by our procedure. This figure was used as a blank in calculating the absorption rates. [b] The average total carboxyl N content of the gastrointestinal tracts of 4 control rats was 3.80 mg. This figure was used as a blank. [c] The average total carboxyl N content of the gastrointestinal tracts of 4 control rats was 3.40 mg. This figure was used as a blank.

TABLE II

GLYCOGEN CONTENTS OF LIVERS OF RATS FED DL-HOMOSERINE AND DL-ALANINE

Group	Number of rats in group	Liver glycogen, %
Controls	9	0.26 ± 0.08
Alanine	10	.92 ± .05
Homoserine	11	.22 ± .05

(1) This work was supported in part by a grant from the American Cancer Society.

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(3) R. H. McCoy, C. E. Meyer and W. C. Rose, *ibid.*, **112**, 283 (1935).

(4) M. D. Armstrong and F. Binkley, *ibid.*, **174**, 889 (1948).

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(6) D. D. Van Slyke, R. F. Dillon, D. A. MacFayden and P. Hamilton, *J. Biol. Chem.*, **141**, 627 (1941).

(7) C. A. Good, W. Kramer and M. Somogyi, *ibid.*, **100**, 485 (1933).

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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

BY DORIS E. WALZ, MELVIN FIELDS AND JAMES A. GIBBS

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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CHEMICAL RESEARCH LABORATORY
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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.

CONTRIBUTION NO. 807
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