# THE CONVERSION OF ETHANOLAMINE TO ACETATE IN MAMMALIAN TISSUES *

D. B. Sprinson + and I. Weliky ‡

Department of Biochemistry, College of Physicians and Surgeons, Columbia University, New York, New York 10032

Received July 28, 1969

SUMMARY: Ethanolamine-1-$D_2$, 2-$^{14}C$ was extensively converted in the rat to labeled acetate which contained the $^{14}C$ entirely in the carboxyl carbon, and had approximately the same ratio of $^{14}C/D$ as the administered $CD_2OH \cdot {}^{14}CH_2NH_2$. In view of these findings the recently described pyridoxal phosphate dependent conversion of ethanolamine-O-phosphate to acetaldehyde, ammonia, and $P_i$, may best be interpreted as activation of hydrogen on the amino carbon atom by pyridoxal phosphate, followed by elimination of orthophosphate; hydrolysis of the resulting eneamine then yields acetaldehyde and ammonia (Fig. 1).

It has been shown in previous studies that ethanolamine-2-$^{14}C$ was effectively incorporated into fatty acids, cholesterol, and C-3 to C-18 of sphingosine, thus indicating a conversion of ethanolamine to acetate (1). Further information was sought by administering ethanolamine-1-$D_2$, 2-$^{14}C$ ($CD_2OH \cdot {}^{14}CH_2NH_2$) to rats together with 2-amino-4-phenylbutyric acid, and analyzing the excreted N-acetyl aminophenylbutyrate. The acetate contained $^{14}C$ only in the carboxyl group, and retained the $^{14}C/D$ ratio of the administered $CD_2OH \cdot {}^{14}CH_2NH_2$. These findings are relevant to a recent report that the conversion by an enzyme from rabbit liver of ethanolamine-O-phosphate to acetaldehyde, ammonia, and $P_i$ is an ammonia lyase reaction (2). It is suggested instead that the enzyme

* This work was supported by grants from the American Cancer Society, the American Heart Association, the National Institutes of Health of the U. S. Public Health Service, and The National Science Foundation.

+ Career Investigator of the American Heart Association.

‡ Present address: The Squibb Institute, New Brunswick, New Jersey.

catalyzes a β-elimination of phosphate, rather than of ammonia. A preliminary report of these results has appeared earlier (3).

## Experimental

Ethanolamine-1-$D_2$, 2-$^{14}C$ hydrochloride was prepared as described previously (4). It had 1.96 atoms excess D per mole, and was diluted with unlabeled ethanolamine hydrochloride to a concentration of 0.60 atoms excess D per mole.

Male rats (220 to 250 g) were accustomed to a 15% casein diet (5). The labeled compound plus 250 mg of DL-2-amino-4-phenylbutyric acid were added to 8 g of diet, made into a paste, and placed into a metabdism cage with the animal. In some experiments the lipid components of the diet were replaced by an equivalent weight of starch, and vitamins A, D, and K were added in pure or highly concentrated forms.

After 24 hours N-acetyl L-2-amino-4-phenylbutyric acid was isolated from the urine, recrystallized to constant activity, and analyzed for deuterium (6). A sample was refluxed for 2 to 3 hours in 2 N $H_2SO_4$, and acetic acid was removed by distillation and recovered as sodium acetate. Degradation by the Schmidt reaction gave $CO_2$ from C-1 which was isolated as $BaCO_3$, and methylamine, representing C-2, which was converted to the picrolonate.

## Results and Discussion

The deuterium concentration of the excreted acetate, and the distribution of $^{14}C$ between methyl and carboxyl carbon atoms, are shown in Table I. Ethanolamine-1-$D_2$, 2-$^{14}C$ was converted to acetate in which the $^{14}C$ was entirely in the carboxyl group. The ratio of $^{14}C$/D was approximately the same as in the administered ethanolamine, indicating that the deuterium atoms were retained during this conversion. Utilization of administered ethanolamine for acetate formation must be high, since similar dilutions were obtained in acetyl

TABLE I

Incorporation of Label from $CD_2OH \cdot {}^{14}CH_2NH_2$ into Acetate [a]

| Ethanolamine[b] administered per 100 g | Label in acetate | | | Ratio of |
|---|---|---|---|---|
| | $^{14}C$ in $CO_2H$ [c] | $^{14}C$ in $CH_3$ | D in $CH_3$ [d] | $^{14}C/D$ [b] x $10^{-6}$ |
| mmoles | cpm x $10^{-4}$ | | atoms per mole | |
| 0.11 [e] | 3.25 | 0 | 0.0036 | 9.0 |
| 0.46 [f] | 20.2 | 0 | 0.0236 | 8.6 |

[a] Samples were counted under standard conditions at "infinite" thickness, and the results were expressed as molar activities (7). Atoms excess D per mole is atoms per cent excess D x number of hydrogen atoms in molecule divided by 100.

[b] The ethanolamine hydrochloride had $5.94 \times 10^6$ cpm and 0.60 atoms excess D per mole; $^{14}C/D$, $9.9 \times 10^6$.

[c] Essentially all of the activity of acetyl 2-amino-4-phenylbutyric acid was recovered in $BaCO_3$ from degradation of sodium acetate. The methylamine, representing C-2 of acetate, was inactive.

[d] Obtained by analysis of acetyl 2-amino-4-phenylbutyric acid for D.

[e] Ingested in 15% casein diet.

[f] Ingested in 15% casein fat free diet.

---

2-amino-4-phenylbutyric acid when labeled acetate was ingested under similar conditions (8). As would be expected, the utilization was relatively higher on a fat free diet.

Attempts in this laboratory to demonstrate the formation of ammonia or $P_i$ *in vitro* from ethanolamine, ethanolamine-*O*-P, or CDP-ethanolamine were unsuccessful. However, the purification of a pyridoxal phosphate dependent enzyme from rabbit liver which converts ethanolamine-*O*-P to acetaldehyde, $P_i$ and ammonia (2) enables the results presented above to

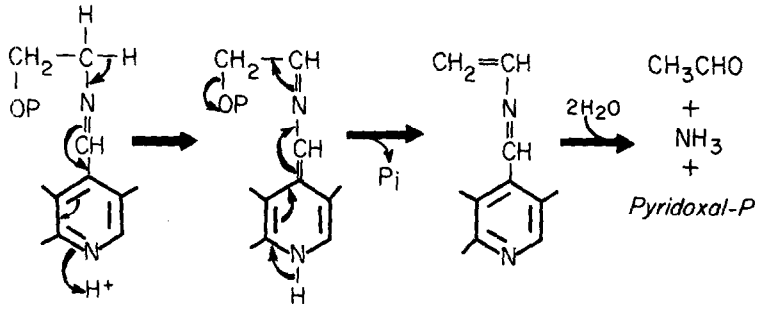


Fig. 1. Hypothetical mechanism for enzymic conversion of ethanolamine-O-phosphate to acetaldehyde, ammonia and $P_i$.

---

be rationalized by the mechanism proposed in Fig. 1. Activation of hydrogen on the amino carbon atom by pyridoxal-P is followed by elimination of $P_i$; the resulting eneamine is hydrolysed to acetaldehyde, ammonia, and pyridoxal phosphate. (In vivo the acetaldehyde would be oxidized to acetate.) Analogous reactions are catalyzed by serine dehydratase and cysteine desulfhydrase. The novel features are the absence of a $\alpha$-carboxyl group, and the $\beta$-elimination of $P_i$. This mechanism is consistent with retention in acetate of the deuterium atoms of $CD_2OH \cdot {}^{14}CH_2NH_2$. On the other hand, an ammonia lyase mechanism (2) would result in loss of one deuterium atom in the conversion of ethanolamine to acetaldehyde. Inherent in the above interpretation are the reasonable assumptions that ethanolamine was degraded in vivo after prior phosphorylation with ATP, and that the reaction observed in rabbit liver occurs in rat tissues. The removal of ethanolamine-O-P by conversion to acetate may exert a regulatory effect on the biosynthesis of phosphatidyl ethanolamine via CDP-ethanolamine (9).

## References

1. Sprinson, D. B., and Coulon, A., J. Biol. Chem., 207, 585 (1954).
2. Fleshood, H. L., Fed. Proc., 28, 727 (1969).

3. Sprinson, D. B., and Weliky, I., Fed. Proc., 13, 302 (1954).
4. Weissbach, A., and Sprinson, D. B., J. Biol. Chem., 203, 1031 (1953).
5. Schoenheimer, R., Ratner, S., and Rittenberg, D., J. Biol. Chem., 130, 703 (1939).
6. Graff, J., and Rittenberg, D., Anal. Chem., 24, 878 (1952).
7. Shemin, D., and Kumin, S., J. Biol. Chem., 198, 827 (1952).
8. Bloch, K., and Rittenberg, D., J. Biol. Chem., 155, 243 (1944); 159, 45 (1945).
9. Kennedy, E. P., and Weiss, S. B., J. Biol. Chem., 222, 193 (1956).