Again radioactivity was incorporated into AMe as judged by its behavior on the two ion-exchange columns. Decomposition was performed as above, the resulting radioactive material was desalted by adsorption on Dowex-1 (OH^-) followed by elution with HCl[6] and was identified by paper chromatography as homoserine. In a third set of experiments, L-[1-^{14}C]methionine was shown also to be incorporated into AMe which on decomposition gave rise to radioactive homoserine.

To confirm and extend these findings, some of the [*Me*-^{14}C]AMe was subjected to treatment with a partially purified yeast enzyme which cleaves AMe to 5'-methylthioadenosine and to α-amino-γ-butyrolactone[3]. The reaction was allowed to proceed until 90% of the AMe was decomposed. The specific activities of the initial AMe and the 5'-methylthioadenosine which was formed were the same within experimental error (15,100 and 16,000 counts/min/μmole respectively). Because of the known substrate specificity of this enzyme, these results confirm not only that the radioactivity was in S-adenosylmethionine[3] but clearly establish the configuration at the asymmetric sulfur and α-amino-carbon atoms of the AMe as—and L[3,7].

The facts that AMe is formed by barley and that it is used by this plant and by millet in a group of alkaloid-forming transmethylations suggests that this compound is the predominant methyl donor in plants as it is known to be in animals and microorganisms.

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[1] S. H. MUDD, *Biochim. Biophys. Acta*, 37 (1960) 164.
[2] S. H. MUDD AND G. L. CANTONI, *J. Biol. Chem.*, 231 (1958) 481.
[3] S. H. MUDD, *J. Biol. Chem.*, 234 (1959) 87.
[4] J. BADDILEY, G. L. CANTONI AND G.A. JAMIESON, *J. Chem. Soc.*, 2662 (1953).
[5] L. W. PARKS AND F. SCHLENK, *J. Biol. Chem.*, 230 (1958) 295.
[6] R. B. ROBERTS, *Studies of Biosynthesis in* Escherichia coli, *Publication No.* 607, Carnegie Institution of Washington, Washington, 1955, p. 186.
[7] G. DE LA HABA, G. A. JAMIESON, S. H. MUDD AND H. H. RICHARDS, *J. Am. Chem. Soc.*, 81 (1959) 3975.

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Inhibition of yeast carboxylase by acetaldehyde

It has been known for some time, in fact since the description of carboxylase by NEUBERG[1], that the velocity of the reaction

$$\text{pyruvate} \longrightarrow \text{acetaldehyde} + CO_2$$

catalyzed by yeast carboxylase quickly decreases even in the presence of an excess of pyruvate[1,2]. The deviation of the curve of the CO_2 evolution from the straight line to be expected at zero-order conditions has generally been attributed to inhibition of the enzyme by the acetaldehyde formed[1–5], especially as the enzyme is relatively stable during incubation without substrate[2,3,5]. There are, however, to our knowledge

no published data deciding the type of inhibition or the question whether the observed decrease is only due to acetaldehyde inhibition.

In order to decide the type of inhibition, the time curve of CO_2 evolution from pyruvate by fresh baker's yeast, which had been made permeable to pyruvate by repeated freezing and thawing, was obtained by the conventional Warburg technique. The reaction velocity at different times was obtained by drawing slopes to the curves at different points or by measuring the CO_2 production at relatively short intervals. The acetaldehyde concentration at any point was assumed to be equal to the amount of CO_2 evolved divided by the volume of the incubation mixture. The amount of pyruvate added was saturating.

In the case of apparent non-competitive or un-competitive inhibition, assuming reversible combination of the enzyme with n molecules of acetaldehyde according to $E + nA \rightleftharpoons EA_n$, the velocity v at any time or acetaldehyde concentration should be proportional to the concentration of uninhibited enzyme at saturating levels of pyruvate, viz. $v = k(e - ea_n)$. This yields

$$v = \frac{da}{dt} = \frac{v_0}{1 + \frac{a^n}{K_n}} \qquad (I)$$

where a is the acetaldehyde concentration, e the total concentration of enzyme ($e \ll a$), K_a the dissociation constant of the acetaldehyde–carboxylase complex, and v_0 ($= ke$) the velocity at $a = 0$. A plot of $1/v$ against a^n should yield a straight line. Fig. 1 shows that this is the case for $n = 1$ and also indicates the correctness of the type of inhibition assumed. From the extrapolated curve in Fig. 1 and other similar curves K_a could be obtained. In 0.1 M acetate buffer of pH 5.1, K_a is about $4 \cdot 10^{-3} M$.

As the calculation of the velocity involved the drawing of slopes to the product-time curve and K_a was obtained by extrapolation a control of the procedure by a

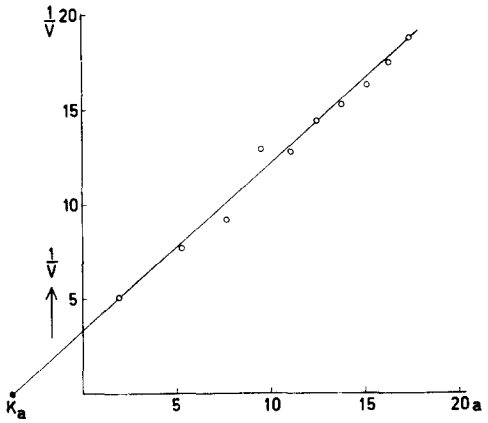

Fig. 1. Plot of reciprocal velocity ($1/v$ in ml·min/μmole) against acetaldehyde concentration (a in μmoles/ml) as calculated from the experiments. The Warburg vessels contained 1.5 ml suspension of four-times frozen and thawed fresh baker's yeast (*Saccharomyces cerevisiae*, Konings-gist) in 0.1 M Na acetate buffer, pH 5.1 (19.2 mg fresh yeast/ml final suspension) and 0.3 ml 3.5% sodium pyruvate (final concn., 53 μmole/ml) in acetate buffer. All points are the mean of triplicate determinations. The drawn curve was obtained by the method of least squares. A K_a value was obtained of $3.7 \cdot 10^{-3}$ M at 27.0°.

more direct use of the experimental data was considered desirable. Integration of the relation in eqn. (I) yields

$$v_0 \cdot t = a + \frac{a^2}{2K_a} \qquad \text{(II)}$$

The right-hand term of this equation divided by t should therefore give a constant value, viz. the initial velocity v_0. Table I shows that this relation holds for the experiment of Fig. 1 which is typical of all experiments done. This relation affords a good method of measuring carboxylase activity, if K_a has first been determined for the experimental conditions used.

TABLE I

Experimental conditions as described in legend to Fig. 1 (for explanation see text).

t (min)	a = acetaldehyde concentration at t (μmoles/ml)	$\frac{a + \frac{a^2}{2Ka}}{t} = v_0$
10	1.98	0.250*
30	5.25	0.296
50	7.61	0.304
70	9.46	0.303
90	11.01	0.299
110	12.49	0.300
130	13.78	0.298
150	15.09	0.300
170	16.28	0.301
190	17.36	0.300

* The value after 10 min in all experiments appeared to be lower owing to insufficient penetration of substrate, and/or incomplete gas equilibration.

It is thus clear that the carboxylase in fresh baker's yeast is inhibited in an apparent non-competitive or uncompetitive manner by the acetaldehyde formed at saturating pyruvate concentrations, with an inhibitor dissociation constant of about $4 \cdot 10^{-3}$ M in 0.1 M acetate buffer, pH 5.1. Further experiments in our laboratory have shown that this constant is pH and/or buffer dependent in the range of carboxylase activity. With air-dried brewer's yeast similar curves were obtained confirming the type of inhibition assumed.

We also tried to obtain the same curves by adding different amounts of acetaldehyde to our incubation mixture. Essentially the same results were obtained, but the added acetaldehyde appeared to be only about 90 % as effective as the same amount formed by the yeast.

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[1] C. NEUBERG AND L. KARCZAG, *Biochem. Z.*, 36 (1911) 76.
[2] H. G. K. WESTENBRINK AND E. P. STEYN-PARVÉ, *Z. Vitaminforsch.*, 21 (1950) 461.
[3] M. GRUBER, *Thesis*, Utrecht, 1952, p. 28.
[4] E. JUNI, *J. Biol. Chem.*, 195 (1952) 727.
[5] H. D. OHLENBUSCH, *Biochem. Z.*, 331 (1959) 18.

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