GLYOXYLATE INTERMEDIATE OF THE DIRECT OXIDATION OF ACETATE AND GLYCOLATE BY E. COLI

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In preceding papers we have demonstrated that a direct oxidation of acetate appears to occur in yeast (BOLCATO et al., 1957) and in E.coli (BOLCATO et al., 1956a) through the following intermediates:

\[
\text{acetate} \rightarrow \text{acetyl} \rightarrow \text{glycolate} \rightarrow \text{glyoxylate} \rightarrow \text{formaldehyde} \rightarrow \text{formyl} \rightarrow \text{CO}_2 + \text{H}_2\text{O}
\]

As to the monocarboxylic acid scheme (MAS), WONG and AJL (1957), AJL (1958) and UTTER (1958) have pointed to the possibility that glyoxylate might arise, not from acetate and glycolate, but by the action of malate synthetase or isocitritase on malate or isocitrate formed by the tricarboxylic acid cycle (TAC) operative in these cells. If this supposition would prove true, our scheme would lose any validity, as it is the result of the connecting of some reactions concerning the activation of acetate and some others concerning the oxidation of glyoxylate. The possibility that glyoxylate might arise from malate or isocitrate had already been considered by us. In fact, experiments with living yeasts cells (BOLCATO and LEGGIERO, 1958) have demonstrated that glyoxylate could only be obtained from acetate or glycolate and not from succinate, fumarate, malate, and citrate.

The importance that the origin of glyoxylate acquires in our experiments for the validity of the MAS, has led us to study the problem also with living cells of E.coli, that are more suitable than the yeast cells for this kind of experiments. In the present
paper we report on the oxidation of malate or citrate (instead of isocitrate) either alone or in presence of acetate or glycolate by proliferating and non-proliferating cells of *E. coli*.

**METHODS.**

**Experiments with proliferating cultures.**

The avirulent C-14 strain of *E. coli* was used in these experiments. It developed well on a mineral medium containing d,l-malate as sole source of carbon: of the acid only the l-moyety was utilized. Citrate and glycolate were assimilated merely slightly for growth, but were readily oxidized in the medium used in the experiments with non-proliferating cultures. The cells of *E. coli* were grown in two glass tubes provided with a sintered diffuser for aeration. Each tube contained 1000 ml of the following medium: H₂O 1000 ml, K₂HPO₄ 9 g, (NH₄)₂SO₄ 3 g, MgSO₄·7H₂O 0.3 g, NaCl 1 g, FeSO₄, MnSO₄ and ZnSO₄ in traces, d,l-malate (Merck) 10 g; pH adjusted about 6.2. When the optical density rose to the half of the maximum obtainable, 2.5 g of glycolate (Fluka) or acetate (Merck) were added in one tube and in both 0.3 g of phenylhydrazine oxalate (PO) as trapping agent for glyoxylic acid (table 1-A). Care was taken to avoid blocking the growth of the cells. For the identification of glyoxylic acid and its increasing formation during the experiments the methods described in a preceding paper (BOLCATO *et al.*, 1956b) were used. The results of a typical experiment are assembled in table 1-A.

**Experiments with non-proliferating cultures.**

The washed cell mass of *E. coli* (22 g w.v.), obtained by following the procedure described elsewhere (BOLCATO *et al.*, 1956a), was suspended in 1600 ml of the following medium: H₂O 900 ml, yeast extract 100 ml, Na₂HPO₄ 3 g, KH₂PO₄ 1.5 g, MgSO₄·7H₂O 0.3 g, d,l-malate (or citrate) 4 g, pH adjusted about 6.2. The suspension was then transferred in equal parts into two glass tubes for aeration. There were added: in one tube 3 g of glycolate and in both tubes 0.3–0.2 g PO salt depending on the pH changes (table 1-B). The results obtained from the experiments with malate alone or associated with acetate or glycolate are assembled in table 1-B).