facts. Thus, a small proportion of the 'succinic oxidase' activity in the supernatant, and the observed decrease in the concentration of this enzyme system in the smaller particles, particularly in the case of ultrasonic treatment, can probably be explained in terms of damage of the membrane structure[14].

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Zusammenfassung

Verschiedene Fraktionen des mit Ultraschall, bzw. mit mechanischer Behandlung freigesetzten Zellinhaltes von *V. cholerae* wurden auf die Bernsteinsäuredehydrogenase, bzw. die Zytochromoxydasekomponente des Bernsteinsäureoxydase-Enzymkomplexes untersucht. Der letztere ist bei *V. cholerae* an Körnchen mit gewissen Teilchengrössen gebunden, deren Verwandschaft mit Mitochondrien besprochen wird.

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Formaldehyde and Formyl Group Intermediates of the Oxidation of Glyoxylate by Living Yeast and *E. coli* Cells

In a preceding series of experiments, it was established that glyoxylate is an intermediate of the direct oxidation of acetate and glycolate by yeast cells[1]. Further experiments[2,3] have excluded the possibility that glyoxylate might arise, instead of from acetate or glycolate, from malate or isocitrate by the action respectively of the malate synthetase or isocitritase, as was questioned by AJL[4] and UTTER[5]. We have now obtained new proofs on the proper position of glyoxylate in the monocarboxylic acid scheme (MAS) of oxidation of acetate by studying the oxidation of glyoxylate by yeast and *E. coli* cells and isolating its direct intermediates. In fact, from such experiments we were able to isolate formaldehyde and formyl group as was to be expected according to the formulation of the MAS:

acetate → acetyl → glycolate → glyoxylate →
→ formaldehyde → formyl → $H_2O + CO_2$

The present paper will report the results of these experiments. Details of the procedures employed for the preparation of yeast[6] and *E. coli*[7] cells suspensions have been described in previous papers. Formaldehyde was trapped by phenylhydrazine[7,8] or by the simultaneous presence of phenylhydrazine and dimedone[9]. The formyl group was identified as benzaldehyde, which is the result of the formylation of the benzene ring of the phenylhydrazine added to the medium[6,7]. The data of two typical experiments with yeast and *E. coli* cells are assembled in the Table.

The tubes for aeration contained: tube A, 1000 ml of a 30% (w.v.) of starved baker's yeast cells suspension. Tube *B*, 1000 ml of a 12% (w.v.) of starved *E. coli* cells suspension. In both tubes 200 mg of glyoxylic acid (Fluka) were added, while the addition of the phenylhydrazine oxalate (PO) occurred as indicated below.

Time	pH	PO g/l	Formaldehyde mg/l	Formyl group mg/l
A: with yeast cells				
8 h 50 min	5·2	0·50		
10 h 00 min	5·2	0·20		
11 h 20 min	5·3	0·20	0·65	
14 h 25 min	5·3		1·80	
18 h 00 min	5·4		0·20	11·40
B: with *E. coli* cells				
11 h 00 min	6·1	0·40		
12 h 30 min	6·2	0·20		
14 h 15 min	6·3	0·20	1·30	
16 h 00 min	6·4		0·20	
20 h 30 min	6·4		0·00	6·60

The findings of the preceding papers, and those referred to in this report, demonstrate that glyoxylate is an intermediate of the direct oxidation of acetate and glycolate. In effect, glyoxylate has been isolated from the oxidation of the two acids[1] and it cannot arise from malate or isocitrate, because of the inactivity, in our experimental conditions, of the malate synthetase and isocitritase[2,3]. In addition, glyoxylate oxidation leads to the same intermediates (formaldehyde and formyl group) obtained from the oxidation of acetate and glycolate, according to the formulation of the MAS.

In the present experiments, it is of interest to note that glyoxylic acid, also in the presence of phenylhydrazine, was oxidized in the form of its phenylhydrazone, as occurred also in the case of the formaldehyde phenylhydrazone[7,8]. This fact explains the impossibility of trapping large amounts of glyoxylate and formaldehyde during the respiration of acetate in presence of phenylhydrazine. Probably these phenylhydrazones, in dilute solution, are in the addition form[10], a form which is certainly labile and easily oxidizable by separation into its components.

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Riassunto

Dall'ossidazione del gliossilato, tanto con cellule intatte di lievito come con cellule di *E. coli*, sono stati isolati l'aldeide formica e il formile, che sono anche intermedi dell'ossidazione dell'acetato e del glicolato. Il risultato costituisce una nuova prova che il gliossilato è, a sua volta, un intermedio dell'ossidazione dell'acetato e del glicolato, dai quali era anche stato isolato in precedenza.

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