OXIDATIVE REACTIONS IN CELL-FREE YEAST EXTRACTS

by

P. M. NOSSAL

Department of Biochemistry, University of Adelaide (Australia)

So far, it has been impossible to prepare actively respiring yeast extracts[1,2] probably because yeast cells are so difficult to disintegrate that, during cell rupture, integrated enzyme systems necessary for respiration are also disrupted. Therefore, respiratory mechanisms have had to be studied in cells frozen with dry ice[3] or liquid air[4] to make the cell wall permeable to suspected organic acid intermediaries.

By using a highly rapid mechanical cell disintegrator[2], we can disrupt, in 10 sec, 35% of the cells in 2 g yeast. The time interval between the commencement of disintegration and the first reading on WARBURG manometers is greatly reduced, and we have now obtained extracts which oxidise aerobically ethanol and substances associated with the KREBS[5] cycle. The oxidations are very unstable, which may explain why previous workers, using much slower disintegrators[1], failed to prepare respiring extracts.

10-sec cell-free baker's yeast extracts were prepared in 0.1 *M* phosphate buffer pH 7.6 as described previously[2,6]. The respiration of 3 ml aliquots of such extracts was measured in standard Warburg equipment at 30°.

The rates of oxidation of various substrates by such extracts are shown in Table I. The main product of ethanol oxidation was acetate. Only about 1% ethanol-1-^{14}C was converted to CO_2, and this proportion was not increased by addition of oxaloacetate. All members of the KREBS cycle were oxidised, succinate most and malate least rapidly.

3.0 ml 10-sec cell-
4.0 ml.

ERRATUM

Biochim. Biophys. Acta, 14 (1954) 154, the heading of the last column of Table I should read

		Maximum Q_{O_2}
Blank, acetate, oxalate	None or DPN, TPN	2
Glycine, L-aspartate	None	2
Formaldehyde	None	2

* See footnote for abbreviations.

The expected products (Table II) were formed from the various acids, as determined by silica gel and paper partition chromatography[7]. We are now conducting quantitative balance studies of the reactions.

TABLE II

PRODUCTS OF THE OXIDATION OF VARIOUS SUBSTRATES IN CELL-FREE YEAST EXTRACTS

Substrate	*Products*
Ethanol	Acetate ($< 1\%$ CO_2) (no acetylphosphate)
Citrate	CO_2, α-ketoglutarate, succinate, malate
α-Ketoglutarate	CO_2, low succinate, "tricarballylate"*, malate
Succinate	Almost *no* CO_2 (unless DPN added), malate + fumarate
Oxaloacetate	CO_2, citrate, α-ketoglutarate, succinate, malate
Oxaloacetate + ethanol	CO_2 (less than oxaloacetate alone), mainly malate + fumarate and acetate

* This is an unidentified acid which on columns coincides with genuine tricarballylic acid, yet on papers behaves like a dicarboxylic acid.

We attempted to obtain the initial condensation of the KREBS cycle by incubating yeast extracts with oxaloacetate plus ethanol, acetaldehyde, acetyl phosphate, acetate ± ATP* or pyruvate ± ATP, adding also such co-factors as DPN, TPN, CoA and A5P. In no case could we observe an *oxidative* condensation. The extracts converted oxaloacetate alone to citrate, α-ketoglutarate, succinate, malate, fumarate, pyruvate, acetate and CO_2. With acetate + ATP as well as oxaloacetate, the formation of KREBS cycle acids was considerably increased, but no oxygen uptake was found. In our extracts, coupled reductions can take the place of oxygen in the oxidative reactions of the KREBS cycle: when oxaloacetate was added to extracts oxidising ethanol, there was an almost immediate reduction in O_2 uptake and a greatly increased formation of malate.

Although it has not been possible to prepare extracts which completely oxidise organic substrates to CO_2, these yeast extracts are the first in which the electron transport system is still largely preserved and all reactions of the KREBS cycle occur.

The individual reactions between citrate and malate in our extracts can transfer electrons to oxygen, some of them very rapidly, but for reasons as yet unknown the complete cycle is impaired. Our findings again[8,9,10] show that the whole KREBS cycle is present in yeast. However, the ultimate proof of whether it is the main respiratory mechanism will probably have to come from tracer studies on the incorporation of labelled glucose, pyruvate or acetate into suspected intermediaries.

The author thanks the Rockefeller Foundation and the National Health and Medical Research Council of Australia for partial support of this work, Mr. D. MORTON for technical assistance and the Effront Yeast Co., Melbourne, for a weekly gift of yeast.

REFERENCES

1 H. M. HIRSCH, *Biochim. Biophys. Acta*, 9 (1952) 674.
2 P. M. NOSSAL, *Austral. J. Exp. Biol.*, 31 (1953) 583.
3 H. A. KREBS, S. GURIN AND L. V. EGGLESTON, *Biochem. J.*, 51 (1952) 614.
4 F. LYNEN AND N. NECIULLAH, *Ann.*, 541 (1939) 203.
5 H. A. KREBS AND W. A. JOHNSON, *Enzymologia*, 4 (1937) 148.
6 P. M. NOSSAL, *Biochem. J.*, 57 (1954) 62.
7 J. N. LADD AND P. M. NOSSAL, *Austral. J. Exp. Biol.*, (1954) in the Press.
8 S. WEINHOUSE AND R. H. MILLINGTON, *J. Am. Chem. Soc.*, 69 (1947) 3089.
9 C. MARTIUS AND F. LYNEN, *Advances Anzymol.*, 10 (1950) 167.
10 E. C. FOULKES, *Biochem. J.*, 48 (1951) 378.

Received March 19th, 1954

* Abbreviations used are: ATP for adenosine triphosphate; A5P for adenosine-5-phosphate; DPN for diphosphopyridine nucleotide; TPN for triphosphopyridine nucleotide; CoA for coenzyme A.