sequently grow on malate only after a period of adaptation. Glucose-trained cells have difficulty in adapting to malate, and on inoculation into glucose-malate media display diauxic growth in which malate is used after glucose. Glycerol-trained cells do not show diauxie in glycerol-malate media, but the degree of malate adaptation depends on the period of growth before glycerol is exhausted. Cells subcultured from glycerol into media containing an excess of malate and various limited amounts of glycerol grow on malate, after glycerol exhaustion, with different rates of growth, O_2 uptake and malate utilization. Whatever the rate of growth on malate, it is stimulated by addition of acetate.

In cells, otherwise physiologically competent, the possible pacemakers in malate utilization are: (1) permeation of malate into the cells; (2) manufacture of biosynthetic precursors derived from phosphoenolpyruvate; (3) production of energy or withdrawal of intermediates from the tricarboxylic acid cycle, which would depend on the production of acetyl-CoA from malate.

As acetate cannot be converted into phosphoenolpyruvate by these cells (Kornberg, 1966), it is unlikely that the conversion of malate into phosphoenolpyruvate limits growth on malate. The provision from malate of C_2 fragments for the tricarboxylic acid cycle depends on 'malic' enzyme to yield pyruvate and thence on pyruvate dehydrogenase to give acetyl-CoA. Measurements of these enzyme systems during adaptation to malate shows that they are not limiting malate metabolism.

It follows that the intracellular supply of malate limits growth and that adaptation to malate depends on synthesis of the mechanism for malate permeation (Kay & Kornberg, 1971). Even maximal malate utilization is limited by permeation, and acetate stimulation reflects a greater total flux of carbon fragments into the cell.

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Preliminary Separation of Enzymes Extracted from *Bacillus stearothermophilus*

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A method has been developed for large-scale enzyme preparation from *Bacillus stearothermophilus*, giving extracts suitable for the eventual isolation of hundred-milligram quantities of several pure enzymes.

A 5 kg batch of *B. stearothermophilus* cell paste

(Sargeant *et al.*, 1971) was suspended in 5 litres of 0.1M-imidazole buffer, pH 8.0, and disrupted by a single pass at 40 litres/h through a Manton-Gaulin homogenizer at 550 kg/cm² (J. Melling, A. Atkinson, B. W. Phillips & W. R. Stones, unpublished work). Then 20 mg of deoxyribonuclease (300 units/mg; BDH Chemicals Ltd., Poole, Dorset, U.K.) was added and the suspension was left at 4°C for 30 min. Cell debris was removed by centrifugation at 23 000 g for 45 min and washed with 2 litres of 0.02M-imidazole buffer, pH 7.0.

The combined supernatants (conductivity 3.5 mmho; pH 7.0 ± 0.2) were then added to 5 kg of DEAE-cellulose (conductivity 8 mmho; pH 7.0) and left for 30 min at 4°C after mixing. Unbound protein was removed and DEAE-cellulose collected by centrifugation in a spin-drier. Batch elution of enzymes was accomplished with stepwise increases in NaCl concentration, with two washes with 6 litres of buffer at each stage, by using a spin-drier to remove the ion-exchanger. The 0.1M-, 0.5M- and 1.0M-NaCl fractions were obtained. The last was treated with ethanol (2.0 vol.) at 4°C to precipitate tRNA, which was further purified by reprecipitation with ethanol. The 66%-(v/v)-ethanol supernatant contained the enzyme triose phosphate isomerase (Fahey *et al.*, 1971), which was adsorbed on hydroxyapatite.

The unbound protein fraction and the 0.1M-NaCl fraction together contained 40% of the total phosphofructokinase activity, free from alcohol dehydrogenase. The 0.5M-NaCl fraction contained glyceraldehyde 3-phosphate dehydrogenase, alcohol dehydrogenase, aminoacyl-tRNA synthetases, trans-formylase, fructose diphosphate aldolase and 60% of the total phosphofructokinase activity. With the exception of the last the recovery of each enzyme in this fraction was 90% of the combined supernatant activity with a purification factor of 2.5, and 70% of the broken-cell activity. The 66%-(v/v)-ethanol supernatant from tRNA precipitation contained 60% of the triose phosphate isomerase activity of the combined supernatant with a purification factor of 40. The tRNA (total yield 7g), which contained little protein or DNA, had a methionyl acceptor activity of 50 pmol/ E_{260} unit.

Further purification of these enzymes and of tRNA from these extracts has been carried out in the Medical Research Council Laboratory of Molecular Biology, Cambridge, U.K., by Dr. C. J. Bruton, Dr. B. F. C. Clark, Dr. R. C. Fahey, Dr. J. I. Harris, Dr. R. Jack, Dr. E. Kolb and Dr. G. Koch.

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Phosphatidylinositol Kinase Activity in *Saccharomyces cerevisiae*

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Phosphatidylinositol kinase catalyses the reaction:

The enzyme has been detected in a variety of animal tissues, and is localized predominantly in the plasma membrane. The function of phosphatidylinositol kinase in animal cell membranes is obscure, but it is possible that diphosphoinositide is involved in the binding of bivalent cations. Phosphatidylinositol kinase has not so far been reported in microorganisms, although its activity has been implicated (Cerbón, 1970) to explain arsenate transport into yeast. In addition, small amounts of diphosphoinositide and triphosphoinositide have been detected in lipid extracts of *Saccharomyces cerevisiae* (Lester & Steiner, 1968). The present communication reports the detection of phosphatidylinositol kinase in a strain of *Sacch. cerevisiae*.

Sacch. cerevisiae N.C.Y.C. 366 was grown aerobically in a glucose-salts-vitamins medium, pH 4.5, and sphaeroplasts were obtained from exponential-phase cells by digestion with a basidiomycete glucanase preparation, in citrate-phosphate buffer, pH 6.0, containing 10 mM-MgCl₂ and 1 M-sorbitol. Phosphatidylinositol kinase activity was assayed by using a reaction mixture containing phosphatidylinositol and [γ -³²P]ATP and measuring the radioactivity of the diphosphoinositide formed after extraction in chloroform-methanol and separation by paper chromatography. Phosphatidylinositol kinase activity was not detected in the supernatant left after sphaeroplasts had been removed from the glucanase digest, which suggests that the enzyme is not located in the periplasm in this organism. Sphaeroplasts were lysed by suspension in ice-cold 10 mM-MgCl₂, pH 6.0, and crude plasma membranes were separated by centrifugation at 45000g-min. Phosphatidylinositol kinase activity was detected in the membranes, but not in the particle-free supernatant obtained by centrifuging the lysate at 6.8 $\times$ 10⁶g-min. Separation of membrane fractions from the yeast on a discontinuous gradient of sucrose has

shown a preferential location of the enzyme in a plasma-membrane-rich fraction. The distribution of phosphatidylinositol kinase in the subcellular fractions is similar to that of the (Na⁺+K⁺)-Mg²⁺-stimulated adenosine triphosphatase. The yeast enzyme, like the mammalian enzyme, is stimulated by Mg²⁺, but differs from mammalian phosphatidylinositol kinase in showing a greater linearity on time-activity plots. Also, it may require a higher concentration of phosphatidylinositol in the reaction mixture.

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The Hexose Phosphate Synthetase of *Methylococcus capsulatus*

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Previous work (Kemp & Quayle, 1966; Lawrence *et al.*, 1970) established the existence in crude extracts of *Methylococcus capsulatus* of an enzyme system catalysing the condensation of [¹⁴C]formaldehyde with D-ribose 5-phosphate to give a hexose phosphate, tentatively identified as allulose phosphate.

The enzyme system has now been fractionated by high-speed centrifugation of ultrasonic extracts. When the soluble fraction was incubated with D-ribose 5-phosphate, a product was formed that could condense with formaldehyde in the presence of the particulate fraction. Various lines of evidence support the identification of the intermediate as D-ribulose 5-phosphate, although the commercial product inhibited the condensation. For instance, the soluble fraction could be replaced by a phosphoriboisomerase preparation purified from spinach.

The nature of the condensation product has been re-examined. The previous work (Kemp & Quayle, 1966) suggested that the sugar moiety was allulose. Further, reduction of the sugar with borohydride gave the two sugar alcohols expected from allulose, allitol and altritol. However, g.l.c. of the trimethylsilyl derivative has clearly differentiated the compound from allulose (kindly given by Dr. F. J. Simpson). Also, neither the sugar phosphate formed in the condensation nor the free sugar isolated by chromatography after treatment with phosphatase gave the colour reactions expected for a 2-hexulose (phosphate).

The possible nature of the product will be discussed and analogy drawn to the condensation of formaldehyde with dihydroxyacetone phosphate catalysed by rat liver (Charalampous & Mueller, 1953).