concentration dependence, and that the partial specific volumes do not differ greatly from the value used in the calculations. Although there is no information available about the partial specific volumes, experimental evidence indicates that there are probably no unexpected concentration effects in the sedimentation behaviour.

The provisional conclusion that guinea-pig albumin differs from the other two albumins, which are closely similar, is supported by the finding that its sedimentation coefficient in the neutral range (pH 6–8) was slightly lower than the 4.4 S found for both rat and rabbit albumins. An additional distinction lay in the sedimentation velocity behaviour in more acid solution (pH 5–2). Here, rat and rabbit albumins followed the trend already established for human, bovine, and horse serum albumins[4], the sedimentation coefficient falling throughout the range pH 5 to pH 2, whereas a more complicated dependence on pH was found for guinea-pig albumin. These observations indicate, therefore, that guinea-pig albumin differs from the others not only in the size of its molecule, but also in some structural feature which remains to be elucidated. Full details of this work will be published later.

National Institute for Medical Research, The Ridgeway, Mill Hill, London (Great Britain) P. A. CHARLWOOD

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Adenosine polyphosphate requirement of baker's yeast phosphopyruvate carboxylase

The enzyme responsible of carbon dioxide fixation by baker's yeast (*Saccharomyces cerevisiae*)[1,2] is a phosphopyruvate carboxylase. It can be extracted in a soluble form from acetone-dried yeast[3] and purified 500 times by a procedure involving treatment with $Ca_3(PO_4)_2$ gel, three precipitations with $(NH_4)_2SO_4$ between 0.42–0.55 satn., dialysis against 0.025 *M* borate buffer pH 8.0, treatment with protamine sulphate, two fractionations with ethanol and a final chromatography on a hydroxylapatite column[4]. If $^{14}CO_2$ is used for the carboxylation of phosphopyruvate, radioactive oxaloacetic acid can be isolated as the 2,4-dinitrophenylhydrazone and the latter identified by paper chromatography and radioautography; further, if the carboxylation takes place in the presence of glutamate-aspartate transaminase and glutamate, or of malic dehydrogenase and reduced diphosphopyridine dinucleotide, the oxaloacetic acid reacts to form respectively aspartic or malic acid, which can be isolated and identified by the chromatographic and radioautographic methods.

The purified enzyme preparations decarboxylate oxaloacetate, catalyse the exchange of $^{14}CO_2$ into the β-carboxyl group of oxaloacetate, and carboxylate phosphopyruvate. The exchange reaction requires ATP and Mn^{++}, but for oxaloacetate decarboxylation, only ATP is necessary. The carboxylation of phosphopyruvate takes

place under the conditions described in Table I, *i.e.*, in the presence of a phosphate acceptor and Mn^{++} (or Mg^{++}). The enzyme is active only with adenosine diphosphate and, accordingly, the exchange and decarboxylations reactions take place with ATP, but not with guanosine, inosine or cytidine triphosphates. The requirement for adenine nucleoside polyphosphates differentiates the enzyme of baker's yeast from nearly all the already known carboxylases, which either are specific for guanosine or inosine polyphosphates[5–8] or irreversibly carboxylate phosphopyruvate without phosphate acceptor[7–10]. Wheat-germ carboxylase can operate also with ATP[8] but it is not clear if this is due to interference by nucleoside diphosphokinase as ocurred with the liver oxaloacetate carboxylase before the latter was sufficiently purified[5]. CO_2-activating enzyme[11] could not be detected in the yeast phosphopyruvate carboxylase preparations.

TABLE I

EFFECT OF NUCLEOSIDE DIPHOSPHATES ON THE ACTIVITY OF BAKER'S YEAST PHOSPHOPYRUVATE CARBOXYLASE

1 ml reaction mixture contains 65 μg enzyme; 2.2 μmoles phosphopyruvate*; 1.25 μmoles $MnCl_2$; 4.31 μmoles $NaH^{14}CO_3$ ($1.09 \cdot 10^5$ counts/min); 1.2 μmoles nucleoside diphosphate (from Sigma Chemical Co.), and 0.1 *M* borate–succinate buffer, pH 6.08. Reaction mixture incubated in a stoppered 10 mm × 90 mm test tube for 15 min at 30°. The reaction was stopped with 0.025 ml H_2SO_4 (sp. gr. 1.84) and samples plated and counted.

Nucleoside diphosphate	*CO_2 fixation (counts/min)*
None	63
Adenosine diphosphate	2080
Inosine diphosphate	126
Guanosine diphosphate	190
Cytidine diphosphate**	126

* 20 μmoles pyruvate and 8 μmoles ATP did not replace phosphopyruvate.
** Uridine diphosphate (kindly supplied by Prof. L. F. LELOIR) was also ineffective.

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Institute of Biochemistry, School of Medicine, University of Buenos Aires, and Laboratory for Cell Metabolism, Atomic Energy Commission, Buenos Aires (Argentina)

J. CANNATA
A. O. M. STOPPANI

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