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New assays for carbamyl phosphate synthetase applicable in the presence of exogenous carbamyl phosphate.

To date one major method of assaying CPS activity has been used. It estimates the amount of carbamyl phosphate (CP) produced from bicarbonate, ammonia, and ATP by converting the carbamyl phosphate produced into either citrulline (via OCTO, ureidosuccinate (US) (via ACT), or chemically to urea, and then colorimetrically assaying the ureido- group (Gerhardt and Pardee 1962 J. Biol. Chem. 237: 891-896, Prescott and Jones 1969 Anal. Biochem. 32: 408-419, and variants).

While investigating the effect of exogenous carbamyl phosphate (CP) on pyrimidine-specific carbamyl phosphate synthetase (CPS) activity, it became necessary to develop an assay which did not depend an the estimation, directly or indirectly, of CP present at the end of the reaction. One method was devised which instead estimated the synthesis of another product of CPS, the glutamate derived from glutamine. This glutamate was used as a substrate for the glutamate dehydrogenase assay. The reaction mixture contains the sample in 100 mMolar tris acetate buffer at pH7.0, 6 mMolar glutamate, 12 mMolar magnesium chloride, 12 mMolar ATP, 30 mMolar potassium bicarbonate, and 0.4 mMo1ar $NADP^+$. Synthesis of NADPH is followed by absorbance at 340 nM over a period of 120 minutes at 37°. using a Unicam SP-800 dual beam spectrophotometer. As a control, ATP, a substrate of CPS,was omitted from the reaction. This still retained some nonspecific reaction involving NADP and glutamine in both the experimental and control mixtures, and the reaction trace produced is shown in the figure. to find that part of the trace corresponding to the CPS activity, a further control was introduced by the use of an extract of the CPS^- strain pyr-3 (KS20), and this trace is also shown in the figure. It can be seen that the positive slope after the initial nonspecific peak corresponds to CPS activity, the initial peak being nonspecific. Comparison of this method with that of Prescott and Jones, assaying in the absence of exogenous carbamyl phosphate, gave identical results.

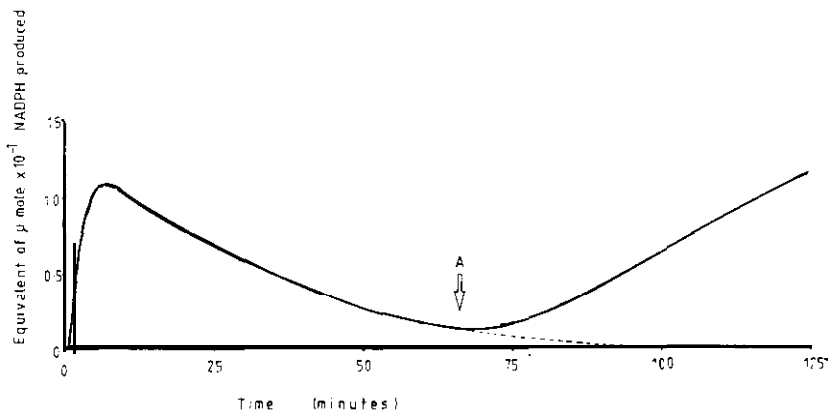

Figure -- Typical reaction curve from the spectrophotometric assay of CPS. Reaction and control are the same upto point A, following which the control is shown as a dotted line. Reaction and control had added wild type and pyr-3 (KS20) extracts respectively.

The second method is based upon the conversion of ^{14}C bicarbonate, to carbamyl phosphate and beyond. The reaction mixture used is basically as for the Prescott and Jones assay, with a 3 ml reaction mixture containing approx. 2 mg/ml of crude Neurospora extract. Duplicate 0.1 ml samples are taken with time. To one sample is added 0.2 ml 50% sulfuric acid, and the mixture is then heated at 90°C to liberate any bicarbonate and CP as CO_2, so that only ureidosuccinate (US) remains. To the second is added 0.1 ml 1M amnonium chloride, followed by 9O°C for 10 minutes, then 0.1 ml 50% sulfuric acid and again 90°C for 10 minutes. This liberates bicarbonate but retains both CP (fixed as urea) and US. Thus the ^{14}C incorporated into CP and US can be estimated by scintillation counting. - - - Department of Genetics, Leeds University, Leeds LS2 9JT, U.K.