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1 M. EBERT, J. P. KEENE, A. J. SWALLOW AND J. H. BAXENDALE, *Pulse Radiolysis*, Academic Press, New York, 1965.
2 T. SANNER AND A. PIHL, *Radiation Res.*, 19 (1963) 12.
3 F. HUTCHINSON AND D. A. ROSS, *Radiation Res.*, 10 (1959) 477.
4 J. A. V. BUTLER, A. B. ROBINS AND J. ROTBLAT, *Proc. Roy. Soc. London, Ser. A.*, 256 (1960) 1.
5 T. SANNER AND A. PIHL, *European J. of Biochem.*, 1 (1967) 33.
6 T. SANNER, *Radiation Res.*, 26 (1965) 95.
7 A. O. ALLEN, in M. HAISSINSKY, *Actions Chimiques et Biologiques des Radiations*, Vol. 5, Academic Press, New York, 1961, p. 11.
8 G. W. SCHWERT AND Y. TAKENAKA, *Biochim. Biophys. Acta*, 16 (1955) 570.
9 P. DESNUELLE, in P. D. BOYER, H. LARDY AND K. MYRBÄCK, *The Enzymes*, Vol. 4, Academic Press, New York, 1960, p. 119.
10 J. K. THOMAS, *Trans. Faraday Soc.*, 61 (1965) 702.
11 G. E. ADAMS, J. W. BOAG, J. CURRANT AND B. D. MICHAEL, p. 131 in ref. 1.
12 J. V. DAVIS, W. GRIFFITHS AND G. O. PHILLIPS, p. 181 in ref. 1.
13 E. J. HART, J. K. THOMAS AND S. GORDEN, *Radiation Res., Suppl. 4*, (1964) 74.
14 G. SCHOLES, P. SHAW, R. L. WILLSON AND M. EBERT, p. 151 in ref. 1.

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Fructose 1,6-diphosphate and 3',5'-cyclic AMP as positive effectors of pyruvate kinase in developing embryos

In the case of feed-back control, the end product inhibits the activity of the first enzyme of a biosynthetic chain and consequently blocks the enzymatic processes leading to its own synthesis. The investigations of YATES AND PARDEE[1] have established the inhibition of aspartate carbamoyltransferase (EC 2.1.3.2) by CTP in the chain of CTP biosynthesis. The inhibition of L-threonine dehydratase (EC 4.2.1.16) by L-isoleucine has been established by UMBARGER AND CHANGEUX[2–4] in the chain of isoleucine biosynthesis. The inhibitory action of ATP and citrate on the activity of phosphofructokinase (EC 2.7.1.11) has been suggested for the mechanism of Pasteur effect[5–8].

In contrast to feed-back control, the "feed-forward" control suggests the activation of the end reaction in a metabolic chain by the product of the first reaction in that metabolic chain. HESS, HAECKEL AND BRAND[9] were the first to report the possibility of this type of regulation in the control of glycolysis in yeast.

In this report, results are presented on the activation of pyruvate kinase (EC 2.7.1.40) by Fru-1,6-P_2 ("feed-forward" control) in developing loach *(Mis-*

gurnus fossilis) embryos. 3',5'-Cyclic AMP appeared to be the activator of this enzyme.

The major part that Fru-1,6-P_2 plays in the regulation of glycolysis in the early embryogenesis of the loach[10], allows one to suggest that Fru-1,6-P_2 may serve as an allosteric effector for certain glycolytic enzymes. In other words, Fru-1,6-P_2 serves in this case not only as substrate for the system converting Fru-1,6-P_2 into lactate but also as a factor controlling enzymic activity in the glycolytic chain.

The pyruvate kinase from loach embryos, free of enzymes which split Fru-1,6-P_2 and react with NADH (such as fructose diphosphate aldolase, glycerol-3-phosphate dehydrogenase, glyceraldehydephosphate dehydrogenase *etc.*)was isolated by the following procedure.

40 g (fresh weight) of developing eggs were extracted by 190 ml of 0.5% EDTA. The nuclei and yolk granules were sedimented by centrifugation at 3000 × *g* for 15 min. 42 g $(NH_4)_2SO_4$ were added to 205 ml of clear supernatant during 90 min. The resulting precipitate was discarded. This sediment contained the fructose diphosphate aldolase, other enzymes reacting with triosephosphates and NADH and only a small amount of pyruvate kinase. 23 g of $(NH_4)_2SO_4$ were added to 245 ml of the resulting supernatant. The precipitate was collected and dissolved in 3.5 ml of 0.02 M acetate buffer (pH 4.6). A sediment of pyruvate kinase was formed during storage at 3°. The resulting preparation was contaminated with traces of lactate dehydrogenase (EC 1.1.1.27).

The method of BEISENHERZ *et al.*[11], generally accepted for the fractionation of certain glycolytic enzymes from rabbit muscle extract, was not suitable for the fractionation of these enzymes from loach embryos. According to the procedure described in the present paper, different ratios of volumes of extracts and different amounts of $(NH_4)_2SO_4$ are necessary for the fractionation of extracts of loach embryos.

Pyruvate kinase activity was determined according to BÜCHER AND PFLEIDERER[12]. The addition of Fru-1,6-P_2 (0.6 mM to 1.2 mM) at pH 7.5 slightly enhanced the pyruvate kinase activity (approx. 20%). The decrease of pH to 6.5 gave more clear-cut results on the activation of pyruvate kinase by Fru-1,6-P_2. The pyruvate kinase activity at pH 6.5 was about one-tenth of the initial activity at pH 7.5. The addition of Fru-1,6-P_2 up to 0.3 mM resulted, in this case, in a three-fold increase

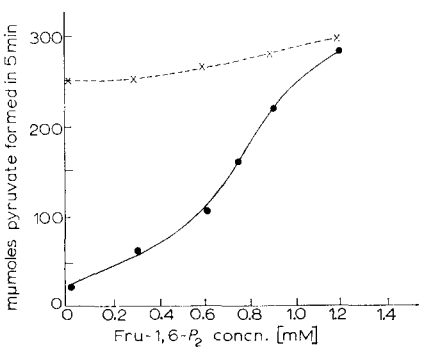

Fig. 1. Activation of pyruvate kinase by Fru-1,6-P_2. Enzyme activity is expressed in mμmoles of pyruvate formed in 5 min. The assay medium was the same as that described in Table I. Dotted line, pyruvate kinase activity in Tris (pH 7.5); solid line, activity in Tris (pH 6.5).

TABLE I

EFFECT OF 3′,5′-CYCLIC AMP AND Fru-1,6-P_2 ON THE ACTIVITY OF PYRUVATE KINASE FROM LOACH EMBRYOS

The assay mixture contained 50 mM Tris (pH 6.5), 0.15 mM NADH*, 0.23 mM ADP, 0.8 mM phosphoenolpyruvate*, 8 mM $MgCl_2$, 75 mM KCl, 0.4 units lactate dehydrogenase* and 0.05 units of pyruvate kinase in a total volume of 3.0 ml. The results are expressed in mμmoles of pyruvate formed in 5 min. The 3′,5′-cyclic AMP and the materials marked * were purchased from Serva.

Nucleotide (mM)	*3′,5′-cyclic AMP*	*3′,5′-cyclic AMP + 0.6 mM Fru-1,6-P_2*
—	12	(121)**
0.1	39	190
0.2	92	310
0.3	100	328

** Pyruvate kinase activity with Fru-1,6-P_2 in the absence of 3′,5′-cyclic AMP.

TABLE II

EFFECT OF ATP ON THE ACTIVATION OF PYRUVATE KINASE BY Fru-1,6-P_2

The conditions for the pyruvate kinase assay are the same as described in Table I.

Control		*0.6 mM Fru-1,6-P_2 added*			
without ATP	*3.3mM ATP*	*without ATP*	*1.7 mM ATP*	*3.3 mM ATP*	*6.0 mM ATP*
24	24	72	72	52	24

TABLE III

CHANGES IN PHOSPHOENOLPYRUVATE AND PYRUVATE LEVELS IN ISOLATED LOACH EMBRYOS DURING ANAEROBIOSIS

Phosphoenolpyruvate and pyruvate were determined in neutralized perchloric extracts of embryos according to procedures of BÜCHER[13] and of CZOCK AND ECKERT[14], respectively. 0.3 ml of extract was added to 4.7 ml of assay mixture in a quartz cell with a light path of 2.5 cm. The assay mixture contained 1.5 mM of ADP, 3.0 mM $MgCl_2$, 90 mM Tris (pH 7.5), 0.3 mM NADH and excess lactate dehydrogenase and pyruvate kinase in a total volume of 5 ml. The ADP and enzyme preparations were from Serva and Boehringer. Amounts are expressed in mμmoles per 100 embryos.

Assay	*Pyruvate*	*Phospho-enolpy-ruvate*	*Pyruvate/ phosphoenol-pyruvate*
Control	13	43	0.3
Anaerobiosis	129	440	0.29

of pyruvate kinase activity; 1.2 mM of Fru-1,6-P_2 caused a 10-fold increase of pyruvate kinase activity (Fig. 1). The effect of Fru-1,6-P_2 was enhanced in the presence of 3′,5′-cyclic AMP: it was effective at concentrations from 0.05 mM to 0.3 mM in the presence of Fru-1,6-P_2. The activating effect of 3′5′-cyclic AMP was also observed

in the absence of Fru-1,6-P_2 (Table I). A slight activating effect could be observed in the presence of 5′-AMP.

The opposite effect was found for ATP. 3–6 mM of ATP did not change the activity of pyruvate kinase, but blocked the activating effect of Fru-1,6-P_2 (Table II). The addition of 3′,5′-cyclic AMP abolished the action of ATP and restored the activating effect of Fru-1,6-P_2.

The opposite actions of ATP on pyruvate kinase activity, on the one hand, and of 3′,5′-cyclic AMP, on the other hand, in the presence of Fru-1,6-P_2, appeared to be the factors controlling the rate of pyruvate formation with increase in the Fru-1,6-P_2 level. This control seems to maintain a constant ratio of phosphoenolpyruvate/pyruvate under both aerobic and anaerobic conditions in loach embryo cells (Table III).

The results we have obtained on the activating effect of Fru-1,6-P_2 on isolated yeast pyruvate kinase are remarkably similar to those obtained by HESS, HAECKEL AND BRAND[9].

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1 R. A. YATES AND A. B. PARDEE, *J. Biol. Chem.*, 221 (1956) 757.
2 H. E. UMBARGER, *Science*, 123 (1956) 848.
3 H. E. UMBARGER, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 301.
4 J. P. CHANGEUX, *Cold Spring Harbor Symp. Quant. Biol.*, 26 (1961) 313.
5 J. V. PASSONEAU AND O. H. LOWRY, *Biochem. Biophys. Res. Commun.*, 7 (1962) 10.
6 J. V. PASSONEAU AND O. H. LOWRY, *Biochem. Biophys. Res. Commun.*, 13 (1963) 372.
7 T. E. MANSOUR, *J. Biol. Chem.*, 238 (1963) 2885.
8 A. H. UNDERWOOD AND E. A. NEWSHOLM, *Biochem. J.*, 95 (1965) 868.
9 B. HESS, R. HAECKEL AND K. BRAND, *Biochem. Biophys. Res. Commun.*, 24 (1966) 824.
10 L. S. MILMAN AND YU. G. YUROWITZKI, *Biokhimia*, 32 (1967) 221.
11 G. BEISENHERZ, H. J. BOLTZE, K. H. GARBADE, T. BÜCHER AND R. CZOK, *Z. Naturforsch.*, 8b (1953) 1955.
12 TH. BÜCHER AND G. PFLEIDERER, in S. P. COLOWICK AND N. O. KAPLAN, *Methods in Enzymology*, Vol. 1, Academic Press, New York, 1955, p. 435.
13 R. CZOCK AND L. ECKERT, in H. U. BERGMEYER, *Methoden der enzymatische Analyse*, Verlag Chemie, Weinheim, 1962, p. 229.
14 TH. BÜCHER, in H. U. BERGMEYER, *Methoden der enzymatische Analyse*, Verlag Chemie, Weinheim, 1962, p. 253.

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