*Biochem. J.* (1980) **190**, 705–710
*Printed in Great Britain*



# Pyruvate dehydrogenase complex of ascites tumour

## ACTIVATION BY AMP AND OTHER PROPERTIES OF POTENTIAL SIGNIFICANCE IN METABOLIC REGULATION

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(*Received 19 December 1979*)

1. AMP is an activator of the pyruvate dehydrogenase complex of the Ehrlich–Lettré ascites tumour, increasing its $V$ up to 2-fold, with $K_a$ of 40$\mu$M at pH 7.4. This activation appears to be an allosteric effect on the decarboxylase subunit of the complex. 2. The pyruvate dehydrogenase complex has a $K_m$ for pyruvate within the range 17–36$\mu$M depending on the pH, the optimum pH being approx. 7.4, with a $V$ of approx. 0.1 unit/g of cells. The rate-limiting step is dependent on the transformation of the enzyme–substrate complex. The $K_m$ for CoA is 15$\mu$M. The $K_m$ for $NAD^+$ is 0.7mM for both the complex and the lipoamide dehydrogenase. The complex is inhibited by acetyl-CoA competitively with CoA; the $K_i$ is 60$\mu$M. The lipoamide dehydrogenase is inhibited by NADH and NADPH competitively with $NAD^+$, with $K_i$ values of 80 and 90$\mu$M respectively. In the reverse reaction the $K_m$ values for NADH and NADPH are essentially equal to their $K_i$ values for the forward reaction, the $V$ for the latter being 0.09 of that of the former. Hence the reaction rate of the complex *in vivo* is likely to be markedly affected by feedback isosteric inhibition by reduced nicotinamide nucleotides and possibly acetyl-CoA.

Tumours have a characteristically high rate of aerobic glycolysis. This property, discovered by Warburg over 50 years ago (Warburg, 1926), is still largely unexplained on a molecular basis (Lynen, 1941; Johnson, 1941; Warburg, 1956; Weinhouse, 1956; Racker, 1976; Sols, 1976; Sols *et al.*, 1978). We have observed that the regulatory enzymes hexokinase and phosphofructokinase in ascites-tumour cells are quantitatively and qualitatively adequate to account for the high rate of glycolysis, but why it does occur still remains to be explained (Lazo *et al.*, 1978; Lazo & Sols, 1979). Pyruvate dehydrogenase complex, at the crossroads between reduction and oxidation of pyruvate, is an enzyme that has been studied intensively as regards metabolic regulation in normal tissues (Reed, 1974; Randle, 1978; Batenburg & Olson, 1976; Cate & Roche, 1978; Olson *et al.*, 1978*a*,*b*; Hansford & Cohen, 1978), but not in tumours.

As part of a systematic study of the enzymic basis of aerobic glycolysis in tumours, we have studied the pyruvate dehydrogenase complex of ascites-tumour cells. We report here its kinetic properties with respect to several parameters of likely physiological significance, and show that this enzyme can be specifically activated by AMP within its physiological concentration range.

### Materials and methods

#### *Cell and enzyme preparations*

A hyperdiploid strain of Ehrlich–Lettré ascites carcinoma (kindly supplied by Dr. M. Gosalvez, Clínica Puerta de Hierro, Madrid) was maintained by weekly inoculation in the abdominal cavity of 2-month-old Swiss mice. The cells were harvested 7–10 days after inoculation, washed in buffer A (140mM-NaCl, 5mM-KCl, 5mM-$MgCl_2$ and 10mM-sodium phosphate, pH 7.4), and homogenized in a mortar with glass beads of 0.11mm diameter in the ratio 3g of beads/5g of cells during 6min at 4°C. The homogenate was diluted with 100ml of buffer B (0.25M-sucrose, 2mM-EDTA, 5mM-$\beta$-mercapto-ethanol and 10mM-sodium phosphate, pH 7.4) and centrifuged at 2500$g$ for 5min. The supernatant was centrifuged at 10000$g$ for 10min and the mito-chondrial pellet suspended in buffer B and washed three times to obtain a preparation without detectable lactate dehydrogenase (EC 1.1.1.27). The washed mitochondrial pellet (approx. 0.5g/5g of



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cells) was suspended in 4ml of buffer B plus 0.4ml of Triton X-100, left for 5min at room temperature, then centrifuged at 39000*g* for 30min, and the supernatant was used for enzyme studies (enzyme preparation I), or was further purified by centrifugation at 150000*g* for 3h, discarding the supernatant and suspending the pellet in 1ml of buffer B; the latter preparation of pyruvate dehydrogenase complex was purified approx. 200-fold (enzyme preparation II). The enzyme preparations were usually utilized within a few hours. If suspended in 50% (v/v) glycerol and stored at −20°C, the lipoamide dehydrogenase, but not the complex, is stable for at least 4 weeks.

Similarly purified preparations of pyruvate dehydrogenase were also obtained from rat liver and heart and from calf adrenal medulla.

### *Reagents*

Biochemicals were obtained from Sigma, St. Louis, MO, U.S.A., except for: citric acid, EDTA and $NH_4Cl$ from Merck, Darmstadt, West Germany; Hyamine, Triton X-100 and Permablend III from Packard Instruments, Downers Grove, IL, U.S.A.; Pipes (1,4-piperazinediethanesulphonic acid) and Tes (2-{[2-hydroxy-1,1-bis(hydroxymethyl)-ethyl]amino}ethanesulphonic acid) from Calbiochem, San Diego, CA, U.S.A.; sucrose from May and Baker, Dagenham, Essex, U.K.; Triton X-100 from BDH, Poole, Dorset, U.K. Dihydrolipoamide was prepared by reduction with $NaBH_4$ as described by Reed *et al.* (1958). [1-$^{14}$C]Pyruvate was from The Radiochemical Centre, Amersham, Bucks., U.K.

### *Enzyme assays*

Pyruvate dehydrogenase complex is composed of: pyruvate decarboxylase (E1), EC 1.2.4.1.; lipoate acetyltransferase (E2), EC 2.3.1.12; lipoamide dehydrogenase (E3), EC 1.6.4.3. Unless specified otherwise, standard conditions used were: 50mM-Tes buffer, pH7.4, 100mM-KCl, 5mM-$MgCl_2$ and 5mM-$\beta$-mercaptoethanol; temperature was 37°C. For the pyruvate dehydrogenase complex, 0.2mM-thiamin pyrophosphate, 2mM-pyruvate, 0.1mM-CoA and 5mM-$NAD^+$ (Brown & Perham, 1976) were added. For the lipoamide dehydrogenase the additions were 2.5mM-dihydrolipoamide and 5mM-$NAD^+$ in the forward reaction and 0.15mM-NADH and 5mM-lipoamide in the reverse reaction. For the lactate dehydrogenase, 1mM-pyruvate and 0.15mM-NADH were added. The reactions were followed at 340nm in a Gilford 2400 or Cary 118 spectrophotometer. Pyruvate decarboxylase was assayed as described by Roche & Reed (1972) with 0.5mM-[1-$^{14}$C]pyruvate (0.125$\mu$Ci/$\mu$mol) and 0.2mM-thiamin pyrophosphate, in 2ml; a blank without enzyme was performed in parallel to correct for the spontaneous decarboxylation of pyruvate. The mixture was incubated for 3min at 37°C, and the reaction stopped by the addition of 0.5ml of 1M-HCl. The $^{14}CO_2$ formed was fixed in 1ml of Hyamine and counted for radioactivity in 10ml of the following mixture: 1 litre of toluene, 0.5 litre of Triton X-100 and 5.5g of Permablend III. The unit of enzyme activity is the amount of enzyme that transforms 1$\mu$mol of substrate/min. $K_a$ is the concentration of activator with which half of the maximal activation is achieved.

In the pH-dependent experiments, a mixture of 50mM-Pipes and 50mM-Tes was used as buffer, adjusted to pH6.5, 7.0, 7.4 or 8.0.

Kinetic parameters were measured with three or four independent preparations.

### *Protein determination*

Protein was determined by Bradford's (1976) dye-binding method.

## Results

### *Activation by AMP*

As shown in Fig. 1, AMP within its physiological range activated by about 2-fold the pyruvate dehydrogenase complex of ascites-tumour cells or its pyruvate decarboxylase (E1); the activity of E1 in the absence of CoA and $NAD^+$ was approx. 1% of that of the complex, which is not significantly different from the 0.35% value reported by Walsh *et*

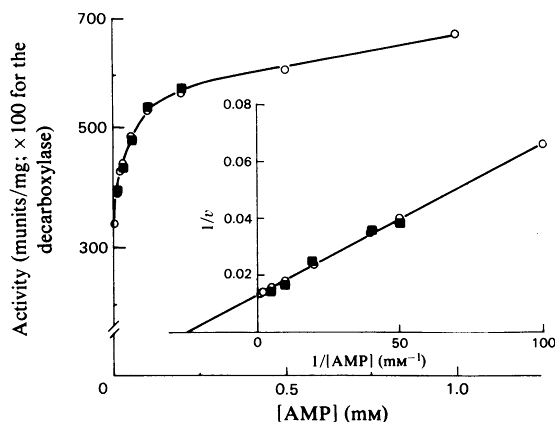


Fig. 1. *AMP activation of pyruvate dehydrogenase complex*

The activity of the complex (○) was assayed spectrophotometrically and that of the pyruvate decarboxylase (■) radioisotopically with preparation II as described in the Materials and methods section. The activity is expressed per mg of protein, except in the double-reciprocal plot (inset) in which $v$ is the percentage activation.

*al.* (1976). The AMP effect is fairly specific, since only AMP, cyclic AMP and GMP are active, with $K_a$ values of 40, 20 and 50$\mu$M respectively, and maximal activation values of 90, 100 and 90% respectively. No effect (<5%) was observed with ADP, ATP, IMP, UMP, GDP, GTP, adenosine 3′-monophosphate, adenosine 5′-monosulphate or adenosine 5′-monophosphoramidate, tested at 0.5mM concentrations. The effect is on the maximal rate, without affecting the affinity for pyruvate. The magnitude of the activation is pH-dependent, as shown in Fig. 2. No effect of AMP was observed on the activity of partially purified pyruvate dehydrogenase complex from rat liver, rat heart and calf adrenal medulla.

$P_i$ (5mM) and $NH_4^+$ (2mM) were tested unsuccessfully as potentially synergistic effectors that could potentiate the activation by AMP. Palmitoyl-CoA (20$\mu$M) and citrate (1mM) were without effect on pyruvate dehydrogenase complex in the presence or absence of AMP. Also they did not affect the affinity of the enzyme for pyruvate.

*Kinetic parameters for substrates and products*

The effect of the concentration of pyruvate on the activity of pyruvate dehydrogenase complex is shown in Fig. 3. The kinetics are hyperbolic and the $K_m$ values obtained were 36, 29, 19 and 17$\mu$M for pH 6.5, 7.0, 7.4 and 8.0 respectively. With saturating concentration of pyruvate the maximal activity was at pH 7.4; at pH 8.0, 7.0 and 6.5, the activities were 54%, 51% and 18% of that at pH 7.4.

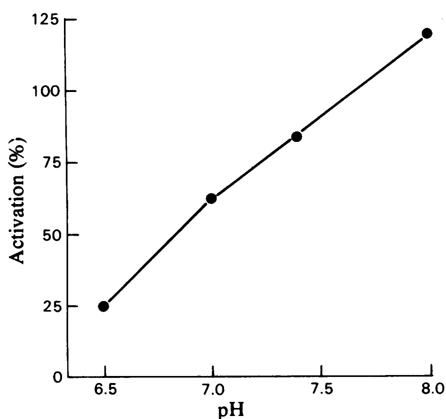


Fig. 2. *pH-dependence of the AMP activation of pyruvate dehydrogenase complex*

The complex was assayed by the spectrophotometric method with preparation II as described in the Materials and methods section. At a given pH, cuvettes with and without 0.2mM-AMP were compared. The reference value is that of the activity in the absence of AMP at a given pH.

In standard assay conditions the activity of pyruvate dehydrogenase in ascites cells is approx. 0.1unit/g of cells. The variation in activity and apparent affinity as a function of pH (Dixon & Webb, 1979) can be seen when represented on a semilogarithmic scale; Fig. 4 shows the variation of

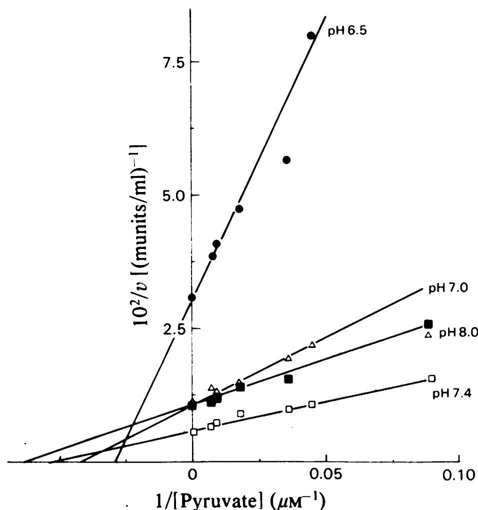


Fig. 3. *Effect of the concentration of pyruvate on the activity of pyruvate dehydrogenase complex within the pH range 6.5–8.0*

Preparation I was assayed spectrophotometrically at saturating concentrations of CoA and $NAD^+$ as indicated in the Materials and methods section: 50mM-Tes/50mM-Pipes was used as the buffer system. The pyruvate concentration was varied as indicated in the Figure.

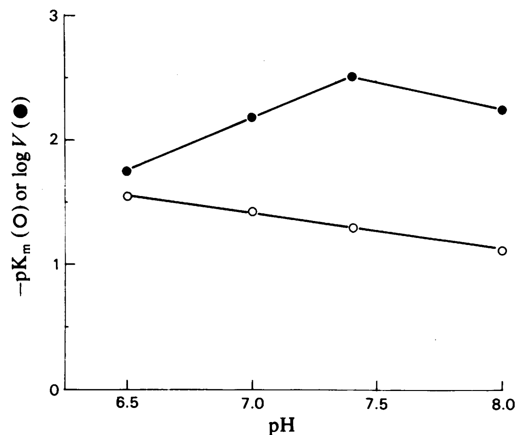


Fig. 4. *Representation of $\log V$ and $-pK_m$ values of pyruvate dehydrogenase complex as a function of pH*

These values were calculated from the data from the experiment described in Fig. 3.

log $V$ and $-pK_m$ at various pH. There is a clear break in the plot for log $V$, which follows the ionization of the enzyme–substrate complex, indicating dependence on the ionization of a specific group within it, whereas the $-pK_m$ values give a straight line.

The effect of the concentration of CoA, a substrate for the lipoate acetyltransferase (E2), on the activity of the complex gave hyperbolic kinetics, with a $K_m$ for CoA of 15μM. The enzyme is inhibited by the product acetyl-CoA competitively with CoA within a wide range of concentration of the inhibitor, as shown in Fig. 5. The $K_i$ for acetyl-CoA is 60μM.

The nicotinamide nucleotide that acts as the final electron acceptor for the pyruvate dehydrogenase-complex overall reaction is a substrate for the lipoamide dehydrogenase (E3). The complex gave hyperbolic behaviour for $NAD^+$, with $K_m$ 0.7mM. To ascertain whether the apparent affinity for $NAD^+$ was influenced by the situation in the complex of the physiological electron donor, e.g. the dihydrolipoyl-lysine arm of the lipoate acetyltransferase (E2), this enzyme was assayed separately with exogenous dihydrolipoamide; the same $K_m$ value was obtained. The activity was 1700-fold greater, because of its known excess within both the pyruvate dehydrogenase complex and the accompanying α-oxo-glutarate dehydrogenase complex. In a separate experiment we found that the apparent affinity for dihydrolipoamide was high enough ($K_m$ 0.4mM) to allow for practical saturation of the enzyme. The similarity in behaviour of the lipoamide dehydrogenase (E3), in both assay conditions, as such or as part of the complex, facilitates the study of possible effectors on the complex acting on the lipoamide dehydrogenase (E3), by direct assay of the latter.

NADH inhibits lipoamide dehydrogenase (E3) competitively with $NAD^+$, within a wide range of concentrations of the inhibitor and with a $K_i$ of 80μM (Fig. 6*a*). NADPH is a similar inhibitor, with a $K_i$ of 90μM (Fig. 6*b*). The possibility that the

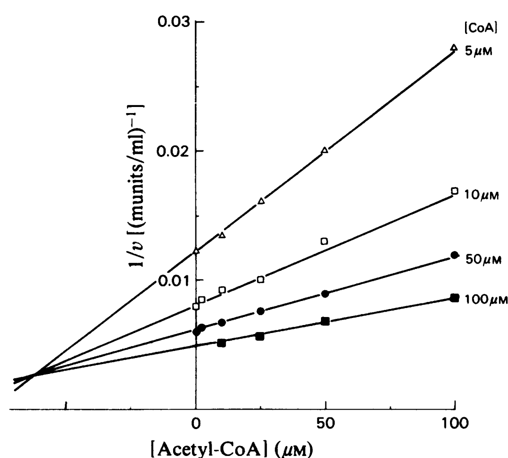


Fig. 5. *Inhibition by acetyl-CoA of pyruvate dehydrogenase complex*

Preparation I was assayed spectrophotometrically as indicated in the Materials and methods section at saturating concentrations of pyruvate and $NAD^+$, with concentrations of CoA and acetyl-CoA varied as indicated in the Figure. The results are represented as a Dixon plot.

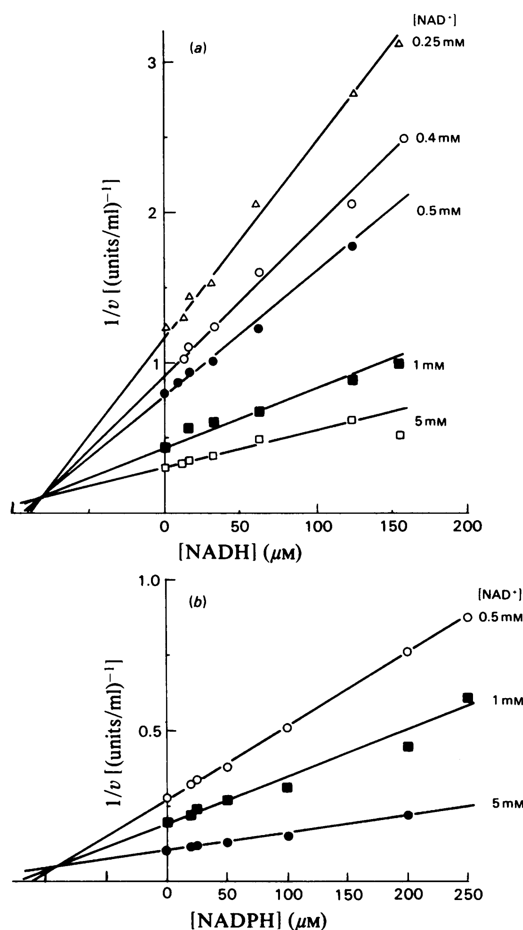


Fig. 6. *Inhibition of lipoamide dehydrogenase by NADH and NADPH*

Preparation I was assayed spectrophotometrically for lipoamide dehydrogenase activity with 2.5mM-dehydrolipoamide as electron donor as indicated in the Materials and methods section, and with concentrations of $NAD^+$ and NADH or NADPH varied as indicated in parts (*a*) and (*b*) respectively. Results are presented as Dixon plots.

overall activity of the complex could be less sensitive to inhibition by NADH (and NADPH) because of the large excess of lipoamide dehydrogenase is unlikely because it has been found not to be the case with liver pyruvate dehydrogenase complex (P. A. Lazo, unpublished work). $NADP^+$ could not be quantitatively studied in the forward reaction because of interference by its spontaneous reduction by dihydrolipoamide. In the reverse reaction both nicotinamide nucleotides in the reduced form can serve as substrates, with $K_m$ values essentially equal to the above $K_i$ values in the forward direction, and at a rate with NADPH of 0.09 of that with NADH. Moreover, in the reverse reaction, both $NAD^+$ and $NADP^+$ are competitive inhibitors versus NADH; the $K_i$ for $NAD^+$ in the reverse reaction is 0.7mM, which is equal to the $K_m$ for the forward reaction, and the $K_i$ for $NADP^+$ is 0.8mM.

## Discussion

The study *in vitro* of the pyruvate dehydrogenase complex of the ascites tumour indicates that its potential activity is indeed small. Ascites-tumour cells have only approx. 0.1 unit of enzyme activity/g of cells, a value similar to the rate that can be estimated from measurements of [1-$^{14}$C]pyruvate decarboxylation by intact cells made by Postius (1978) and ourselves (P. A. Lazo, unpublished work) that gave some 0.05–0.1 μmol/min per g of cells. This activity is two orders of magnitude smaller than the capacity of these cells to make pyruvate from glucose and one order of magnitude smaller than the maximal rate of pyruvate transport into their mitochondria as reported by Eboli *et al.* (1977). Although pH changes within the tumours (Navon *et al.*, 1977) could alter some kinetic parameters, this is not likely to improve significantly the low activity of the enzyme. Furthermore, since the enzyme can be strongly inhibited by physiological concentrations of the reduced nicotinamide nucleotides and possibly by acetyl-CoA, its actual activity *in vivo* is likely to be not much more than 0.01 unit/g. This would lead to the aerobic production of little more than 0.2 μmol of ATP/min per g of cells, which is a minor fraction of the glycolytic potential of approx. 5 μmol of ATP/min per g formed during the glycolytic production of an equimolar amount of lactate.

Preliminary attempts to detect metabolic interconversion of the tumour pyruvate dehydrogenase by phosphorylation–dephosphorylation have been largely unsuccessful. Incubation with 0.1 mM-$CaCl_2$ (Siess *et al.*, 1976) led to activations of no more than 20%, suggesting that most of the enzyme in our preparations was not in a phosphorylated form. Moreover, as mentioned above, the activity measured *in vivo* agrees well in order of magnitude with the *V* obtained *in vitro*. Hence it is unlikely that interconversion *in vivo* could markedly alter the conclusion that the energetic potential of pyruvate dehydrogenase complex in ascites tumour is well below that of the glycolytic chain. This fact can help to account for the high aerobic glycolysis of the tumour and its low Pasteur effect.

Bresters *et al.* (1975) showed that in pyruvate dehydrogenase complex from *Azotobacter vinelandii* the rate-limiting step of the overall reaction was at the level of the pyruvate decarboxylase (E1)–substrate complex, suggesting that it could be in the transfer of the hydroxyethyl group from thiamin pyrophosphate to protein-bound lipoate. Our data show that one of the limiting steps within the physiological pH range studied is at the level of some ionization of the enzyme–substrate complex. However, we cannot rule out completely the involvement of the ionization of one group in the free enzyme that is required for substrate binding; depending on the method used (Dixon & Webb, 1979), its appearance was evident, but this effect was much less striking than the rate-limiting effect of the enzyme–substrate complex.

The lipoamide dehydrogenase behaves in this preparation as a single population according to its kinetic parameters for $NAD^+$, dihydrolipoamide, NADH and NADPH, despite the fact that the preparation used in the present work was a mixture of pyruvate dehydrogenase and α-oxoglutarate dehydrogenase complexes, in which the activity of the latter was 4 times that of the former. This suggests that lipoamide dehydrogenase can be a common subunit of both complexes, in support of the principle of the economy of molecules postulated by Perham (1975), exemplified by a common subunit playing the same function but taking no part in the initial substrate specificity; these data make good sense for the argument in favour of a single structural gene (Brown & Perham, 1972, 1974; Pettit & Reed, 1967). Our observation that the $K_i$ values for the reduced nicotinamide nucleotides are much lower than the $K_m$ for $NAD^+$ stands in marked contrast with the report of Roche & Cate (1977) for the liver enzyme. This makes easy a feedback inhibition of the tumour enzyme. Moreover, NADPH could be an important regulator of lipoamide dehydrogenase *in vivo*, if its content in tumour mitochondria is as high as that reported for liver mitochondria (Glock & McLean, 1956).

The activation of pyruvate dehydrogenase complex by AMP is a novel finding, and its specificity and apparent affinity suggest an allosteric effect by a potentially very significant metabolite, since allosteric activation by AMP is a well-known feature of several key enzymes of energy metabolism (Sols, 1979). That its maximal effect appears to be relatively small in this case could

depend on some as yet unrecognized factor, or perhaps it could affect the enzyme in some additional way (e.g. by affecting a metabolic interconversion). As for the apparent insensitivity to AMP of the enzyme in other tissues examined, it could be that this activation is restricted to, or only easy to detect in, a particular type of isoenzyme previously unrecognized. In any case, allosteric activation of a pyruvate dehydrogenase complex by AMP is of obvious potential significance for metabolic regulation. The concentration of AMP in ascites cells is approx. 0.1 mM (Sauer, 1978). To our knowledge the corresponding value is not known for mitochondria, but it is reasonable to assume that it will not be very different from that in whole cells. Quantitatively it is well established that the NAD-dependent isocitrate dehydrogenase of yeast mitochondria is allosterically activated by AMP (Hathaway & Atkinson, 1963). It is also known that there are in the mitochondria AMP-producing enzymes (aminoacyl-tRNA synthetases). Hence adenylate kinase is likely to be present, although the difficulty of distinguishing between matrix and intermembrane space confuses the picture (Marco *et al.*, 1974). Cyclic AMP is presumed to mimic AMP since, as Sols (1979) says in relation with the activation of phosphofructokinase, it is likely to be merely an analogue of AMP acceptable *in vitro* but unable to act *in vivo*, because it can operate only in a concentration range which it cannot reach for thermodynamic limitation. For this reason there never was selective evolutionary pressure to have an AMP-binding site discriminating against cyclic AMP; just the opposite argument excludes cyclic AMP as the physiological effector.

We acknowledge the advice of Dr. R. N. Perham and Dr. C. H. Henderson on the handling of pyruvate dehydrogenase, the criticism of the manuscript by Dr. S. P. Mistry and Dr. C. Gancedo, and the technical assistance of Ms Renée Clarys. P. A. L. had a predoctoral fellowship from the Ministerio de Educatión y Ciencia. This work was partly supported by a grant from the Comisión Asesora para la Investigación Científica y Técnica.

### References

Batenburg, J. J. & Olson, M. S. (1976) *J. Biol. Chem.* **251**, 1363–1370

Bradford, M. (1976) *Anal. Biochem.* **72**, 248–254

Bresters, T. W., DeKok, A. & Veeger, C. (1975) *Eur. J. Biochem.* **59**, 347–353

Brown, J. P. & Perham, R. N. (1972) *FEBS Lett.* **26**, 221–224

Brown, J. P. & Perham, R. N. (1974) *Biochem. J.* **137**, 505–512

Brown, J. P. & Perham, R. N. (1976) *Biochem. J.* **155**, 419–427

Cate, R. L. & Roche, T. E. (1978) *J. Biol. Chem.* **253**, 496–503

Dixon, M. & Webb, E. C. (1979) *Enzymes*, 3rd edn., pp. 138–163, Longmans, London

Eboli, M. L., Paradies, G., Galeotti, T. & Papa, S. (1977) *Biochim. Biophys. Acta* **460**, 183–187

Glock, G. E. & McLean, P. (1956) *Exp. Cell Res.* **11**, 234–236

Hansford, R. G. & Cohen, L. (1978) *Arch. Biochem. Biophys.* **191**, 65–81

Hathaway, J. A. & Atkinson, D. E. (1963) *J. Biol. Chem.* **238**, 2875–2881

Johnson, M. J. (1941) *Science* **94**, 200–202

Lazo, P. A. & Sols, A. (1979) *Abstr. IUB Int. Congr. Biochem. 11th* abstr. 04-3-S138

Lazo, P. A., Castaño, J. G. & Sols, A. (1978) *Abstr. FEBS Meet. 12th* abstr. 2736

Lynen, F. (1941) *Justus Liebigs Ann. Chem.* **546**, 120–141

Marco, R., Pestaña, A., Sebastian, J. & Sols, A. (1974) *Mol. Cell. Biochem.* **3**, 53–70

Navon, G., Ogama, S., Schulman, R. G. & Yamane, T. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 87–91

Olson, M. S., Dennis, S. C., DeBuysere, M. S. & Padma, A. (1978*a*) *J. Biol. Chem.* **253**, 7369–7375

Olson, M. S., Dennis, S. C., Routh, C. A. & DeBuysere, M. S. (1978*b*) *Arch. Biochem. Biophys.* **187**, 121–131

Perham, R. N. (1975) *Philos. Trans. R. Soc. London Ser. B* **272**, 123–136

Pettit, F. H. & Reed, L. J. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **58**, 1126–1130

Postius, S. (1978) *Anal. Biochem.* **90**, 534–542

Racker, E. (1976) *A New Look at Mechanism in Bioenergetics*, pp. 153–175, Academic Press, New York

Randle, P. J. (1978) *Trends Biochem. Sci.* **3**, 217–219

Reed, L. J. (1974) *Acc. Chem. Res.* **7**, 40–46

Reed, L. J., Koike, M., Levitch, M. E. & Leach, F. R. (1958) *J. Biol. Chem.* **232**, 143–158

Roche, T. E. & Cate, R. L. (1977) *Arch. Biochem. Biophys.* **183**, 664–677

Roche, T. E. & Reed, L. J. (1972) *Biochem. Biophys. Res. Commun.* **48**, 840–846

Sauer, L. A. (1978) *Cancer Res.* **38**, 1057–1063

Siess, E. A., Nimmannit, S. & Wieland, O. H. (1976) *Cancer Res.* **36**, 55–59

Sols, A. (1976) in *Reflections in Biochemistry* (Kornberg, A., Horecker, B. L., Cornudella, L. & Oró, J., eds.), pp. 199–206, Pergamon Press, Oxford

Sols, A. (1979) in *Covalent and Noncovalent Modulation of Protein Function* (Atkinson, D. E. & Fox, C.F., eds.), pp. 27–43, Academic Press, New York

Sols, A., Aragon, J. J., Lazo, P. A. & Gonzalez, J. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1428

Walsh, D. A., Cooper, R. H., Denton, R. M., Bridges, B. J. & Randle, P. J. (1976) *Biochem. J.* **157**, 41–67

Warburg, O. (1926) *Uber den Stoffwechsel der Tumoren*, Springer-Verlag. Berlin

Warburg, O. (1956) *Science* **123**, 309–314

Weinhouse, S. (1956) *Science* **124**, 267–269