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Properties of Phosphofructokinase from the Mucosa of Rat Jejunum and their Relation to the Lack of Pasteur Effect

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1. The properties of phosphofructokinase after its slight purification from the mucosa of rat jejunum were studied. 2. The enzyme is inhibited by almost 100% by an excess of ATP (1.6mM), with 0.2mM-fructose 6-phosphate. AMP, ADP, P_i and NH_4^+ at 0.2, 0.76, 1.0 and 2mM respectively do not individually prevent the inhibition of phosphofructokinase activity by 1.6mM-ATP with 0.2mM-fructose 6-phosphate to any great extent, but all of them together completely prevent the inhibition of phosphofructokinase by ATP. 3. One of the effects of high concentrations of ATP on the enzyme was to increase enormously the apparent K_m value for the other substrate fructose 6-phosphate, and this increase is largely counteracted by the presence of AMP, ADP, P_i and NH_4^+. At low concentrations of ATP the above effectors individually decrease the concentration of fructose 6-phosphate required for half-maximum velocity and when present together they decrease it further, in a more than additive way. 4. When fructose 6-phosphate is present at a saturating concentration (5mM), 0.3mM-NH_4^+ increases the maximum velocity of the reaction 3.3-fold; with 0.5mM-fructose 6-phosphate, 4.5mM-NH_4^+ is required for maximum effect. The other effectors do not change the maximum reaction velocity. 5. The results presented here suggest that NH_4^+, AMP, ADP and P_i synergistically decrease the inhibition of phosphofructokinase activity at high concentrations of ATP by decreasing the concentration of fructose 6-phosphate required for half-maximum velocity. Such synergism among the effectors and an observed, low 'energy charge' $[(ATP+\frac{1}{2}ADP)/(AMP+ADP+ATP)]$ in conjunction with the possibility of a relatively high NH_4^+ and fructose 6-phosphate concentration in this tissue, may keep the mucosal phosphofructokinase active and uninhibited by ATP under aerobic conditions, thus explaining the high rate of aerobic glycolysis and the lack of Pasteur effect in this tissue.

The rate of conversion of fructose 6-phosphate into fructose 1,6-diphosphate catalysed by phosphofructokinase (ATP–D-fructose 6-phosphate 1-phosphotransferase, EC 2.7.1.11) represents a major control point of the glycolytic pathway in a variety of tissues and organisms (Atkinson, 1966; Stadtman, 1966). Facilitation of this reaction during anoxia has been demonstrated in mouse brain (Lowry, Passonneau, Hasselberger & Schulz, 1964), in muscle (Passonneau & Lowry, 1962), rat heart (Williamson, 1966), yeast (Lynen, Hartmann, Netter & Schuegraf, 1959; Salas, Viñuela, Salas & Sols, 1965), rat heart and diaphragm (Newsholme & Randle, 1961) and in slices of kidney cortex, Novikoff hepatoma and in adenocarcinomas (Wu, 1964), as well as in many other systems. Enhancement of phosphofructokinase reaction was considered as a primary step involved in the Pasteur effect. The salient features of the phosphofructokinase regulation are the inhibition of the enzyme activity by high concentrations of one of its substrates, i.e. ATP (Lardy & Parks, 1956) and the relief of this inhibition by various effector metabolites (Passonneau & Lowry, 1964). The activation of the phosphofructokinase reaction under anaerobic conditions was thought to be effected by a decrease in the ATP concentration (a negative effector) and increases in the concentrations of ADP, AMP and P_i, all positive effectors of the enzyme in the cell (Stadtman, 1966). However, there are certain tissues, e.g. the rat jejunum, where the Pasteur effect was not observed either in the intact jejunum or in the epithelial cells (Dickens & Weil-Malherbe, 1941; Wilson & Wiseman, 1954; Stern & Reilly, 1965). In view of the association of activation of phosphofructokinase with the Pasteur effect, it was decided to investigate whether the kinetic properties of phosphofructokinase from rat jejunal mucosa are in any way uniquely different from the enzyme of

other tissues and organisms, thus explaining the lack of Pasteur effect in this tissue. Preliminary results of this work have been presented (Tejwani & Ramaiah, 1970).

MATERIALS AND METHODS

Animals. Normal female albino rats of the All India Institute of Medical Sciences strain weighing 150–200g were used.

Chemicals and enzymes. ADP was obtained from Nutritional Biochemical Corp., Cleveland, Ohio, U.S.A. KF was obtained from Riedel–De Häenag, Seelze/Hanover, Germany. The following chemicals and enzymes were obtained from Sigma Chemical Co., St Louis, Mo., U.S.A.: sodium salt of fructose 6-phosphate, fructose 1,6-diphosphate, AMP, β-NADH, EDTA, tris, bovine serum albumin and an $(NH_4)_2SO_4$ suspension of aldolase, triose phosphate isomerase and α-glycerophosphate dehydrogenase. All other chemicals used were of analytical reagent grade.

Partial purification of phosphofructokinase from the mucosa of rat jejunum. Normal female albino rats were killed by decapitation and the jejunum was removed. The jejunal lumen was washed thoroughly by flushing it with a cold solution containing 100mM-tris–HCl, pH7.5, 30mM-KF and 1.0mM-EDTA. All further operations were performed at 0–4°C unless otherwise stated. The mucosa was collected by scraping the jejunum with a plastic spatula on a glass plate. The mucosa was weighed and homogenized with 1.5vol. of a cold solution containing 100mM-tris–HCl, pH7.5, 30mM-KF, 1.0mM-EDTA, twice for 0.5min in a Polytron homogenizer (type *PT* 10 *OD*, Kinematica G.m.b.H., Lucerne, Switzerland) at 22000rev./min. The resulting homogenate was centrifuged at 15000*g* for 40min at 0–2°C. The specific activity of phosphofructokinase in the supernatant was 0.029 when assayed immediately after centrifugation, but this value decreased to 0.001 after 10h. The supernatant obtained by centrifuging the homogenate at 105000*g* had less phosphofructokinase activity as compared with the supernatant obtained at 15000*g*, indicating that enzyme may be present in both the soluble and the particulate fractions; therefore centrifugation at 15000*g* was done as a routine and the supernatant was taken as the source of enzyme.

Step I. The supernatant was immediately filtered through cheesecloth and was treated with 0.1vol. of propan-2-ol at 0°C. The mixture was stirred for 40min at 0°C and was then centrifuged at 75000*g* for 30min at 0–2°C. The precipitate contained negligible phosphofructokinase activity and was discarded.

Step II. The supernatant from step I was cooled to −4°C to −6°C and 0.15vol. of propan-2-ol (cooled to −4°C to −6°C) was added. The mixture was kept at −4°C to −6°C for 40min with occasional stirring and was then centrifuged at 75000*g* at −4°C to −6°C for 30min. The precipitate was dissolved in a solution of 50mM-tris–HCl–10mM-K_2HPO_4–4mM-KF–0.33mM-EDTA and 0.02% bovine serum albumin, buffered to pH8.0. The specific activity of phosphofructokinase in this fraction was 0.05–0.07. This preparation was almost free of adenosine triphosphatase and adenylate kinase activities.

Step III. The supernatant from step II was treated with 0.2vol. of propan-2-ol under conditions similar to those described in step II. The precipitate was collected and dissolved in the same solution used for dissolving the precipitate in step II. The phosphofructokinase in this fraction had a specific activity of 0.07. The enzyme preparations from steps II and III were similar in properties. These fractions were used as the source of phosphofructokinase for all the studies described in the present paper. The enzyme was stored at −70°C and it was stable for at least 4 months under these conditions.

Assay system. The phosphofructokinase was measured spectrophotometrically at room temperature (25–27°C) as described by Ramaiah, Hathway & Atkinson (1964). Unless otherwise mentioned the assay mixture, in a total volume of 1.0ml, contained 50mM-tris–HCl, pH7.5, 3.0mM-$MgCl_2$, 0.15mM-NADH, 0.36 unit of aldolase, 0.6 unit of triose phosphate isomerase and 1.12 units of α-glycerophosphate dehydrogenase. The concentrations of ATP and fructose 6-phosphate used are given in the legends to the Tables and Figures. The auxiliary enzymes were freed of $(NH_4)_2SO_4$ by dialysis against 1.5 litres of 0.01M-tris–HCl, pH8.0, for 16h with one change of buffer. The contamination of $(NH_4)_2SO_4$ from dialysed auxiliary enzymes was less than 5nM. The concentration of NADH used in the assay of phosphofructokinase was in the range that does not inhibit the auxiliary enzymes used in the assay system (Newsholme & Sugden, 1970). A unit of phosphofructokinase activity corresponds to the production of 1μmol of fructose 1,6-diphosphate/min. The specific activity of phosphofructokinase is defined as the number of units/mg of protein. Protein was determined by the method of Lowry, Rosebrough, Farr & Randall (1951) with bovine serum albumin as standard.

The reaction was initiated by adding the enzyme. The reaction velocity was directly proportional to the enzyme concentration.

The reaction rate was linear for at least the first 2.5min in the presence of positive effector(s) and at or above a fructose 6-phosphate concentration of 0.2mM. With 0.1mM-fructose 6-phosphate or less, the reaction velocity decreased with time and in such cases, the decrease in extinction between 0.5 and 1.5min after the enzyme was added was taken as the initial rate of the reaction.

RESULTS

*Synergistic action of AMP, ADP, P_i and NH_4^+ in decreasing the $(F6P)_{0.5}$ value.** The ratio of velocity (v) at any concentration of fructose 6-phosphate, to the velocity at saturating concentration of fructose 6-phosphate ($V_{max.}$ under the particular conditions) was plotted against fructose 6-phosphate concentration, at a fixed concentration of ATP (0.195mM), in the presence and absence of AMP, ADP, P_i and NH_4^+ or SO_4^{2-}, as shown in Fig. 1.

The fructose 6-phosphate saturation curve in the absence of any other effector was very sigmoid and

* $(F6P)_{0.5}$ value: the concentration of fructose 6-phosphate required to give half-maximum velocity under any particular conditions of assay.

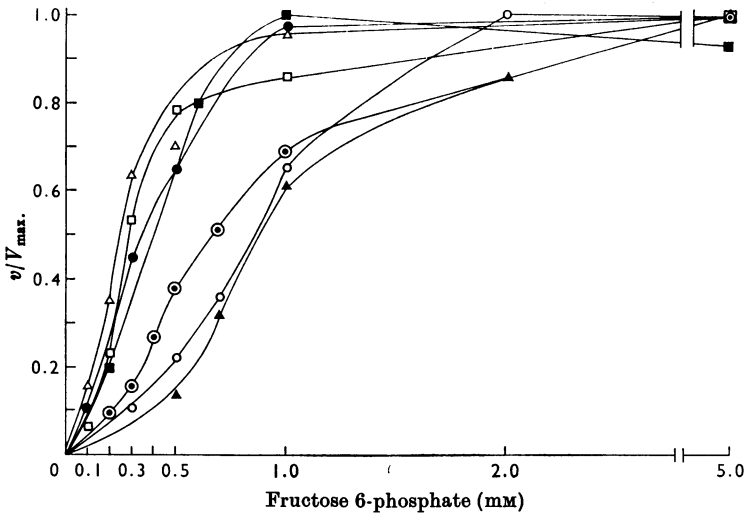

Fig. 1. Effect of AMP, P_i, NH_4^+, SO_4^{2-} or ADP on the fructose 6-phosphate saturation curve of phosphofructokinase. The ATP concentration in each case was 0.195 mM. P_i, NH_4^+ or SO_4^{2-} were added as sodium phosphate buffer, pH 7.4, as NH_4Cl, or as Na_2SO_4 respectively. The other conditions of the assay are as described in the Materials and Methods section. ○, No addition; △, 1 mM-AMP; □, 0.5 mM-P_i; ▲, 0.3 mM-NH_4^+; ●, 0.5 mM-SO_4^{2-}; ■, 0.76 mM-ADP; ⊙, 10 mM-NH_4^+.

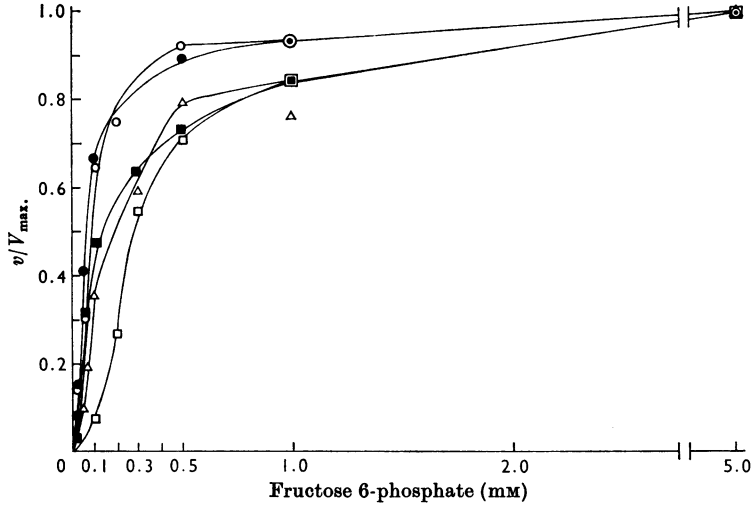

Fig. 2. Effect of NH_4^+, SO_4^{2-}, P_i, AMP and ADP in various combinations, on the sigmoidicity of the fructose 6-phosphate saturation curve. The ATP concentration in every case was 0.195 mM. The other conditions of the assay are as described in the Materials and Methods section. ○, 0.95 mM-ADP–0.3 mM-NH_4^+–0.5 mM-P_i; ●, 1 mM-AMP–0.3 mM-NH_4^+–0.5 mM-P_i; ■, 1 mM-AMP–0.5 mM-P_i; △, 0.3 mM-NH_4^+–0.5 mM-P_i; □, 0.3 mM-NH_4^+–0.5 mM-SO_4^{2-}.

the sigmoidicity and the $(F6P)_{0.5}$ value were decreased in the presence of saturating concentrations of AMP, P_i, SO_4^{2-}, ADP or NH_4^+, in that order of effectiveness. When these effectors were added in various combinations the sigmoidicity and $(F6P)_{0.5}$ value were further decreased. AMP or ADP instead of AMP, together with P_i and NH_4^+, decreased the $(F6P)_{0.5}$ value in a synergistic manner, as shown in Fig. 2 and Table 1.

Although AMP, P_i and SO_4^{2-} decreased the

Table 1. *Effect of AMP, ADP, P_i, NH_4^+ and SO_4^{2-} separately and in combination on the $V_{max.}$ and $(F6P)_{0.5}$ value of phosphofructokinase from the mucosa of rat jejunum*

The conditions of assay are as described in the text. Maximum activity is the maximum possible activity obtained when the same amount of enzyme was assayed in the presence of 5mM-fructose 6-phosphate, 1mM-ATP, 10mM-NH_4^+, 5mM-P_i and 0.38mM-AMP; other conditions of assay were the same. The $(F6P)_{0.5}$ value is the amount of fructose 6-phosphate required to give half-maximum velocity under the given conditions of assay.

AMP (mM)	ADP (mM)	P_i (mM)	NH_4^+ (mM)	SO_4^{2-} (mM)	Percentage of maximum activity	$(F6P)_{0.5}$ value (mM)	$(F6P)_{0.5}$ value Relative to control
0	0	0	0	0	26.1	0.870	1.000
0	0	0	0.30	0	76.3	0.885	1.0
0	0	0	10.0	0	73.9	0.670	0.770
0	0.76	0	0	0	10.9	0.402	0.462
0	0	0	0	0.50	27.0	0.348	0.400
0	0	0.50	0	0	30.0	0.280	0.321
1.0	0	0	0	0	27.4	0.240	0.275
0	0	0	0.30	0.50	80.9	0.275	0.316
0	0	0.50	0.30	0	67.8	0.195	0.224
1.0	0	0.50	0	0	27.4	0.135	0.155
0	0.95	0.50	0.30	0	43.0	0.078	0.089
1.0	0	0.50	0.30	0	76.1	0.068	0.078

$(F6P)_{0.5}$ value from 0.87 to 0.24, 0.28 and 0.348mM respectively, the velocity in the presence of any one of them or in the presence of AMP and P_i together, at saturating concentration of fructose 6-phosphate (5mM), was about 26–30% of the maximum activity (the maximum possible activity of the same amount of enzyme, determined in the presence of 5mM-fructose 6-phosphate, 1mM-ATP, 10mM-NH_4^+, 5mM-P_i and 0.38mM-AMP) of the enzyme. ADP decreased the $(F6P)_{0.5}$ value from 0.87 to 0.4 but inhibited the activity of enzyme by 50% at saturating concentration of fructose 6-phosphate; the explanation for this inhibition at higher concentration of fructose 6-phosphate is not clear at present. NH_4^+ by itself at 0.3mM did not alter the $(F6P)_{0.5}$ value but the velocity at a saturating concentration of fructose 6-phosphate (5mM) was about 80% of the maximal activity (Figs. 1 and 3) and remained essentially so in the presence of other effectors except ADP, where a 50% decrease in activity was observed (Table 1).

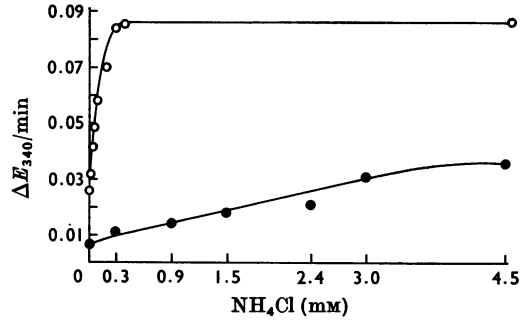

Fig. 3. Effect of increasing the concentration of NH_4^+ on the activity of phosphofructokinase at saturating (5.0mM; ○) and at non-saturating (0.5mM; ●) fructose 6-phosphate concentrations. The ATP concentration in both cases was 0.195mM. The other conditions of the assay are as described in the Materials and Methods section.

Effect of AMP, ADP, P_i and NH_4^+ on reversing the ATP inhibition of the enzyme activity. The mucosal phosphofructokinase, like the enzyme from other sources, is inhibited at higher concentrations of ATP. The extent of inhibition of phosphofructokinase activity at 1mM-fructose 6-phosphate and 3.12mM-ATP was 94 and 90% at an ATP/Mg ratio of 1:1.6 and 1:5 respectively (results not shown), suggesting that ATP inhibits, but not simply by chelating Mg^{2+}, and that both ATP as well as ATPMg are equally effective inhibitors of enzyme activity. The concentration of ATP at which it is inhibitory and the extent of inhibition is a function of the fructose 6-phosphate concentration and the presence of effectors as shown in Fig. 4.

With 0.05 and 0.1mM-fructose 6-phosphate and with 1.56mM-ATP in the presence of ADP, P_i and NH_4^+ the enzyme activity was about 16 and 25% respectively, of the activity obtained with 0.195mM-ATP. When ADP was replaced by AMP, the activity of the enzyme with 0.1mM-fructose 6-phosphate was two- and five-fold more with

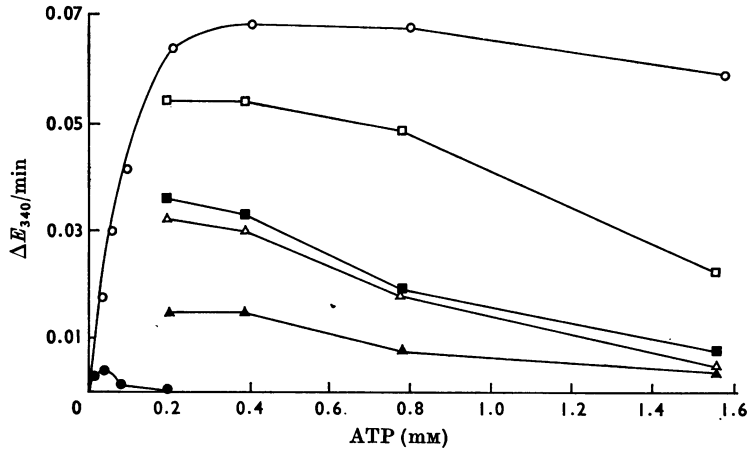

Fig. 4. Effect of various combinations of the positive effectors NH_4^+, P_i, ADP and AMP on the activity of phosphofructokinase at different concentrations of ATP and various fixed concentrations of fructose 6-phosphate. The other conditions of assay are as described in the Materials and Methods section. ●, 0.2 mM-fructose 6-phosphate; ▲, 0.05 mM-fructose 6-phosphate–0.95 mM-ADP–0.5 mM-P_i–0.3 mM-NH_4^+; △, 0.1 mM-fructose 6-phosphate–0.95 mM-ADP–0.5 mM-P_i–0.3 mM-NH_4^+; ■, 0.05 mM-fructose 6-phosphate–1.0 mM-AMP–0.5 mM-P_i–0.3 mM-NH_4^+; □, 0.1 mM-fructose 6-phosphate–1.0 mM-AMP–0.5 mM-P_i–0.3 mM-NH_4^+; ○, 0.2 mM-fructose 6-phosphate–0.2 mM-AMP–0.76 mM-ADP–1.0 mM-P_i–2 mM-NH_4^+.

Table 2. *Effect of AMP, ADP, P_i and NH_4^+ separately and in combination on the activity of phosphofructokinase from the mucosa of rat jejunum with 0.2 mM-fructose 6-phosphate and 0.195 mM- or 1.56 mM-ATP*

Conditions of assay are as described in the text. Maximum activity is defined in Table 1.

AMP (mM)	ADP (mM)	P_i (mM)	NH_4^+ (mM)	Percentage of maximum activity: With 0.195 mM-ATP	Percentage of maximum activity: With 1.56 mM-ATP
0	0	0	0	0.0	0.0
0.20	0	0	0	4.3	0.0
0	0.76	0	0	3.5	1.7
0	0	1.0	0	8.7	1.7
0	0	0	2.0	3.5	0.0
0.20	0	1.0	2.0	53.5	40.9
0.20	0.76	1.0	2.0	55.2	51.3

0.195 mM-ATP and 1.56 mM-ATP respectively than the corresponding activities in the presence of ADP, indicating that AMP is more effective than ADP in decreasing the $(F6P)_{0.5}$ value and the ATP inhibition (Fig. 4 and Table 1). About 40% of the enzyme activity was inhibited by 1.56 mM-ATP with 0.1 mM-fructose 6-phosphate in the presence of AMP, P_i and NH_4^+. In the presence of AMP, ADP, P_i and NH_4^+ together there was almost no inhibition of phosphofructokinase activity by 1.56 mM-ATP with 0.2 mM-fructose 6-phosphate. The concentrations of AMP and ADP used in this experiment correspond to values reported for these nucleotides in the intact jejunum, and the highest concentration of ATP reported for the jejunum of rat was 1.56 mM (Parsons, 1959).

Synergism among AMP, ADP, P_i and NH_4^+ in decreasing the ATP inhibition of the phosphofructokinase activity. The synergistic effect of AMP, ADP, P_i and NH_4^+ in decreasing the inhibition of phosphofructokinase activity at higher concentrations of ATP is shown in Table 2. The activity of phosphofructokinase with 0.2 mM-fructose 6-phosphate was zero at ATP concentrations of 0.195 mM and 1.56 mM. In the presence of any single effector the activity of the enzyme with 0.195 mM-ATP was a small fraction of the maximum possible activity and in the presence of 1.56 mM-ATP it was only 0–2% of the maximum possible activity. However, in the presence of AMP, ADP, P_i and NH_4^+ together, the enzyme activity with 0.195 mM-ATP was about 55% of the maximum

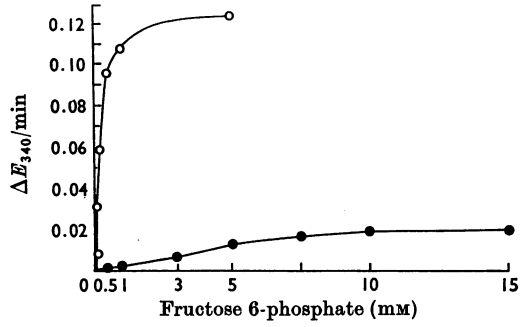

Fig. 5. Effect of NH_4^+, P_i, ADP and AMP on the activity of phosphofructokinase with 1.56mM-ATP and with different concentrations of fructose 6-phosphate. Other conditions of assay are as described in the Materials and Methods section. ●, 1.56mM-ATP; ○, 1.56mM-ATP–0.2mM-AMP–0.76mM-ADP–1mM-P_i–2mM-NH_4^+.

possible activity, which remains almost unaltered with 1.56mM-ATP.

Thus it is clear that positive effectors not only increase the total enzyme activity from 0 to 55% of the maximum possible activity in a synergistic manner but also abolish the ATP inhibition completely.

The effect of an inhibitory concentration of ATP (1.56mM) on the $(F6P)_{0.5}$ value in the presence and absence of AMP, ADP, P_i and NH_4^+ at concentrations that abolish ATP inhibition completely is shown in Fig. 5. In the absence of the above effectors the $(F6P)_{0.5}$ value with 1.56mM-ATP was 4mM and the maximum velocity was about 15% of the maximum possible activity whereas in their presence the $(F6P)_{0.5}$ value was only 0.2mM and the maximum velocity at the saturating concentration of fructose 6-phosphate was 100% of the maximum possible activity.

DISCUSSION

As in many tissues and organisms, the phosphofructokinase of jejunal mucosa is one of the rate-controlling enzymes of glycolysis in this tissue (Srivastava & Hübscher, 1966).

The phosphofructokinase of jejunal mucosa is not inhibited by up to 1.6mM-ATP, with 0.2mM-fructose 6-phosphate in the presence of NH_4^+, P_i, AMP and ADP, but its activity is almost zero in their absence or in the presence of any one of them, suggesting that these effectors act synergistically in overcoming inhibition of the phosphofructokinase reaction by excess of ATP (Fig. 4 and Table 2). These positive effectors also decrease the $(F6P)_{0.5}$ value in a synergistic manner, in that the $(F6P)_{0.5}$ value obtained in the presence of all the positive effectors at their saturating concentrations is less than the value expected if these effectors were to act independently of each other (Figs. 1 and 2 and Table 1). The increase in the $(F6P)_{0.5}$ value in the presence of excess of ATP is largely prevented by the presence of NH_4^+, AMP, ADP and P_i (Fig. 5), showing that the synergism among the effectors that decrease the $(F6P)_{0.5}$ value is related to their effect in decreasing the inhibition by ATP. These effectors acted synergistically in decreasing the inhibition of phosphofructokinase by excess of ATP and in decreasing the $(F6P)_{0.5}$ value for phosphofructokinase of sheep brain (Lowry & Passonneau, 1966) and phosphofructokinase of liver, heart, skeletal muscle and brain of rabbit (Ramaiah, Passonneau & Lowry, 1967). Among these effectors only NH_4^+ at an optimum concentration of 0.3mM increases the maximum velocity by about 3.3-fold at a saturating concentration of fructose 6-phosphate (5mM). However, this concentration of NH_4^+ did not cause either an increase in the phosphofructokinase activity at a non-saturating concentration of fructose 6-phosphate (0.5mM) or a lowering of the $(F6P)_{0.5}$ value, although at much higher concentrations NH_4^+ increases the velocity of the phosphofructokinase reaction at non-saturating concentrations of fructose 6-phosphate by about fivefold and decreases the $(F6P)_{0.5}$ value from 0.87 to 0.67mM (Figs. 1, 2 and 3; Table 1).

The synergism of these effectors in decreasing the $(F6P)_{0.5}$ value and the inhibition of phosphofructokinase activity by excess of ATP, as well as the differential effect of NH_4^+ at saturating and non-saturating concentrations of fructose 6-phosphate may be interpreted in terms of the model for allosteric enzymes existing in two conformational states R and T, proposed by Monod, Wyman & Changeux (1965).

At non-saturating concentrations of fructose 6-phosphate, which serves both as a substrate and a positive effector, the enzyme is expected to be predominantly in one conformational state, say T, with less affinity for fructose 6-phosphate and high affinity for the negative effector, ATP. Addition of positive effectors under these conditions would shift the equilibrium to the R state, to which fructose 6-phosphate and other positive effectors bind tightly. In the presence of two or more positive effectors the shift towards the R state would be more than additive since, under these conditions, the species of R bind not only individual effectors, but also effectors in various combinations, thus explaining the more than additive effect of NH_4^+, AMP, P_i and ADP in decreasing the ATP inhibition and the $(F6P)_{0.5}$ value as shown in Figs. 1, 2 and 5.

Irrespective of the mechanism by which the positive effectors bring about a more than additive

decrease in the $(F6P)_{0.5}$ value and the inhibition by excess of ATP, this phenomenon may be one of the factors associated with the lack of Pasteur effect in this tissue.

The small intestine of the rat is highly populated with the intestinal flora that produce ammonia from urea and other nitrogenous sources and the mucosal cells themselves contribute to NH_4^+ production from urea by the urease present in these cells (Summerskill, Aoyagi & Evans, 1966; Kettering & Summerskill, 1967). The other possible source of NH_4^+ is from the relatively high content of adenosine deaminase activity in the intestinal mucosa (Schaedal, Waldvogel & Schlenk, 1947), which could serve as a part of the degradative system for nucleosides and perhaps adenosine 3′-phosphate released by digestion of RNA.

Thus it is likely that the concentration of NH_4^+ in this tissue may be higher than in other tissues such as brain, liver and resting muscle of rat, which have 41.4 or less, and 1.1 μmol of NH_4^+/100g fresh wet wt. of tissue respectively (Brown, Duda, Korkes & Handler, 1957).

The concentration of fructose 6-phosphate, the substrate and the positive effector of phosphofructokinase, is also likely to be relatively high in this tissue because the jejunum is the major site of absorption of glucose and fructose from the diet (Reynell & Spray, 1956) and the capacity of tissue to phosphorylate glucose and fructose is commensurate with their absorption rates (Hele, 1953). For instance the fructose 6-phosphate concentration in the intestinal mucosa of starved rat jejunum was 46–52.50 μmol/kg wet wt. of tissue and rose to 677.25 μmol at 30min after feeding the rats with 600mg of fructose/100g of rat (Papadopoulos & Roe, 1957). This value is high compared with the 16 μmol of fructose 6-phosphate/kg wet wt. of tissue in mouse brain (Lowry *et al.* 1964) or 30 μmol/kg wet wt. in mouse skeletal muscle, based on the concentration of glucose 6-phosphate present and assuming that the ratio of glucose 6-phosphate to fructose 6-phosphate would be in the equilibrium proportion of 3:1 in the intact tissue (A. Ramaiah, J. V. Passonneau & O. H. Lowry, unpublished work).

In addition, the 'energy charge' of the adenylate system $[(ATP + \frac{1}{2}ADP)/(AMP + ADP + ATP)]$, as defined and proposed by Atkinson & Walton (1967) as a fundamental metabolic control parameter that inversely alters phosphofructokinase activity (Shen, Fall, Walton & Atkinson, 1968), is only 0.46 in the mucosa of rat jejunum (Iemhoff, van den Berg, de Pijper & Hülsmann, 1970) as compared with 0.77 in muscle, 0.70 in brain, 0.65 in heart and 0.78 in the diaphragm of the rat (Parker, 1954).

It is therefore likely that a low 'energy charge' and the possibility of the existence of relatively high concentrations of NH_4^+ and fructose 6-phosphate in this tissue, together with synergism (among the positive effectors NH_4^+, AMP, P_i and ADP) in decreasing the inhibition of phosphofructokinase activity by excess of ATP, may keep phosphofructokinase uninhibited under aerobic conditions. This would explain the high aerobic glycolytic rate and the lack of Pasteur effect in this tissue.

There are other systems, such as intestine of mouse, hamster, chicken and sparrow, where the Pasteur effect was not observed (see Lohman, Graetz & Langen, 1966, for references), and situations similar to that found in rat jejunal mucosa might exist there.

It would be of particular interest to test this possibility by measuring the concentrations of the adenine nucleotides and other effector metabolites of phosphofructokinase, as well as glycolytic intermediates, in this tissue during aerobic and anaerobic conditions.

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