The role of AMP deaminase (EC 3.5.4.6) reaction in the stimulation of the regulatory enzymes of glycolysis was investigated using permeabilized yeast cells. 1) The addition of polyamine activated AMP deaminase in situ, resulting in the subsequent increase in ammonium production, which can stimulate the activity of 6-phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40). 2) Zn²⁺ inhibited AMP deaminase activity, followed by a decrease in ammonium ion concentration which reduced the activity of phosphofructokinase. 3) Polyamine and Zn²⁺ did not activate or inhibit directly the activity of phosphofructokinase and pyruvate kinase. 4) A simple Michaelis-Menten relationship was observed between the various levels of ammonium ion and fructose 1,6-biphosphate formed in situ, indicating that phosphofructokinase activity, or glycolytic flux was dependent upon the level of ammonium ion produced through the action of AMP deaminase. 5) The increase in P₄ concentration resulted in the decreased magnitude of activation by NH₄⁺ and marked stimulation by P₄ itself of phosphofructokinase, and further reduced the production of NH₄⁺ through the inhibition of AMP deaminase, suggesting that phosphofructokinase activity may not be regulated by the level of NH₄⁺ but by P₄ concentration under conditions of increased P₄ levels.

The AMP deaminase-ammonium system shows a regulatory function in glycolysis of yeast cells in the presence of physiological P₄ levels, whereas glycolysis may be principally controlled by P₄ level under the conditions of elevated P₄ concentration. Polyamines may play a part in the stimulation of glycolysis through the elevated level of ammonium ion under the conditions of increased ATP utilization during cell proliferation, and can participate in the catabolic processes as well as anabolic processes through the stimulation of the AMP deaminase-ammonium system.

The glycolytic pathway is controlled by a large amount of metabolites, many of which reflect the energy status of the cells (1). 6-Phosphofructokinase (EC 2.7.1.11) and pyruvate kinase (EC 2.7.1.40) are nonequilibrium reactions under in situ conditions. These enzymes, which are considered the regulatory enzymes of glycolysis, are activated by ammonium and potassium ions, and phosphofructokinase activity is stimulated by phosphate (2-7). In keeping with their origin as the major effectors of the glycolytic pathway, the increase in the concentration of ammonium ion and P₄, under conditions of increased ATP utilization in certain tissues, is suggested to play a part in the stimulation of glycolysis through the effect on these glycolytic key enzymes (8-12). The role of AMP deaminase (EC 3.5.4.6) in the production of NH₄⁺ from AMP was discussed in relation to the control of phosphofructokinase by Sugden and Newsholme (8) and Winder et al. (13), although direct evidence was not presented. AMP deaminase may be important in the regulation of adenylate energy charge, adenylate pool size (14-18), and the control of the purine nucleotide cycle (19): the importance of the cycle has been discussed in relation to the control of glycolysis in some tissues. Recently, we presented a permeabilization method which allows the assay of intracellular enzymes within the boundaries of yeast cells (20), and the yeast AMP deaminase was demonstrated to be regulated in situ by a variety of effectors. Furthermore, preliminary evidence was presented suggesting that ammonium ion, produced through the activation of AMP deaminase, effectively stimulates yeast phosphofructokinase in the presence of polyamine (21). In the present study, we demonstrate that the levels of NH₄⁺ produced through the action of AMP deaminase, qualitatively and quantitatively correlate with the activity of phosphofructokinase and pyruvate kinase in situ, which can stimulate glycolysis. The specificity and physiological significance of polyamine effect is discussed in relation to the participation in the stimulation of glycolysis through the activation of AMP deaminase-ammonium system under the conditions of increased ATP utilization during cell proliferation.

**Experimental Procedures**

**Materials**

Nucleotides were obtained from Yamasa Co. (Tokyo, Japan). Spermine and firefly lantern extract (FLE-50) were products of Sigma. Enzymes used for the determination of glycolytic intermediates were purchased from Boehringer. Commercial baker's yeast (Saccharomyces cervisiae) was obtained locally.

**Methods**

Procedures for Incubation—Permeabilized yeast cells were prepared according to the method described previously (20). (A) Glycolytic activity was examined at 37 °C in the reaction mixture containing 4.5 mM glucose, 4.5 mM ATP, 10 mM MgCl₂, 2 or 10 mM P₄, 0.1 mM NAD, 10 mM cacodylate buffer (pH 6.5), and toluenized yeast cells (40 mg/ml) in the absence and presence of 1 mM spermine or 0.2-0.5 mM Zn²⁺ in a total volume of 4 ml. (B) When only the activity of phosphofructokinase in situ was quantitatively examined, P₄ and NAD were excluded in the reaction mixture so that Fru-P₄ and triose...
phosphates cannot be further metabolized. Aliquots of 0.2 ml were
deproteinized by 0.6 ml of 7.5% perchloric acid at appropriate inter-
vals. After standing for 15 min at 0 °C, 0.6 ml of potassium carbonate
was added and the potassium perchlorate formed was removed by
centrifugation. The supernatant was used for the determination of
adenylates, ammonia, and glycolytic intermediates.

(D) Activation of pyruvate kinase was studied in the reaction mixture containing 5 mM
P-enolpyruvate, 4 mM ATP, 10 mM deoxyglucose, 10 mM MgCl₂, 10
mM cacodylate buffer (pH 6.5), 1 mM spermine, and the tolenuized
yeast (40 mg/ml) in a total volume of 3 ml. After aliquots of 0.5 ml
were deproteinized at appropriate intervals and neutralized, the su-
pernatant was used for the determination of adenylates, ammonia,
and pyruvate.

**Determination of Metabolites**—ATP was analyzed by luciferin-
luciferase reaction, and ADP and AMP were measured as the
difference after enzymatic conversion to ATP (22). Ammonia was estimated
by the method of Chaney and Marbach (23). Glucose was estimated
by the method of Bergmeyer et al. (24), glucose 6-phosphate (Glc-6-
P) and fructose 6-phosphate (Fru-6-P) by the method of Lang and
Michal (25). Fru-6-P and combined triose phosphates by the method
of Michal and Beutler (26), pyruvate, P-enolpyruvate, and glycerate
2-phosphate by the method of Czok and Lämprecht (27), and ethanol
by the method of Bernt and Guttmann (28).

**RESULTS**

**Stimulation of Glycolysis**—Fig. 1 shows the variation of the
concentrations of hexose 6-phosphates, Fru-P₂, and pyruvate
in permeabilized yeast cells. After the addition of ATP and
glucose, hexose 6-phosphates rapidly increased and then gradu-
ally decreased (Fig. 1A), followed by an increase in Fru-P₂
(Fig. 1B) and further by a formation of pyruvate (Fig. 1C). Glucose recovery as glycolytic intermediates including Glc-6-
P, Fru-6-P, Fru-P₂, triose phosphates, and pyruvate is above
95 and 85% 10 and 20 min after initiation of the reaction,
respectively (Fig. 1C); thus, we can evaluate the effects of these
ligands on the glycolytic activity under these conditions.
As can be seen in Fig. 1, the addition of spermine and Zn²⁺
stimulated and inhibited the formation of Fru-P₂ and pyru-
vate, respectively, although glucose was decreased with the
identical rate under the experimental conditions.
Adenine nucleotide concentrations were also determined
under the same conditions (Fig. 2). After the addition of ATP and
glucose, ATP decreased rapidly, and AMP and ADP² decreased
in amplitude (Fig. 2A), resulting in a drastic drop in the adenylate
energy charge (Fig. 2B). The decrease in total adenylates, which
had occurred before the energy charge began to rise,
was essentially ascribed to the degradation of AMP through
the action of AMP deaminase. This assumption was also
verified by the stoichiometric production of ammonium ion
(corresponding to the decrease in total adenylates (Fig. 2C).
The 5'-nucleotidase (EC 3.1.3.5) and adenosine deaminase
(EC 3.5.4.4) cannot be responsible for the degradation of
adenylates and for the production of NH₃ because of the
lower activity of adenosine deaminase as discussed previously
(17, 18). Recovery of the energy charge and the depletation of
total adenylates were significantly enhanced by the addition
of spermine, the activator of AMP deaminase (29), whereas the
energy charge was more slowly recovered with a slower
decline in total adenylates in the presence of Zn²⁺, the
powerful inhibitor of AMP deaminase (29, 30) (Fig. 2, B and
C).

A graphical representation of the changes in the concentrat-
ion of glycolytic intermediates and adenylates is given in Fig.
3. As noted above, glucose added was almost completely
recovered as glycolytic intermediates including Glc-6-P, Fru-
6-P, Fru-P₂, triose phosphates, and pyruvate after the first 10
min of the reaction (Fig. 1C); the level of phosphoglycerate
was negligible. The values after 10 min of the reaction are
given in % change of the control values. Using the theory of
the crossover theorem (31), these results indicate that the
addition of spermine or Zn²⁺ can affect the activity of phos-
phofructokinase. Variation of the levels of adenylates also
indicates that AMP deaminase reaction was influenced by
the presence of spermine or Zn²⁺. Equilibrium of adenylate kinase
(EC 2.7.4.3) reaction was scarcely affected by these ligands.

**Activation of Phosphofructokinase Activity**—The mecha-
nism of the effects of spermine and Zn²⁺ on the phosphofruc-
tokinase activity and adenylate metabolism was studied. The
experiment was designed to examine the activity of phos-
phofructokinase in situ; thus, P₃, and NAD were excluded in
the reaction mixture so that Fru-P₂, the reaction product of
the enzyme and the triose phosphates formed from it, cannot
be further metabolized. When ATP was used as phosphoryl
donor, the production of Fru-P₂ increased with the increase in
the concentration of spermine; however, NH₃ formed through
the adenylate kinase-AMP deaminase system as a result of
ATP hydrolysis was also increased under these conditions
(Fig. 4A). It was still an open question whether the activity of
phosphofructokinase was stimulated by NH₃ or by spermine
directly. When ITP was used as phosphoryl donor, the system
was still not able to produce NH₃; the increase in the concen-
tration of spermine did not stimulate the formation of Fru-P₂
under these conditions, although the phosphofructokinase
Activation of Glycolysis by AMP Deaminase-Ammonia System

FIG. 2. Effects of spermine and Zn\(^{2+}\) on the changes in adenine nucleotides, NH\(_4^+\), and the adenylate energy charge in permeabilized yeast cells. Toluenedized yeast cells (40 mg/ml) were incubated at 37 °C as described in the legend to Fig. 1. Adenine nucleotides and NH\(_4^+\) were determined according to the luciferin-luciferase method (22) and phenol-hypochlorite reagent (23), respectively. A, ATP (open symbols) and AMP (closed symbols). B, adenylate energy charge. The values were calculated as follows: energy charge = [ATP] + 1/2.[ADP]/[ATP] + [ADP] + [AMP]. C, NH\(_4^+\) (open symbols) and total adenylates (closed symbols). ○, no addition; △, 1 mM spermine added; ▽, 0.2 mM Zn\(^{2+}\) added; ▼, 0.5 mM Zn\(^{2+}\) added.

Activity remained 25–30% of the activity obtained for ATP. The addition of NH\(_4^+\) to the ITP system gave a full activity of phosphofructokinase without stimulation by spermine (data not shown). The effect of Zn\(^{2+}\) on the in situ phosphofructokinase activity was also examined. The inhibition of Fru-P\(_2\) formation by Zn\(^{2+}\) paralleled inhibition of NH\(_4^+\) production under the conditions where ATP was used as phosphoryl donor, and no inhibitory effect of Zn\(^{2+}\) on the phosphofructokinase was observed when excess NH\(_4^+\) was included (Fig. 4B). From these results, Zn\(^{2+}\) action on phosphofructokinase is suggested to be indirect, as in the case of spermine action. Furthermore, Zn\(^{2+}\) showed no inhibitory effect on the phosphofructokinase activity under the conditions where ITP was used as phosphoryl donor: the formation of Fru-P\(_2\) remained 25–30% of the activity obtained for ATP, but the full activity of phosphofructokinase without inhibition by Zn\(^{2+}\) was obtained when excess NH\(_4^+\) was included (data not shown). These results suggest that the effects of spermine and Zn\(^{2+}\) on phosphofructokinase in situ are indirect, via the activation and the inhibition of AMP deaminase, respectively.

A relationship between the level of ammonium ion increased and the production of Fru-P\(_2\) was quantitatively examined after 10 min of the reaction in the absence of F\(_2\) and NAD similar to the experiment of Fig. 4. When spermine or Zn\(^{2+}\) was included in the reaction mixture at various concentrations, the levels of ammonium and Fru-P\(_2\) varied from 0 to 2 mM, and 0.1 to 1.2 mM, respectively. A relationship between the levels of NH\(_4^+\) and Fru-P\(_2\) represents a simple Michaelis-Menten type saturation curve (Fig. 5). Furthermore, NH\(_4^+\) added was able to activate phosphofructokinase under the conditions where AMP deaminase was completely inhibited by the addition of Zn\(^{2+}\): the same response was obtained in Fig. 5 with NH\(_4^+\) added as with the NH\(_4^+\) produced through the AMP deaminase reaction in situ. The apparent K_s value of phosphofructokinase for NH\(_4^+\) was calculated to be 1.43
Activation of Glycolysis by AMP Deaminase-Ammonia System

**Fig. 5 (left).** A relationship between the level of ammonium ion and Fructose-1,6-bisphosphate formation in permeabilized yeast cells. Incubation conditions were similar to those of Fig. 4 except that the concentrations of spermine and Zn²⁺ were varied from 0 to 1 mM, and from 0 to 0.5 mM, respectively, and that 5 mM ATP and 10 mM glucose were used as substrates. Fructose-1,6-bisphosphate and NH₄⁺ were determined after the reaction was terminated at 10 min. The values of Fructose-1,6-bisphosphate concentrations were plotted against those of NH₄⁺ (circle). Triangle symbols show the values of Fructose-1,6-bisphosphate produced by NH₄⁺ added under the conditions where AMP deaminase was completely inhibited by the addition of 0.2 mM Zn²⁺. The theoretical line is drawn from the following equation and values $v = \frac{V[A]}{K_0 + [A] + v_0}$, where $[A]$ is the concentration of NH₄⁺, $K_0$, the concentration required for 50% activation. $K_0$ was determined to be 3.43 mM from the double reciprocal plot (inset). $v$ is represented as the concentration of Fructose-1,6-bisphosphate formed/10 min. $v_0$, the Fructose-1,6-bisphosphate formed under the conditions where AMP deaminase is completely inhibited by the addition of 0.2 mM Zn²⁺, is 0.1 mM (per 10 min).

**Fig. 6 (center).** Effect of spermine on the changes in adenine nucleotides and the adenylyl energy charge in permeabilized yeast cells. The reaction mixture contained 5 mM F-enolpyruvate, 4 mM ATP, 10 mM deoxyglucose, 10 mM MgCl₂, 10 mM cadoxylate buffer (pH 6.5), 1 mM spermine, and the toluenized yeast (40 mg/ml) in a total volume of 3 ml. After aliquots of 0.5 ml were deproteinized at appropriate intervals and neutralized, the supernatant was utilized for the determination of adenylates. A, adenine nucleotides (Δ, A, ATP, □, ■, AMP). B, adenylyl energy charge (○, ●). Open and closed symbols show the values in the absence and presence of spermine, respectively.

**Fig. 7 (right).** Effect of spermine on the changes in total adenine nucleotides, NH₄⁺ and pyruvate. The reaction conditions were similar to those of Fig. 6. A, total adenine nucleotides (Δ, A, AMP). B, pyruvate (○, ●). Open and closed symbols show the values in the absence and presence of spermine, respectively.

mm (Fig. 5, inset), a value which coincided well with that calculated from the kinetics of the purified enzyme (32).

**Activation of Pyruvate Kinase Activity**—The effect of AMP deaminase-ammonium system on the activity of pyruvate kinase, one of the regulatory enzymes of glycolysis, was investigated. The reaction system consisted of P-enolpyruvate, MgATP, and deoxyglucose-P-enolpyruvate and ADP, produced through the hexokinase reaction, can form ATP and pyruvate. ATP rapidly decreased and then slowly recovered, while ADP and AMP inversely increased; increased AMP was slowly recovered, in particular, in the presence of spermine (Fig. 6). The decrease in total adenylates, which is ascribed to the action of AMP deaminase, and the stoichiometric production of NH₄⁺ were also observed (Fig. 7A). Depletion of total adenylates, and production of NH₄⁺ (Fig. 7A) as well as recovery of the energy charge (Fig. 6B) were remarkably enhanced by the presence of spermine. Formation of pyruvate, which was effectively stimulated by the addition of spermine (Fig. 7B), can be correlated with the activation of AMP deaminase since spermine itself did not affect the activity of pyruvate kinase.

**Effect of P₃ on the Activity of Phosphofructokinase**—Conditions of increased ATP utilization and of decreased ATP formation, that is, hypoxia and ischemia, have been demonstrated to increase the levels of ATP and NH₄⁺ in several organisms and tissues (33-38). P₃ can act as an effective activator and inhibitor of phosphofructokinase (2, 4, 6, 7) and AMP deaminase (39), respectively. We thus examined the effect of increased P₃ on the activation by NH₄⁺ of phosphofructokinase and glycolysis. Ammonium ion exhibited a powerful activating effect on the activity of phosphofructokinase in the absence of P₃; however, addition of up to 5 mM NH₄⁺ produced only little enhancement of the activation of the enzyme produced by higher P₃ concentrations (Fig. 7). The data in Fig. 8 were replotted as the relationship between the formation of Fructose-1,6-bisphosphate and P₃ concentration in the presence of different levels of NH₄⁺ (Fig. 8, inset); P₃ can act as an effective activator of phosphofructokinase in the presence of lower concentration of NH₄⁺; but this ligand showed little or no stimulating effect when the enzyme was activated by excess NH₄⁺.

The effect of spermine on the adenylate regulation and the production of NH₄⁺ was examined under the conditions where 10 mM P₃ was included (Fig. 8A): spermine showed little effects on the synthesis of ATP, the recovery of the energy charge (Fig. 9, B and C), or on the glycolytic activity (data not shown). The depletion of total adenylates and production of NH₄⁺ were significantly lower in comparison with those observed for the conditions of lower P₃ (Fig. 9C). These results suggest that P₃ appears to affect glycolysis and adenylate regulation system in two fashions: 1) NH₄⁺ ion shows much less magnitude of activation of phosphofructokinase in the presence of higher P₃ levels, which can activate phosphofructokinase, and 2) P₃ at higher concentration reduces the production of NH₄⁺ through the inhibition of AMP deaminase.
FIG. 8. Effect of the concentration of \( \text{NH}_4^+ \) on the activity of phosphofructokinase in situ in the presence of different levels of \( P_i \). The reaction mixture contained 5 mM glucose, 5 mM ATP, 10 mM MgCl\(_2\), 15 mM KCl, 10 mM cacodylate buffer (pH 7.1), the indicated concentration of \( P_i \), and of ammonium ion as chloride salt, 0.2 mM Zn\(^{2+}\) and the toluenized yeast cells (10 mg/ml). Fru-P\(_2\) was determined after incubation for 10 min. \( \bigcirc \) no addition; \( \bigtriangleup \) 2 mM \( P_i \) added; \( \square \), 5 mM \( P_i \) added; \( \triangledown \), 10 mM \( P_i \) added. Inset, effect of concentration of \( P_i \) on the activity of phosphofructokinase in the presence of different levels of \( \text{NH}_4^+ \). \( \bigcirc \), 0.5 mM \( \text{NH}_4^+ \) added; \( \bigtriangleup \), 5 mM \( \text{NH}_4^+ \) added. Closed symbols show the values of Fru-P\(_2\) and \( \text{NH}_4^+ \) which were produced from the ATP-glucose system as shown in Fig. 1 in the absence (\( \bigcirc \)) and presence of 1 mM spermine (\( \bigtriangleup \)).

Thus, the AMP deaminase-NH\(_4^+\) system does not stimulate glycolysis when glycolysis is fully activated in the presence of higher \( P_i \) levels.

DISCUSSION

AMP deaminase, which is widely distributed in various eukaryotes such as animals (39), yeast (17), and higher plants (40), may be important in stabilization of adenylate energy charge (14-18) and the interconversion of adenine, inosine, and guanine nucleotides (19). AMP deaminase as an allosteric enzyme can be regulated by a variety of effectors (19); kinetic properties suggest that the enzyme may be related to the regulation of glycolysis (19) and fatty acid metabolism (15, 41). The purine nucleotide cycle, which consists of AMP deaminase, adenylosuccinate synthetase (EC 6.3.4.4), and adenylosuccinate lyase (EC 4.3.2.2), is closely correlated with the control of glycolysis (19). Ammonium ion and \( P_i \), which increase under conditions of increased ATP utilization, are considered to be responsible for the stimulation of glycolysis (8-12). The increase in the concentration of \( \text{NH}_4^+ \), \( P_i \), and AMP, and decrease in ATP, citrate, and the energy charge values may play a role in the activation of phosphofructokinase, a regulatory enzyme of glycolysis (2, 4, 6, 7). Munro was the first to show that \( \text{NH}_4^+ \) ion activated phosphofructokinase in yeast extract (10) and rat brain homogenates (11, 12). The participation of AMP deaminase in the production of \( \text{NH}_4^+ \), from AMP was discussed in relation to the activation of phosphofructokinase by Sugden and Newsholme (8), although direct evidence had not been presented. The permeabilized system of yeast cells, which was presented by us (20), can offer an excellent experimental system for the study on the control of glycolysis. It is of particular interest to examine the effects of various regulatory ligands on the glycolytic activity under the conditions where intracellular levels of ATP and glucose were included, although some previous in situ studies on glycolysis were carried out in a high concentration of glucose and catalytic amount of ATP (42). The results presented in this paper show that \( \text{NH}_4^+ \) produced through the action of AMP deaminase correlates with the activation of phosphofructokinase and pyruvate kinase in situ, which may stimulate glycolysis. Much less Fru-P\(_2\) was formed under the conditions where AMP deaminase activity was inhibited by the addition of Zn\(^{2+}\), although the phosphofructokinase activity could be stimulated by the marked decrease in the energy charge and the increase in AMP concentration (see Figs. 1 and 2). Furthermore, pyruvate kinase activity was also stimulated by increased \( \text{NH}_4^+ \) level rather than by the decrease in the energy charge and the increase in AMP or ADP (see Figs. 6 and 7). Thus, the AMP deaminase-NH\(_4^+\) system or the level of \( \text{NH}_4^+ \) produced can play a principal part in the activation of phosphofructokinase and pyruvate kinase.

The average concentrations of \( \text{NH}_4^+ \) in some tissues and organisms were found to be 0.24-0.34 mM (43-45). The concentration of \( \text{NH}_4^+ \) is increased under conditions that cause an increase in glycolytic flux. In the rat muscle and mammalian brain the concentration of \( \text{NH}_4^+ \) is increased from 0.2-0.3 mM to 1 mM and 5 mM by a tetanic stimulation (45) and prolonged ischemia (46), respectively. The increase in \( \text{NH}_4^+ \) as well as \( P_i \) levels under conditions where glycolytic flux is stimulated may be a general phenomenon. The apparent \( K_a \) of phosphofructokinase for \( \text{NH}_4^+ \) calculated in this paper was 1.43 mM, a value which agreed well with that observed for the purified enzyme (32), and which was within the range of physiological concentration of this ion. The results of the present work, concerning the stimulation of phosphofructokinase and pyruvate kinase by the AMP deaminase-NH\(_4^+\)
system, can account for the experimental results on some tissues or cells in vivo (10-12, 45, 46).

It should be noticed, however, that the AMP deaminase-NH₄⁺ system is not necessarily responsible for the stimulation of glycolysis in the presence of higher concentration of P₄, which results in a decreased magnitude of activation by NH₄⁺, a remarkable stimulation by P₄ itself of phosphofructokinase, and a reduced production of NH₄⁺ through the inhibition of AMP deaminase. Glycolysis may not be regulated by the level of NH₄⁺ under the conditions where P₄ is significantly elevated: little or no effect of spermine on the recovery of the energy charge was noticeable under these conditions (Fig. 9).

Inorganic ions such as K⁺, Mg²⁺, and P⁴, were demonstrated to accumulate mainly in vacuoles of yeast cells: their concentrations are 60, 5, and 1 mM in the cytoplasm and 470, 73, and 110 mM in vacuoles, respectively (47). Efflux of these ions from vacuolar pool in the presence of glucose or fructose (48) may contribute to the stimulation of glycolysis or ATP synthesis.

Monovalent cations participate in biological processes in a variety of ways. One of the important functions of these cations is as activators or inhibitors of enzymes (49, 50). As noted from tables of monovalent cation-activated enzymes compiled by Evans and Sorger (51) and Sweeter (49), most of these enzymes show relatively strict specificity toward activating cations. 1) Those enzymes activated by K⁺ are also usually activated by NH₄⁺ and Rb⁺, but are activated little by Na⁺ and Li⁺. 2) A few enzymes activated by Na⁺ and also activated by Li⁺ and Cs⁺ can be activated much less by K⁺, NH₄⁺, and Rb⁺. However, 3) some enzymes are activated equally efficiently by K⁺ and Na⁺ (52). AMP deaminase (53-55) and AMP nucleosidase (EC 3.2.2.4) (56, 57), which are typical enzymes of this group, show broad specificity toward activating cations: monovalent cations of both K⁺-NH₄⁺ and Na⁺-Li⁺ groups are equally efficient, and alkaline earth metals and transition metals also affect the enzyme activity. Polyamine is the most effective activator of these enzymes (52, 55, 58, 59).

The first group of monovalent cation-activated enzymes, K⁺-NH₄⁺ type, has been classified as a group of the enzymes which can be activated by both K⁺ and NH₄⁺. However, the enzymes of this group should be divided into the following subgroups: a) K⁺-type: enzymes which are activated equally efficiently by K⁺ and NH₄⁺ with A₀.₅ values of 50-100 mM; b) NH₄⁺ type: enzymes which are activated by NH₄⁺ with A₀.₅ values of 0.5-2.5 mM, and by K⁺ with A₀.₅ values of 50-100 mM. Ammonium ion at physiological level can be responsible for the control of the enzyme activities of the NH₄⁺-type, and K⁺ ion may act as a physiological activator of the enzymes of K⁺-type. Of particular interest is the fact that phosphofructokinase and pyruvate kinase, the regulatory enzymes of glycolysis belong to NH₄⁺-type: glycolysis may be one of the best targets for AMP deaminase-NH₄⁺ system. Enzymes belonging to this group are listed in Table 1 (3, 9, 60-71).

It should be noticed that polyamines can stimulate glycolytic activity through the enhancement of AMP deaminase-NH₄⁺ system. Polyamines, which accumulate accompanying cellular proliferation, are responsible for the increase in the rate of RNA and protein synthesis (72-74), and participate in the control of the activation of many enzymes (52). Recently, a new role of polyamines related to control of glucose metabolism was demonstrated: spermine and spermidine stimulate conversion of glucose to carbon dioxide and inhibit epinephrine-stimulated lipolysis in isolated adipocytes (75, 76). Polyamines bind to fat cell membrane at a site distinct from the insulin receptor sites, resulting in the insulin-like effects of facilitating glucose transport and inhibiting lipolysis by suppressing cyclic AMP levels (77). The function of increased polyamines on glucose catabolism in cells has remained unknown, although polyamines may be responsible for the direct activation of several glucose-catabolizing enzymes (78-81). The results presented here will serve as a guide to the analysis of the role of polyamines in vivo: polyamines can stimulate glycolysis by the activation of phosphofructokinase and pyruvate kinase through the increased NH₄⁺ produced from AMP, as well as facilitating glucose transport or direct activation of several enzymes in some tissues.

AMP deaminase, as an NH₄⁺-producing system, can participate in the regulation of glycolysis as well as the supply of some amino acids through the stimulation of threonine dehydratase activity in yeast (82). Polyamines and NH₄⁺ may play a key role in anabolic processes: polyamine can be responsible for the increase in the rate of nucleic acid synthesis and protein synthesis (69-71), and ammonium ion is utilized for the synthesis of amino acids (83). The present results as well as insulin-like effects of polyamines suggest that polyamines can participate in catabolic processes, that is, glucose degradation. Polyamines and NH₄⁺ can participate in catabolic processes as well as anabolic processes through the stimulation of the AMP deaminase-ammonium system. Thus, these ligands may be termed as "amphibolic ligands."

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REFERENCES

### Table 1

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<th>Enzymes activated by monovalent cations</th>
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<tr>
<td>I. K⁺-NH₄⁺ type</td>
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<tr>
<td>a. K⁺-type</td>
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<tr>
<td>b. NH₄⁺-type</td>
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<tr>
<td>Enzymes (references)</td>
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<tr>
<td>Phosphofructokinase (3, 9, 60), pyruvate kinase (61), threonine dehydratase (62), tryptophanase (63), phosphotransacetylase (64), panepadiol dehydratase (65), myoinositol 1-phosphate synthase (66), formyltetrahydrofolate synthetase (67), glycerol dehydrogenase (68), malic enzyme (69), aspartokinase (70, 71)</td>
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<td>II. Na⁺-Li⁺ type</td>
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<tr>
<td>III. Nonspecific type (polymine type)</td>
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<td>Polyamine &gt; K⁺, NH₄⁺ = Na⁺, Li⁺</td>
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<td>AMP deaminase (53-55), AMP nucleosidase (56, 57), glucose 6-phosphate dehydrogenase (78), UDP glucose 4-epimerase (79), glyco- gen phosphorylase (80), phosphoenolpyruvate carboxylase (81)</td>
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AMP deaminase reaction as a control system of glycolysis in yeast. Activation of phosphofructokinase and pyruvate kinase by the AMP deaminase-ammonia system.
M Yoshino and K Murakami


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