Adenine nucleotide degradation during energy depletion in human lymphoblasts

**ADENOSINE ACCUMULATION AND ADENYLATE ENERGY CHARGE CORRELATION***

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Adenine nucleotide breakdown to nucleosides and purine bases was measured in cultures of human lymphoblastoid cells following: 1) the inhibition of oxidative phosphorylation in the absence of glucose or 2) the addition of 2-deoxyglucose. A mutant cell line, deficient in adenosine kinase, in the presence of an adenosine deaminase inhibitor was used to measure utilization of the two pathways of AMP catabolism involving initial action of either purine 5'-nucleotidase or AMP deaminase. In such a system the appearance of adenosine induced by the oxidative phosphorylation inhibitor, rotenone, implies that approximately 70% of AMP breakdown occurs via dephosphorylation. By the same method, deamination accounts for 82% of AMP breakdown when 2-deoxyglucose is added. The occurrence of AMP dephosphorylation is not correlated with elevated concentrations of substrate or with decreased concentrations of the inhibitors of 5'-nucleotidase, ATP and ADP. Dephosphorylation occurs if, and only if, the adenylate energy charge decreases to about 0.6 in these experiments. In cultures deprived of glucose and oxygen, adenine nucleotide degradation via dephosphorylation results in recovery of normal energy charge values.

Breakdown of the adenine nucleotides to nucleosides and bases follows the rapid utilization of ATP in mammalian cells incubated with glucose or 2-deoxyglucose in vitro (1-6). This phenomenon may be the counterpart in vitro of the elevated uric acid production induced by fructose infusion in vivo and in perfused organs (7-9). The mechanism of nucleotide degradation following fructose infusion involves accumulation of a hexose phosphate, depletion of intracellular inorganic phosphate, relief of the phosphate inhibition of AMP deaminase, deamination of AMP to IMP, and subsequent conversion of IMP to nucleosides and bases (Equation 1).

$$AMP = IMP + \text{NH}_3$$
$$IMP = \text{inosine} + \text{PO}_4^{3-}$$

(1)

Changes in GTP and ATP concentrations and the adenylate energy charge may also play a role in the regulation of AMP deaminase activity (9-11).

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The function of adenine nucleotide degradation may be the stabilization of the adenylate energy charge, (ATP + %ADP)/ (ATP + ADP + AMP). The adenylate energy charge coordinates many metabolic reactions and is a major factor in maintaining cellular homeostasis (12). Following a metabolic stress that lowers the energy charge, recovery of normal values of the energy charge is achieved by degrading the AMP formed during periods of high ATP utilization (10). In extracts of ascites cells AMP deaminase is activated by a decrease in energy charge (11). However, in intact ascites cells nucleotide degradation is correlated with a drop in intracellular inorganic phosphate concentration and is not correlated with changes in energy charge (6).

An alternative pathway of adenine nucleotide breakdown exists beginning with dephosphorylation of AMP in the sequence of reactions catalyzed by 5'-nucleotidase and adenosine deaminase (Equation 2).

$$AMP = \text{adenosine} + \text{PO}_4^{3-}$$
$$\text{Adenosine} = \text{inosine} + \text{NH}_3$$

(2)

When ascites are incubated with 2-deoxyglucose, up to 18% of the adenine nucleotide breakdown proceeds through this alternative pathway (4). The present work demonstrates that the dephosphorylation of AMP accounts for the major part of nucleotide degradation induced by the inhibition of oxidative phosphorylation in the absence of a glycolytic substrate. Examples of the close correlation between the adenylate energy charge and adenine nucleotide degradation after oxidative phosphorylation inhibition are also presented.

**EXPERIMENTAL PROCEDURES**

*Chemicals—Rotenone, 2,4-dinitrophenol, 6-aminonicotinamide, methylene blue, and antimycin A were obtained from Sigma. 2-Deoxyglucose, oligomycin, and carbonyl cyanide m-chlorophenylhydrazone were obtained from Calbiochem. Deoxycoformycin was obtained from the Developmental Therapeutics Program, Chemotherapy, the National Cancer Institute. The following were obtained from New England Nuclear: [8-14C]adenine (44 mCi/mmol), [8-14C]hypoxanthine (42.5 mCi/mmol), and L-[U-14C]leucine (320 mCi/mmol). Sodium [14C]formate (61.3 mCi/mmol) was obtained from Amer sham/Searle.

*Cell Culture—The human splenic lymphoblast cell line, WI-L2, and the adenosine kinase-deficient mutant, MTF-107, derived from WI-L2 have been previously described (13). The mutant has less than 1% of the adenosine kinase activity of WI-L2. Both cell lines were maintained in RPMI 1640 medium supplemented with 20% fetal calf serum and 2 mM glutamine under an atmosphere of 5% carbon dioxide in air with shaking in a 37°C incubator. The cell densities were maintained between 0.5 to 1 x 10^5 cells/ml. The doubling times were 12 to 14 h. Cell numbers were determined with a Coulter counter. The cultures were screened at regular intervals for uracil incorporation and were thereby shown to be free of mycoplasma contamination.

*Experimental Medium—Because of the presence of purine metab-
olizing enzymes in fetal calf serum, all experiments were carried out in RPMI 1640 medium supplemented with 1% (w/v) bovine serum albumin, 2 mM glutamine, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer, pH 7.4 (Ultrotpe, Calbiochem). The latter is hereafter referred to simply as “medium.” In this medium cell viability as determined by trypan blue exclusion was well maintained up to at least 30 h, but the cells underwent less than one population doubling before ceasing to divide. A few experiments were carried out in medium containing in addition 0.1% fetal calf serum which supports continued division of these cells (14), and similar results were obtained. Medium lacking glucose was used where indicated.

**Incubation and Extraction of Cells**—Purine nucleotides were labeled by incubating cells at a density of about 5 × 10^6 cells/ml in medium with 0.5 or 100 μM [8-^14^C]adenine for 20 to 24 h with shaking at 37°C. At the end of this period cell viability remained at 85 to 95%. Labeled cells were washed three times in fresh medium before resuspension in the medium to a density of 5 to 10 × 10^6 cells/ml. Aliquots of 0.4 ml were pipetted into plastic tubes (12 × 75 mm, Falcon) with the additions indicated, gassed with 5% carbon dioxide in air, and shaken in a horizontal position within a 37°C incubator. Extraction was usually performed by adding 40 μl of cold 4 M perchloric acid directly to a tube containing the cell suspension. Larger volumes of cell suspension were centrifuged for 4 min at 270 × g and perchloric acid was added separately to cells and medium. After centrifugation to remove the acid-insoluble material the supernatants were neutralized by treatment with Alamine 336 (General Mills) in a 1-to-1 volume ratio. The radioactivity in this medium was determined by liquid scintillation counting. A comparison with high pressure liquid chromatographic separations points out two limitations of the thin layer chromatographic separation. In the thin layer chromatography method, AMP values are systematically overestimated, possibly due to contamination with the faster running NAD, so that adenylate energy charge values are low. Also in thin layer chromatography, hypoxanthine is not completely separated from inosine, so that the reported results combine the radioactivity of both these compounds.

**High Pressure Liquid Chromatography**—Nucleotides were separated on polyethyleneimine-paper sheets (Polygram Cel 300 PEI, Brinkmann Instruments) in a single dimension with a stepwise formate gradient (15). Nucleotides and bases were also separated on polyethyleneimine-paper sheets using the solvent system: n-butyl alcohol/propanoic acid/water (46:23:32) (17). Following visualization under ultraviolet light, the areas corresponding to the various compounds were cut out and their radioactivity was determined by liquid scintillation counting. A comparison with high pressure liquid chromatographic separations points out two limitations of the thin layer chromatographic separation. In the thin layer chromatography method, AMP values are systematically overestimated, possibly due to contamination with the faster running NAD, so that adenylate energy charge values are low. Also in thin layer chromatography, hypoxanthine is not completely separated from inosine, so that the reported results combine the radioactivity of both these compounds.

**Purine Synthesis de Nove**—The incorporation of formate into the purine ring was measured as previously described (18) with the following modifications. Cells were preincubated in medium plus 106 units/ml of penicillin and streptomycin, and 50 μg/ml of deoxyribonuclease. After 4 h with shaking at 37°C, the cell samples represent the amount of de novo-synthesized purine incorporated into nucleotides, and the media samples represent the amount of de novo purine incorporated into nucleotides and bases.
Adenine Nucleotide Degradation

the guanine nucleotides. Virtually all of the nucleotide was intracellular. Less than 1% of any labeled nucleotide could be detected in media samples obtained by centrifugation of cell cultures after 4 h of incubation under any of the conditions used. On the other hand at least 96% of the nucleosides and bases were present in the extracellular medium. Hypoxanthine and adenosine were the major compounds excreted by the cells in these experiments, but smaller amounts of inosine and adenosine were also present. At the end of the labeling period approximately two-thirds of the total intracellular radioactivity existed as acid-insoluble material.

Table I shows the results of an experiment in which both molar amount and radioactivity of the nucleotides, nucleosides, and bases were determined by high pressure liquid chromatography. In general the radioactivity measurements accurately reflect the molar proportions. Initially the cells had an adenylate energy charge of 0.90. After 4 h of incubation with 11 mM glucose, the energy charge was maintained at a normal value, although the sum of the adenylates had dropped to about 75% of their initial level. The number of viable cells remained constant during this period. The decline in the total adenylate level may have been caused by renormalization of a nucleotide pool which was expanded by preincubation with adenosine or by the stress of resuspension in fresh media. The amount of inosine plus adenosine that accumulated was approximately equivalent to the total nucleotide material lost. Hypoxanthine and adenosine did not form and may have been used. On the other hand at least 95% of the nucleosides and bases were present in the extracellular medium. Hypoxanthine was present along with the inhibitors (see for example Figs. 3 and 5). However, the absence of glucose was not sufficient by itself to cause such a large degree of degradation (Fig. 2). In mutant cells deficient in adenosine kinase, in the presence of the adenosine deaminase inhibitor deoxycoformycin, large amounts of adenosine accumulated during inhibition of oxidative phosphorylation. Dephosphorylation of AMP, therefore, must have constituted one step of the induced degradation. Adenosine also accumulated to a lesser extent in normal cells that possessed adenosine kinase when incubated with deoxycoformycin and an oxidative phosphorylation inhibitor, suggesting that a functional deficiency of adenosine kinase activity was produced. Quantitative aspects of the degradation effect are shown in Table I. After incubation with 100 nM rotenone for 4 h, the total adenylate level dropped to 12% of the level in control cells incubated with glucose. The ATP dropped even more drastically to 4% of the control level. The adenylate energy charge dropped to a low value. Although GTP was also dephosphorylated, thin layer chromatographic separations showed that GMP accumulated and the sum of

<table>
<thead>
<tr>
<th>INCRUBATION CONDITION</th>
<th>ATP</th>
<th>ADP</th>
<th>AMP</th>
<th>GTP</th>
<th>GDP</th>
<th>Hyp</th>
<th>Ino</th>
<th>Ade</th>
<th>Ado</th>
<th>ZMP</th>
<th>EC</th>
<th>IMP</th>
<th>IMP</th>
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<tbody>
<tr>
<td>INITIAL</td>
<td>2.60</td>
<td>0.44</td>
<td>0.11</td>
<td>0.70</td>
<td>0.15</td>
<td>0.12</td>
<td>0.17</td>
<td>0.05</td>
<td>0.04</td>
<td>-</td>
<td>-</td>
<td>3.15</td>
<td>0.90</td>
</tr>
<tr>
<td>Glucose</td>
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<td>0.15</td>
<td>0.59</td>
<td>0.13</td>
<td>3.01</td>
<td>3.01</td>
<td>-</td>
<td>-</td>
<td>2.62</td>
<td>3.14</td>
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</tr>
<tr>
<td>Rumenone</td>
<td>0.08</td>
<td>0.08</td>
<td>0.06</td>
<td>0.04</td>
<td>0.13</td>
<td>0.13</td>
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<td>0.31</td>
<td>0.48</td>
<td>0.48</td>
<td>0.48</td>
</tr>
<tr>
<td>2-deoxyglucose</td>
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<td>0.32</td>
<td>0.21</td>
<td>0.04</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
<td>0.16</td>
<td>0.25</td>
<td>0.33</td>
<td>0.44</td>
<td>0.44</td>
<td>0.44</td>
</tr>
</tbody>
</table>

a. ATP + ADP + AMP.
b. Adenylate energy charge = ATP + ZMP/ATP + ADP + AMP.
c. Sun of all purine nucleotides.
d. Sun of all purine nucleosides. e. ATP + ADP + AMP.

Incubation with Oxidative Phosphorylation Inhibitors—
The inhibition of oxidative phosphorylation at any of several sites caused nucleotide breakdown to nucleosides and bases (Fig. 2). This effect was not observed when 11 mM glucose was present along with the inhibitors (see for example Figs. 3 and 5). However, the absence of glucose was not sufficient by itself to cause such a large degree of degradation (Fig. 2). In mutant cells deficient in adenosine kinase, in the presence of the adenosine deaminase inhibitor deoxycoformycin, large amounts of adenosine accumulated during inhibition of oxidative phosphorylation. Dephosphorylation of AMP, therefore, must have constituted one step of the induced degradation. Adenosine also accumulated to a lesser extent in normal cells that possessed adenosine kinase when incubated with deoxycoformycin and an oxidative phosphorylation inhibitor, suggesting that a functional deficiency of adenosine kinase activity was produced. Quantitative aspects of the degradation effect are shown in Table I. After incubation with 100 nM rotenone for 4 h, the total adenylate level dropped to 12% of the level in control cells incubated with glucose. The ATP dropped even more drastically to 4% of the control level. The adenylate energy charge dropped to a low value. Although GTP was also dephosphorylated, thin layer chromatographic separations showed that GMP accumulated and the sum of

Table I

High pressure liquid chromatographic determination of purine nucleotides, nucleosides, and bases in lymphoblasts

Lymphoblast mutant cells, deficient in adenosine kinase, were prelabeled with [14C]adenine, incubated for 4 h with 1 μM deoxycoformycin plus the indicated additions, extracted, and analyzed as described under "Experimental Procedures."

<table>
<thead>
<tr>
<th>NUCLEOTIDE</th>
<th>GMP</th>
<th>IMP</th>
<th>ZMP</th>
<th>IMP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td></td>
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</tr>
<tr>
<td>Rumenone</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-deoxyglucose</td>
<td></td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>
Fig. 2. Accumulation of nucleosides and bases during stress of energy metabolism. Lymphoblast mutant cells, deficient in adenine kinase, were prelabeled with [14C]adenine. The cells were resuspended in RPMI 1640 medium without glucose and containing 1 mM deoxycoformycin plus the indicated additions. In all but the anaerobic case the cell suspensions were shaken in an atmosphere of air plus 5% carbon dioxide. In the anaerobic case the cells were allowed to settle into a pellet. At zero time (initial), or after 4 h of incubation at 37°C, the entire cell suspension was extracted and samples separated by thin layer chromatography to determine the combined radioactivity in adenine (Ado) plus adenine (Ade) (cross-hatched bar) and the combined radioactivity in hypoxanthine (Hyp) plus inosine (Ino) (clear bar). Error bars give standard deviations for three determinations. AN, 6-aminonicotinamide; MB, methylene blue; CI-CCP, carbonylcyanide m-chlorophenylhydrazone; 2,4-DNP, 2,4-dinitrophenol.

Variation of the Adenylate Pool Size—The stimulus for nucleotide degradation may be a high concentration of the substrate AMP, low concentrations of inhibitory ATP or ADP, or a variation in some other parameter. To test these possibilities a cell culture was stressed once when it contained adenine kinase-deficient cells. The nucleotide degradation and hypoxanthine accumulation reached a maximum rate at 1 h, at which time the adenylate energy charge had dropped to a value of 0.24. During continued incubation from 3 to 6 h, the energy charge began to recover spontaneously. As the energy charge approached the control value of 0.8, the nucleotide degradation slowed. When cells were incubated with 100 nM rotenone, in less than 10 min the energy charge dropped to a value of 0.24 and the ATP content was reduced to 15% of the original level (Fig. 4). This was accompanied by an initial rapid increase in the concentration of AMP which then began to fall after 30 min. The decrease in the sum of the adenine nucleotides paralleled the decrease of AMP. Rotenone and other strong inhibitors of oxidative phosphorylation had an irreversible effect on the energy charge. Although some increase in the energy charge occurred at low inhibitor concentration, spontaneous recovery was not observed. The lowering of the energy charge by 2-deoxyglucose was also rapid and irreversible.

Inhibitor Effects on Purine Synthesis de Novo—The contribution of the de novo synthetic pathway to nucleotides, nucleosides, and bases in the presence of rotenone and 2-deoxyglucose was assessed by [14C]formate incorporation into the purine base moiety. Adenosine kinase-deficient cells were incubated with [14C]formate for 2 h and then exogenous label was washed away. The resulting radioactive nucleotide profiles and their changes on incubation with deoxycoformycin plus glucose, rotenone, or 2-deoxyglucose, as well as the accumulation of labeled nucleosides and bases, were identical to the observations for [14C]adenine-labeled cells. A different labeling pattern was observed when [14C]formate and inhibitors were added simultaneously. After 4 h of incubation with [14C]formate plus glucose, nucleotides were the main end products of de novo synthesis, while 8% of the total de novo purine was in the medium as nucleosides and bases. During incubation with [14C]formate plus rotenone or 2-deoxyglucose, the total de novo synthesis was reduced to 2 or 3% of the level in glucose and of that total 90 or 81%, respectively, was excreted into the medium. Thus, most of the IMP formed by de novo synthesis during energy depletion was broken down and excreted as soon as it was formed. Similar results were obtained with a cell line deficient in both adenosine kinase and hypoxanthine phosphoribosyltransferase.

Time Course of Degradation—As cells settled into a pellet, anaerobic conditions slowly developed. When the glucose concentration was low, adenine nucleotide degradation occurred (Fig. 3). The nucleotide degradation and hypoxanthine accumulation reached a maximum rate at 1 h, at which time the adenylate energy charge had dropped to a value of 0.54. During continued incubation from 3 to 6 h, the energy charge began to recover spontaneously. As the energy charge approached the control value of 0.8, the nucleotide degradation slowed. When cells were incubated with 100 nM rotenone, in less than 10 min the energy charge dropped to a value of 0.24 and the ATP content was reduced to 15% of the original level (Fig. 4). This was accompanied by an initial rapid increase in the concentration of AMP which then began to fall after 30 min. The decrease in the sum of the adenine nucleotides paralleled the decrease of AMP. Rotenone and other strong inhibitors of oxidative phosphorylation had an irreversible effect on the energy charge. Although some increase in the energy charge occurred at low inhibitor concentration, spontaneous recovery was not observed. The lowering of the energy charge by 2-deoxyglucose was also rapid and irreversible.

the guanylates did not decrease. If it is assumed that the labeled adenosine resulted from AMP dephosphorylation and the labeled hypoxanthine and inosine resulted from AMP deamination, then 70% of the total purine nucleotide degradation must have occurred by way of dephosphorylation and 25% by way of deamination during the inhibition of oxidative phosphorylation. The molar amount of nucleosides and bases formed was twice the decrease of total moles of nucleotides from their initial level. The radioactivity in accumulated nucleosides and bases was only slightly greater than the concentration of AMP which then began to fall after 30 min. The decrease in the sum of the adenylates to 16% of the level in cells incubated with glucose (Table 1). GDP was dephosphorylated and GDP accumulated, but the total guanine nucleotide level did not decrease. Hypoxanthine was the major degradation product formed. Control experiments indicated that the presence of 2-deoxyglucose reduced the incorporation of added [14C]hypoxanthine to 5.1%, compared to incubation in glucose. In the adenosine kinase-deficient cells with deoxycoformycin the amount of labeled hypoxanthine and inosine indicates that 85% of the AMP was deaminated and the amount of labeled adenosine indicates that 18% of the AMP was dephosphorylated during 2-deoxyglucose-induced degradation. Incubation with 6-aminonicotinamide plus methylene blue also caused an increase in hypoxanthine along with a smaller increase in adenosine.

Incubation with 2-Deoxyglucose—In human lymphocytes, degradation of nucleotides was caused by incubation with 2-deoxyglucose (Fig. 2). Incubation with 5.5 mM 2-deoxyglucose led to a decrease in the sum of the adenylates to 16% of the level in cells incubated with glucose (Table 1). GDP was dephosphorylated and GDP accumulated, but the total guanine nucleotide level did not decrease. Hypoxanthine was the major degradation product formed. Control experiments indicated that the presence of 2-deoxyglucose reduced the incorporation of added [14C]hypoxanthine to 5.1%, compared to incubation in glucose. In the adenosine kinase-deficient cells with deoxycoformycin the amount of labeled hypoxanthine and inosine indicates that 85% of the AMP was deaminated and the amount of labeled adenosine indicates that 18% of the AMP was dephosphorylated during 2-deoxyglucose-induced degradation. Incubation with 6-aminonicotinamide plus methylene blue also caused an increase in hypoxanthine along with a smaller increase in adenosine.
Adenine Nucleotide Degradation

FIG. 3. Adenine nucleotide degradation and adenylate energy charge recovery during anaerobic stress. WI-L2 lymphoblasts were prelabeled with \(^{14}C\)adenine, then resuspended at a density of 10^6 cells/ml in media containing either 11 mM glucose (○), □, △) or 1 mM glucose (Low GLC, ●, ■, ▲). The cells settled into a pellet in the test tube and were incubated without shaking at 37°C. At the indicated times the total contents of the tubes were extracted and separated by thin layer chromatography. Panel A, adenylate energy charge = (ATP + \(\frac{1}{2}\)ADP)/(ATP + ADP + AMP). Panel B, \(\Sigma AXP\) = ATP + ADP + AMP (○, ■, ▲); Hyp, hypoxanthine (△, ▲).

FIG. 4. Variation of nucleotide pools during energy depletion. WI-L2 lymphoblasts were prelabeled with \(^{14}C\)adenine, resuspended, and incubated in RPMI 1640 medium with 100 nM rotenone and without glucose. After 3 h of incubation with rotenone, the cells were washed free of rotenone and exogenous radioactive material. The cells were incubated in 11 mM glucose for 30 min, washed, and resuspended in glucose-free medium, and then reincubated with 100 nM rotenone. Samples were extracted at the indicated times and separated by thin layer chromatography as described under “Experimental Procedures.” Panel A, adenylate energy charge. Panel B, \(\Sigma AXP\) = ATP + ADP + AMP (○, ■, ▲); ATP, △; ADP, □; AMP, ○.

Protein Synthesis and Adenylate Levels—Adenine nucleotide levels and the incorporation of leucine into protein were simultaneously measured in cells that had been incubated with rotenone (Fig. 5). The adenosine kinase-deficient cells with deoxycoformycin added could not reincorporate any adenosine formed during adenylate degradation. Duplicate nucleosides and bases formed from nucleotide degradation. The cells then were incubated with 11 mM glucose for 30 min to stop adenylate degradation and to restore the energy charge to a value of 0.78. Then the cells were resuspended in medium without glucose and were stressed once more with rotenone. The adenine nucleotides were further degraded to 40% of the level following the glucose incubation and the energy charge again dropped to a low value.

FIG. 5. Protein synthesis and adenylate levels during energy stress and recovery. Two cultures of adenosine kinase-deficient mutant lymphoblasts were incubated with \(^{14}C\)leucine (Panel A) or with \(^{14}C\)adenine (Panels B, C, and D) and samples were treated as described under “Experimental Procedures.” Incubation mixtures contained: Control, 22 mM glucose (○); +rotenone, 100 nM rotenone without glucose (▲); +glucose, 100 nM rotenone and after 2 h (arrow) 22 mM glucose was added (△).
cultures of cells were used. One culture had been prelabeled with $[^{14}C]$adenine before suspension in medium containing unlabeled leucine. The other had been preincubated with unlabeled adenine before suspension in medium containing $[^{14}C]$leucine. When rotenone was added in the absence of glucose, adenylate degradation and the decrease in energy charge occurred as described before. In the presence of rotenone, leucine incorporation into protein was abolished. Other control experiments have shown that if oxygen and amino acids were provided, cells synthesized protein in media without glucose. After 2 h of incubation, glucose was added to some of the rotenone-containing cultures. On addition of glucose, adenylate degradation ceased and the adenylate pool remained constant at 56% of its initial size. The adenylate pool did not increase in size because the adenosine formed during degradation could not be reincorporated into nucleotides in the adenosine kinase-deficient cells. After glucose addition, ATP was formed by phosphorylation of ADP and AMP, but because the adenylate pool was one-half its original size, ATP could rise to only 51% of its original level. The energy charge rose immediately above 0.6 and approached the normal control rate within 1 h after glucose addition. This experiment indicates that protein synthesis could occur at a nearly normal rate even though the ATP level and the sum of the adenylates were reduced to about half of their original values. A normal rate of protein synthesis was observed only when the energy charge was 0.70 or higher.

**Discussion**

The present experiments show that when cultured human lymphoblasts can use neither glycolysis nor respiration to fulfill their energy requirements, ATP is rapidly converted to AMP through normal metabolic use and AMP is slowly degraded to nucleosides and bases. The main mechanism of degradation is dephosphorylation of AMP followed by deamination of adenosine. The onset of degradation is correlated with a decrease in the adenylate energy charge and, in some cases, degradation leads to a subsequent recovery of normal energy charge values.

In cultured cells the rapid decrease of ATP levels following the inhibition of oxidative phosphorylation is well known, although varying degrees of subsequent degradation of the adenine nucleotides have been reported. Following the inhibition of respiration in vitro, the sum of the adenosine nucleotides has been reported to decrease in Ehrlich ascites cells (1-3) and in human platelets (19, 20) or has been reported to remain unchanged in Ehrlich ascites cells (5, 6). The relatively slow rate of adenosine nucleotide degradation resulting from inhibition of oxidative phosphorylation as compared to the effect of 2-deoxyglucose may partly account for the discrepancies. In the present experiments, AMP degradation following inhibition of oxidative phosphorylation by rotenone proceeded approximately 70% by dephosphorylation and 23% by deamination of AMP. On the other hand, the finding that deamination of AMP was the major route of the adenosine nucleotide catabolism induced by 2-deoxyglucose in human lymphoblasts is consistent with previous studies of cultured cells (1-6). The values of 82% deamination and 18% dephosphorylation estimated here are identical to those reported by Lomax and Henderson for Ehrlich ascites cells (4). The small amount of dephosphorylation that did occur may have been the result of partial inhibition of oxidative phosphorylation by the inorganic phosphate depletion caused by 2-deoxyglucose (6). The fact that both hypoxanthine and adenosine accumulated to some extent in every case of degradation suggests that both pathways could operate simultaneously. Treatment with 6-aminonicotinamide plus methylene blue produced an approximately equivalent utilization of both pathways. Cultured human lymphoblasts in a glutamine-supplemented medium were able to maintain their energy supply either by glycolysis alone (when glucose and oxidative phosphorylation inhibitors are present together) or by respiration alone (when glucose and oxidative phosphorylation inhibitors are absent). This is indicated by the lack of nucleotide degradation under either condition. Perhaps the two pathways of degradation allow the cells to respond to perturbations of one or the other pathway of energy production.

The experiments described here may constitute a cell culture model for anoxia in whole tissues and organs. Adenine nucleotide degradation accompanied by a transient accumulation of adenosine has been observed in ischemic rat and rabbit tissues (21-25). The hyperuricemia and gout associated with glycogen storage disease type I has been attributed to increased nucleotide catabolism via AMP deamination (26, 27). AMP dephosphorylation during hypoglycemia may be another mechanism of increased uric acid formation in this disease. In the present experiments, when insufficient ATP was available, normal cells possessing adenosine kinase behaved similarly to adenosine kinase-deficient mutant cells. In the presence of inhibitors of energy metabolism, reductions in both de novo purine synthesis and in the salvage of purine bases occurred, suggesting that the phosphoribosylpyrophosphate concentration was decreased. Thus, a cell possessing normal enzyme activities may become functionally deficient in several of those activities when its energy supply is lowered.

The dephosphorylation of AMP is catalyzed by the enzyme 5'-nucleotidase. The properties of the membrane-associated 5'-nucleotidase (28-32), together with the nucleotide concentrations measured here (Table I), imply that the enzyme is exposed to saturating concentrations of substrate, AMP, and inhibitors ADP and ATP. It should also be mentioned that a soluble form of the enzyme from rat liver has a 100- to 1000-fold lower affinity for AMP, is activated by ATP and GTP, and is inhibited by inorganic phosphate (33). The effect of varying nucleotide pools upon nucleotide degradation was determined (Fig. 4). This experiment shows that AMP degradation proceeded in a similar fashion whether the adenylate pool was at a normal level or about one third that size. In the first phase of stress, the AMP concentration increased up to 3.5-fold. However, in the second phase of stress, the AMP concentration never did rise much above the concentration in the unstressed cells at the start of the experiment, yet the degradation continued even after AMP fell below the starting concentration. This implies that an increased substrate concentration was not necessary for degradation to take place. During the intermediate period of incubation with glucose, the AMP concentration was not more than one-third of its initial value, yet no degradation of the adenylate pool was observed. In the first phase of rotenone stress, degradation began while the ATP concentration was well above one-third of the initial value. Therefore, the relief of ATP inhibition of 5'-nucleotidase cannot be the sole stimulus for the observed effect. The AMP concentration rose during the initial period of nucleotide degradation. Therefore, the relief of AMP inhibition cannot be the sole stimulus for degradation. However, the nucleotide activity may still be responsive to a net effect of simultaneously changing substrate and inhibitor concentrations. The adenylate energy charge may summarize the net effect of the adenine nucleotides on 5'-nucleotidase activity. This hypothesis is consistent with the observation in these experiments that adenylate degradation occurred if, and only if, the energy charge dropped to a value of about 0.6 or lower.
No degradation occurred when the energy charge had a high value no matter what the AMP, ADP, or ATP concentrations were.

What is the metabolic role of adenine nucleotide breakdown during stress of energy metabolism? One proposal is that adenosine released during myocardial hypoxia serves as a coronary vasodilator (21). Another hypothesis is that nucleotide degradation acts to replenish the intracellular inorganic phosphate pools required for glycolytic and oxidative phosphorylations (6). The degradation of deoxynucleotides by the nucleotidase pathway may play a role in the immune response (32). A fourth possible function of adenine nucleotide degradation is the stabilization of the adenylate energy charge (10, 11). Some additional support for the last proposal is given in the present work. In these experiments a decrease in energy charge was associated with nucleotide degradation (Fig. 4) and, for cells deprived of glucose and oxygen, degradation resulted in a re-establishment of normal energy charge values (Fig. 3). Such reciprocal effects are characteristic of a well regulated system (12). Once the energy charge reattained normal values, so did metabolic processes, such as protein synthesis, even though nucleotide pools were reduced (Fig. 5). Similar observations have been made in Escherichia coli cultures (34), in human platelets (20), and in Ehrlich ascites cells (35).

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Adenine nucleotide degradation during energy depletion in human lymphoblasts. Adenosine accumulation and adenylate energy charge correlation.

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