lated to acceleration of heart rate or increase in blood pressure.

From ancient to modern times, medical thinking and folklore shared the notion that sudden death may be provoked by psychological factors (*9*). The present study suggests a model for analyzing the neurophysiologic pathways by which stress may alter electrical stability of the heart.

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## Pyrimidine Starvation Induced by Adenosine in Fibroblasts and Lymphoid Cells: Role of Adenosine Deaminase

Abstract. *In the presence of $10^{-4}$ to $10^{-5}$ molar adenosine, established cell lines of fibroblastic or lymphoid origin die of pyrimidine starvation. Less than lethal concentrations inhibit cell growth. Over a broad concentration range, the effects of adenosine are prevented by providing a suitable pyrimidine source. We suggest that the recently described immune deficiency disease associated with absence of adenosine deaminase may be the result of pyrimidine starvation induced by adenosine nucleotides in cells of the lymphoid system.*

Study of the purine salvage pathways of mammalian cells has shown that the action of the two enzymes adenosine kinase and adenosine deaminase must be precisely balanced. Excessive deamination of adenosine sets in motion the adenosine cycle (*1*), and leads to loss of purines from the cell (*2*). Excessive phosphorylation of adenosine by the kinase results in a lethal interruption of pyrimidine synthesis at a late stage of the biosynthetic pathway (*3*). Ordinarily the adenosine concentration in the cell must be sufficiently low and the activity of each enzyme so regulated as to prevent both of these effects.

The addition of exogenous adenosine to fibroblast cultures leads rapidly to nearly total depletion of the cellular pyrimidine nucleotide pool (Fig. 1). To one of two cultures of 3T6 cells growing exponentially in medium containing 10 percent horse serum [a serum free of adenosine deaminase (*3*)], adenosine was added to $10^{-4}M$. Three hours later, the medium was removed from both cultures, the cell layers were extracted with perchloric acid, and the proteins were removed by centrifugation. The perchlorate was precipitated with dilute KOH, and the supernatant was analyzed quantitatively for nucleotides by high pressure liquid chromatography (Varian Aerograph LCS 1000), and the elution method of Brown (*4*), with minor modifications. Figure 1A shows the nucleotide pattern of the cell extract of control cultures, to which no adenosine was added. All the ribonucleoside diphosphates and triphosphates except cytidine diphosphate (CDP) are readily identified. Figure 1B shows the pattern given by the adenosine-treated cells. The adenosine diphosphate (ADP) and adenosine triphosphate (ATP) peaks are obviously larger, and the uridine diphosphate (UDP), cytidine triphosphate (CTP), and uridine triphosphate (UTP) peaks are drastically reduced.

Table 1 shows the amounts of the nucleoside diphosphates and triphosphates obtained by measurement of the areas under the peaks. For the less abundant nucleotides, larger samples of extract were used so as to increase the accuracy of measurement. The presence of adenosine in the medium led to a 40 to 60 percent expansion of the pools of ADP and ATP; guanosine triphosphate (GTP) was also increased, although guanosine diphosphate (GDP) was not. UDP was reduced by 79 percent, and UTP and CTP were reduced by more than 90 percent. These results are in accord with the conclusion reached earlier that the addition of exogenous adenosine results in an interruption of pyrimidine synthesis in the fibroblast; at adenosine concentrations below $2 \times 10^{-4}M$ this was the only cause of cell death, since the lethality was prevented by the addition of a suitable pyrimidine source (*3*).

The toxicity of adenosine depends on its direct conversion to adenosine monophosphate (AMP) by adenosine kinase (*3*). Adenosine deaminase should have a protective effect, since the deamination reaction is not reversible, and the products—inosine and its free base, hypoxanthine—do not affect cell growth even when present in high concentration (*3*). The recently reported association between absence of adenosine deaminase and a human disease of the lymphoid system manifested by a greatly reduced number of lymphocytes and impaired immunity (*5*) naturally suggested to us that the absence of deaminase in cells of the lymphoid system

Table 1. Nucleoside diphosphate and triphosphate pool in cells exposed to adenosine.

| Nucleotide | 3T6 | | | MGL-5 | | |
|---|---|---|---|---|---|---|
| | Absorbance at 254 nm | | | Absorbance at 254 nm | | |
| | Control | Ar ($10^{-4}M$) | Change (%) | Control | Ar ($2 \times 10^{-5}M$) | Change (%) |
| ADP | 57.9 | 93.5 | + 61 | 5.6 | 8.5 | + 52 |
| GDP | 16.1 | 15.3 | − 5 | 3.7 | 4.0 | + 8 |
| UDP | 32.2 | 6.8 | − 79 | 5.3 | 0.9 | − 83 |
| ATP | 94.8 | 131.5 | + 39 | 26.1 | 53.0 | + 103 |
| GTP | 18.5 | 26.0 | + 40 | 5.3 | 8.0 | + 51 |
| UTP | 22.2 | 2.0 | − 91 | 9.0 | 0.9 | − 90 |
| CTP | 18.4 | 1.5 | − 92 | 3.0 | 0.8 | − 73 |

would result in greatly increased susceptibility to pyrimidine starvation induced by adenosine nucleotides.

To determine the susceptibility of lymphoid cells to exogenous adenosine, we tested its effect on two cultured cell lines of lymphoid origin—L-5178y, a long-established mouse lymphoma line (*6*), and MGL-5, a human lymphoblastoid line recently established from the blood cells of an 18-year-old girl with infectious mononucleosis by Drs. R. Zielki and J. Littlefield of the Massachusetts General Hospital, and kindly provided by Dr. Lee Jacoby. Cultures growing exponentially in medium supplemented with 10 percent horse serum were harvested, and $10^4$ cells were inoculated into fresh medium containing adenosine at various concentrations, with or without $10^{-3}M$ uridine. Table 2 shows that both cell lines were killed by an adenosine concentration of $10^{-5}M$. At $10^{-6}$ to $3 \times 10^{-6}M$ there was marked inhibition of growth, although some cells of normal appearance remained after 8 days, and could be grown out when transferred to medium free of adenosine. It is clear that these lines are at least as sensitive to adenosine as the fibroblasts described earlier (*3*), and probably are more sensitive. The presence of uridine permitted growth of MGL-5 at adenosine concentrations up to $10^{-4}M$, but in the case of L-5178y the uridine was effective only at the lower adenosine concentrations. For the few cell lines tested, $10^{-5}M$ uridine was nearly as effective as $10^{-3}M$ in reversing the effects of adenosine.

The effect of adenosine on the pyrimidine nucleotide pool of the human lymphoblastoid line MGL-5 was also examined by high-pressure liquid chromatography. After 6 hours of exposure to $2 \times 10^{-5}M$ adenosine, the pyrimidine nucleoside diphosphates and triphosphates were reduced to about the same degree as that found for 3T6 fibroblasts (Table 1).

These experiments show that cellular adenosine deaminase is not able to protect cells against pyrimidine starvation induced by even quite low external adenosine concentrations; in at least some species, including the human, cells are evidently protected from exogenous adenosine by a deaminase in the serum (*3*, *7*), and by a very active enzyme in the intestinal epithelium (*8*). On the other hand, only cellular deaminase can protect a cell against its own endogenously generated adenosine nucleotides. Absence of adenosine deaminase might raise adenosine nucleotides to a concentration which, if not lethal, could prevent cells from proliferating. This possibility is of special interest for the immune deficiency disease, since uridine or another pyrimidine source should then have a favorable effect on the disease.

Table 2. Effect of adenosine on two established cell lines of lymphoid origin. Cultures were examined 8 days after inoculation. Abbreviations: G, growth to saturation density; V, little or no growth, but cells appearing viable remained.

| Adenosine ($M$) | L-5178y | | MGL-5 | |
|---|---|---|---|---|
| | No uridine | Uridine ($10^{-3}M$) | No uridine | Uridine ($10^{-3}M$) |
| 0 | G | G | G | G |
| $10^{-7}$ | G | G | G | G |
| $10^{-6}$ | V | G | G | G |
| $3 \times 10^{-6}$ | | | V | G |
| $10^{-5}$ | Killed | V | Killed | G |
| $10^{-4}$ | Killed | Killed | Killed | G |

There remains the problem of why the lymphoid system should be specially sensitive to absence of adenosine deaminase. There exists one other enzyme—AMP deaminase—whose activity would reduce the concentration of adenosine nucleotides. The importance of this enzyme is difficult to estimate (*9*). In some cell types such as muscle, it is very active (*10*), while adenosine deaminase is not (*8*). In other cell types the relative importance of the two enzymes probably varies. Cells of the lymphoid system, which normally have high levels of adenosine deaminase (*8*), may be unable to control their content of adenosine nucleotides with AMP deaminase alone.

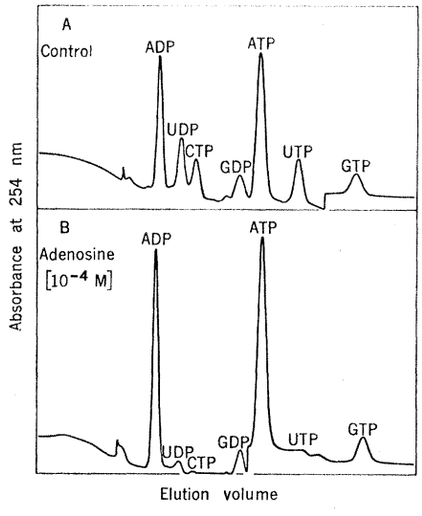


Fig. 1. Depletion of pyrimidine nucleotide pool of growing 3T6 cells by the addition of adenosine. To one of two identical cultures containing $7 \times 10^5$ cells per milliliter of medium, adenosine was added to $10^{-4}M$. After 3 hours, the cell layers were extracted, and equal volumes of extract were analyzed chromatographically.

The consequences of adenosine deaminase deficiency for different cell populations even within the lymphoid system would also depend on the relative growth rates of the populations. We have examined the lethal effects of adenosine only for rapidly growing cells. Slowly growing or nongrowing cells should be much more resistant since they have little or no requirement for pyrimidine synthesis. If a cell type with high growth rate originates from a precursor cell with lower growth rate, the rapidly growing cell type should be more sensitive to elevated levels of adenosine nucleotides than its slowly growing precursor, even if none of the cells of the lineage possessed adenosine deaminase. In this way it might be possible for precursor cells to remain relatively unaffected, and able to give rise to normal progeny if an adequate pyrimidine source were provided.

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