is what consumption might be if no measures were taken to slacken its growth. It is the pattern of the past projected into the future. If anything, it is a low estimate of unrestricted growth, since the growth rate of the few years preceding the embargo was considerably higher than the 3.4 percent average growth rate of the 1950–1973 period. Curve 2 is an estimate, which I adapted from the PIES demand model of the Department of Energy (DOE), of what can be expected as a result of the automobile efficiency standards mandated by Congress and now existing in law (Table 1) together with expected gasoline price increases resulting from market actions, but otherwise no new legislative action. Curve 2 represents a significant decrease in consumption relative to curve 1.

Curve 4 in Fig. 2 is the year-by-year national goal established by President Carter in his energy proposal to Congress (*17*). It calls for 10 percent less gasoline consumption nationwide in 1985 than in 1977. A large gap remains between this goal and the best present forecast of future sales based on existing legislation.

How much additional conservation would the proposed tax stimulate? The elasticity is used to answer this question. Consumption without the tax is taken from curve 2. Price without the tax is extrapolated from the current average price for regular gasoline at full-service retail outlets, estimated [by extrapolating data from the last 4 years (*22*)] to be 66.5 cents per gallon. According to a DOE forecast (C forecast, middle values of supply and demand) the price will rise at nearly 1 percent per year, in 1978 dollars; at the highest, it may rise at an average rate of 1.93 percent (their F forecast, high import price) (*23*). Extrapolation using the two forecasts leads to 1988 prices of 73.5 and 80.5 cents per gallon without tax (123.5 and 130.5 cents per gallon with tax), respectively, bracketing the reasonably expected range of future prices. (Results obtained with lowest price forecast of DOE, their D forecast, are not significantly different from those obtained with the C forecast.)

The savings induced by the tax are shown by year in the lower right corner of Fig. 2. Curve a corresponds to the C forecast and curve b to the F forecast. When these savings are subtracted from the sales without tax, the results are curves 3a and 3b of Fig. 2. The savings induced by the tax are substantial; in 1988, for example, they amount to more than 1 million barrels per day, 14 percent of what would otherwise have been consumed. Futhermore, the savings are not very sensitive to the assumed future price without the tax. Thus, contrary to popular opinion, a tax of the kind proposed can be expected to provide a substantial reduction in gasoline consumption.

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References and Notes

1. S. I. Hayakawa, personal communication.
2. A. M. Schneider, *Energy* **2**, 1 (1977).
3. ———, *Energy Systems and Policy* **1**, 3 (1975).
4. G. E. P. Box and G. M. Jenkins, *Time Series Analysis, Forecasting and Control* (Holden-Day, San Francisco, 1970).
5. J. A. Roberts, unpublished memorandum.
6. *Estimation of 1974 California Monthly Travel Distribution* (California Department of Transportation, Sacramento, 1974).
7. For each month in the forecast interval, the actual price is deflated relative to the price for October 1973 by dividing by the ratio of the deflator for that month to the deflator for October 1973. The result is the "actual real price" relative to the price for October 1973. The quarterly implicit price deflators for the gross national product were obtained from *Survey of Current Business* (Department of Commerce, Washington, D.C., 1976), vol. 56, No. 1, p. 85; No. 4, p. 3.
8. It was assumed that personal income rose in proportion to inflation. Although this was not strictly true month by month, the real disposable income rose almost 2 percent from 1973 through the third quarter of 1976. Thus real income must have risen slower than prices at some times and faster at other times, approximately balancing out overall. The net effect on the elasticity estimate could not be appreciable.
9. P. Verleger and D. Sheehan, *A Study of the Quarterly Demand for Gasoline and Impacts of Alternative Gasoline Taxes* (Data Resources, Inc., Lexington, Mass., 1973).
10. E. Hirst, *Science* **192**, 15 (1976).
11. Alternatively, short- and long-term elasticities were calculated by making a least-squares estimate of β and γ in the dynamic consumption function $y_i = \beta y_{i-1} + \gamma x_i$, where x_i is the fractional change in price and y_i is the fractional change in consumption in month i. The results gave a long-term elasticity $\hat{\gamma}/(1 - \hat{\beta}) = -0.2007$ —essentially the same value; the system responds such that it has essentially reached its long-term value by the end of the fourth month following a step change in price. A copy of this analysis is available on request.
12. *San Diego Regional Carpool Demonstration Project, Quarterly Report* (California Department of Transportation, Sacramento, 1976).
13. "Sin and the diamond lanes," Los Angeles *Times*, 24 June 1976, part II, p. 6.
14. "Fuel crisis not serious, most in poll say," *ibid.*, 21 September 1977, part I, p. 2.
15. *The California Public Appraises the "Energy Crisis" and Steps to Deal with It* (Field Institute, San Francisco, 1977).
16. "55 MPH limit: Many don't care," Los Angeles *Times*, 4 December 1977, part I, p. 1.
17. Executive Office of the President, *The National Energy Plan* (White House, Washington, D.C., 1977), p. 39.
18. *Petroleum Facts and Figures* (American Petroleum Institute, Washington, D.C., 1967), p. 155.
19. *Minerals Yearbook* (Bureau of Mines, Washington, D.C., 1973), p. 934.
20. *Mon. Energy Rev.* (May 1977), p. 12.
21. *Ibid.* (April 1978), p. 10.
22. *Ibid.*, p. 62.
23. *Annual Report to Congress* (Department of Energy, Washington, D.C., 1977), vol. 2, p. 111.
24. *Ibid.*, p. 117.

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S-Adenosylhomocysteine Hydrolase Is an Adenosine-Binding Protein: A Target for Adenosine Toxicity

Abstract. *When adenosine deaminase activity is inhibited, low concentrations of adenosine are toxic to human lymphoblast mutants that are unable to convert adenosine to intracellular nucleotides. In order to identify the mediator of this cytotoxicity, we searched for a cytoplasmic protein capable of binding adenosine with high affinity. Such a protein was identified in extracts of human lymphoblasts and placenta as the enzyme* S*-adenosylhomocysteine hydrolase.*

The toxicity of adenosine to cultured mammalian cells, first described in the early 1960's (*1*), is now of interest largely because of its possible role in causing the severe combined immune defect in children with autosomal recessive deficiency of adenosine deaminase (ADA; E.C. 3.5.4.4) (*2*). Beyond relevance to this specific condition, study of this phenomenon with inhibitors of ADA activity has led to recognition of unexpected interrelationships between aberrant adenosine metabolism and several other metabolic processes. Thus when ADA is blocked, adenosine can induce pyrimidine starvation in cultured cells (*1, 3–5*), can increase adenosine 3′,5′-monophosphate (cyclic AMP) concentrations (*6*), and can interfere with *S*-adenosylmethionine–dependent methylation (*7*) and with the hexose monophosphate pathway of carbohydrate metabolism (*8*). Deoxyadenosine, also a substrate for ADA, has long been known to inhibit DNA synthesis in cultured mammalian cells after conversion to deoxyadenosine 5′-triphosphate, an allosteric inhibitor of ribonucleoside diphosphate reductase (*9–14*). Which of these mechanisms contributes to the immune deficit in ADA-deficient children is still unknown.

In an attempt to identify an intracellular mediator of adenosine toxicity, we searched directly for a protein capable of binding adenosine with high affinity. Two lines of reasoning led to this approach. First, it appears that only small amounts of adenosine may be generated by cultured human lymphoid cells (*15*), and very little adenosine has been found in the plasma and urine of ADA-deficient children (*16*). Indeed, some have proposed that adenosine itself plays no role in causing the immune dysfunc-

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tion in ADA deficiency (*12, 13*). It seemed, however, that even at low concentration, a high-affinity "target" might still account for significant adenosine-mediated effects, particularly since cytotoxic effects have been observed with $10^{-6}M$ adenosine when ADA activity is inhibited. The second reason is related to proposals (*4, 16–20*) that the toxic effects of adenosine derive only from adenine nucleotides to which it is converted via the enzyme adenosine kinase (AK; E.C. 3.7.1.20). We reported previously (*21*) that adenosine remained toxic to adenosine kinase–deficient mutants of the WI-L2 human lymphoblast line, and that adenine remained toxic to other mutants lacking adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) which cannot convert adenine to nucleotides. Exogenous pyrimidines did not reverse growth inhibition caused by adenine, nor that of adenosine to the AK$^-$ mutant, suggesting the presence of some mechanism of

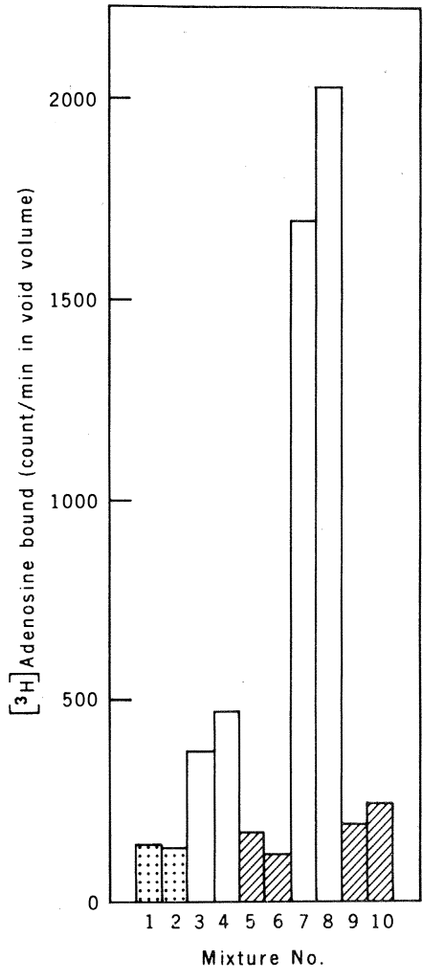

Fig. 1 (left). Assay for binding of [^{3}H]adenosine in lymphoblast and placental extracts. Portions of cell extracts were incubated in 25 m*M* potassium phosphate, *p*H 7.0, 1 m*M* 2-mercaptoethanol, 1 m*M* Na_2-EDTA (the disodium salt of ethylenediaminetetraacetate) (reaction buffer), and [2-^{3}H]adenosine (Amersham), 1 μM, specific activity 5 Ci/mmole in a final volume of 100 μl. After incubation at 30°C for 60 minutes, tubes were placed on ice and 50-μl portions were applied to 0.7 by 20 cm columns of Sephadex G-25 equilibrated with reaction buffer at 4°C. Columns were washed first with 2.5 ml and then 1.3 ml of reaction buffer. The latter fraction, containing the excluded volume (protein), was collected in a scintillation counter vial and mixed with 10 ml of a toluene-based scintillation mixture containing 33 percent by volume Triton X-100 for measuring protein-bound radioactivity. WI-L2 human splenic lymphoblasts were grown and extracts prepared, centrifuged, and dialyzed as described (*21*). An extract was prepared from a freshly obtained human placenta by disrupting a piece of tissue in a Waring blender with a volume of 0.01*M* tris-HCl, *p*H 7.4, 1 m*M* Na_2-EDTA, 5 m*M* 2-mercaptoethanol equal to its weight. The suspension was then centrifuged at 10,000*g* for 30 minutes at 4°C. The supernatant was further purified by chromatography on carboxymethyl and then diethylaminoethyl (DEAE) cellulose (*22*). In the experiments presented in this figure, numbers along the horizontal axis refer to individual [^{3}H]adenosine-binding mixtures containing the following in addition to reaction buffer and 1 μM [^{3}H]adenosine: 1 and 2, no source of binding protein (control); 3 and 4, lymphoblast extract; 5 and 6, lymphoblast extract and 100 μM unlabeled adenosine; 7 and 8, placental extract; 9 and 10, placental extract plus 100 μM unlabeled adenosine. All even-numbered tubes also contained 10 μM EHNA to inhibit ADA activity.

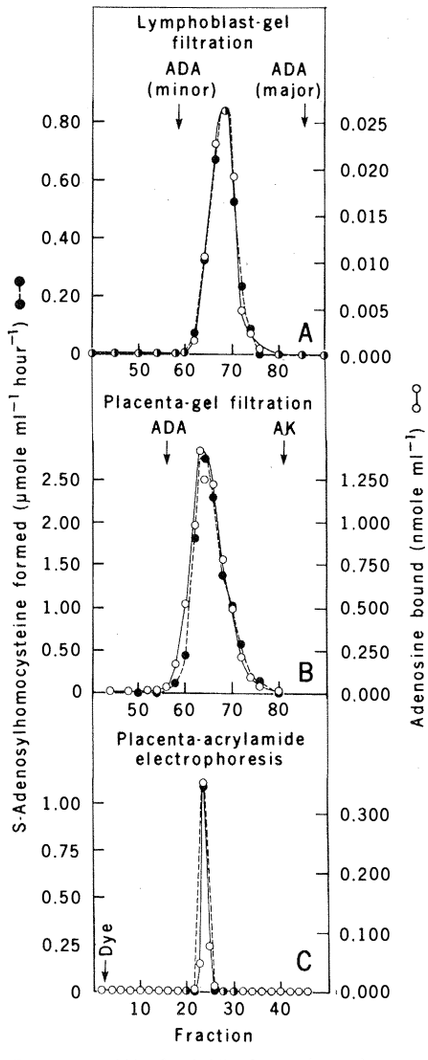

Fig. 2 (right). Coincidence of adenosine-binding and AdoHcy-synthesizing activities during purification procedures. Binding of [^{3}H]adenosine (1 μM) was measured as described for Fig. 1, with 10 μM EHNA present and after incubation at 37°C for 20 minutes. Two assays were used to measure synthesis of AdoHcy. In assay 1 (A), the 0.05-ml reaction mixtures contained 25 m*M* potassium phosphate, *p*H 7.0; 1 m*M* Na_2-EDTA; 1 m*M* 2-mercaptoethanol; 10 μM EHNA; 5 m*M* L-homocysteine (prepared immediately before use by mild alkaline hydrolysis of the thiolactone); and 0.1 m*M* [8-^{14}C]adenosine, 27 mCi/mmole (Amersham). Incubation was for 5 minutes at 37°C followed by addition of 5 μl of 8*M* formic acid. A 20-μl portion was then applied to a cellulose thin-layer chromatography plate (Eastman) along with unlabeled AdoHcy marker and developed in a mixture of butanol, methanol, water, and NH_4OH (60:20:20:1, by volume). The AdoHcy spot was identified under ultraviolet light, cut out, and counted in a toluene-based scintillation fluid. In assay 2 (B and C), the 0.20-ml reaction contained 0.1*M* potassium phosphate, *p*H 7.3; 0.5 m*M* Na_2-EDTA; 1 μM EHNA; 1 m*M* L-homocysteine; and [8-^{14}C]adenosine, 2 mCi/mmole, 0.2 m*M*. After incubation at 37°C for 30 minutes, tubes were heated at 100°C for 2 minutes, cooled, and sufficient purified ADA (0.2 unit, Sigma type I) added to overcome inhibition by EHNA. Tubes were then incubated at 37°C for 10 minutes to convert unutilized adenosine to inosine. Portions (50 μl) were then applied to 2.5 by 2.5 cm squares of Whatman P-81 phosphocellulose paper, which were then washed in three changes of 1 liter of 10 m*M* ammonium formate, *p*H 2.5. Papers were blotted dry and assayed for radioactivity in a toluene-based scintillation fluid containing, by volume, 26 percent Triton X-100 and 7 percent water. (A) A centrifuged extract of WI-L2 lymphoblasts was prepared as described (*21*) but with 1 ml of lysis buffer per 10^8 cells. Portions (4 ml) of the extract were applied to a 2.5 by 100 cm gel filtration column of AcA 34 Ultrogel (LKB). The column was equilibrated and eluted with 25 m*M* tris-HCl, *p*H 7.4, 5 m*M* 2-mercaptoethanol, 1 m*M* Na_2-EDTA, and 25 m*M* KCl. Arrows indicate the positions of elution of the peaks of ADA activity [assayed as described (*36*)] in the extract. Protein (absorbance at 280 nm) was found in fractions 30 through 95 but, for clarity, is not shown. (B) A partially purified preparation of human placental adenosine-binding protein was concentrated and applied to the gel filtration column described for (A) and eluted in a similar fashion. Arrows indicate the position of ADA and AK (*21*) present in the material applied to the column. (C) A more extensively purified, but not homogeneous, fraction of the preparation of placental binding protein used in (B) was subjected to analytical polyacrylamide gel electrophoresis (*37*). The gel cylinder was cut into 2-mm slices which were extracted into 25 m*M* tris-HCl, *p*H 7.4; 5 m*M* 2-mercaptoethanol; 1 m*M* EDTA. Portions were then assayed for adenosine-binding and AdoHcy-synthesizing activities.

Table 1. Inhibition of [^{3}H]adenosine binding by purine compounds. Partially purified preparations of adenosine-binding protein from lymphoblast and placenta were incubated with 1 μM [^{3}H]adenosine and the indicated test compounds at 37°C for 20 minutes. Binding of [^{3}H]adenosine was measured as described in Fig. 1. In all experiments 10 μM EHNA was present. Results are expressed as percentage of inhibition relative to control (no additions).

Compound added	Concentration (μM)	Inhibition of [^{3}H]adenosine binding (%)
Lymphoblast extract		
Inosine	10	−13
Adenine	10	66
S-Adenosylhomocysteine	10	69
Placental extract		
Adenine	50	73
S-Adenosylhomocysteine	50	80

growth inhibition that was not due to the well-known ability of these purines to inhibit pyrimidine biosynthesis via conversion to adenine nucleotides. We proposed (*21*) the existence of an adenine nucleotide–independent mechanism of toxicity that might be mediated by purine receptors or by intracellular enzymes that could interact with adenosine and adenine. We have now identified such a high-affinity adenosine- and adenine-binding protein in human tissues as the enzyme *S*-adenosylhomocysteine (AdoHcy) hydrolase (E.C. 3.3.1.1), a result consistent with other evidence pointing to involvement of this enzyme in mediating adenosine cytotoxicity (*7*).

We assayed adenosine binding by incubating cell extracts with [^{3}H]adenosine and then passing portions of the reaction mixtures over Sephadex G-25 gel filtration columns to separate bound from unbound adenosine. As shown in Fig. 1, a 100-fold excess of unlabeled adenosine abolished binding of 1 μM [^{3}H]adenosine by extracts of human lymphoblasts and human placenta. The radioactivity appearing in the G-25 excluded volume was increased slightly by erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA), an ADA inhibitor, suggesting that the binding activity was not due to ADA. When crude extracts of these tissues (subjected only to low-speed centrifugation) were fractionated by gel filtration chromatography (shown in Fig. 2A for a lymphoblast extract and in Fig. 2, B and C, for later stages in purification of the placental protein), all the binding activity was accounted for in a single peak. The binding activity is distinct from AK as well as ADA activities, but coelutes with an activity which converts L-homocysteine and adenosine to AdoHcy. The results in Tables 1 and 2 are also consistent with both adenosine-binding and AdoHcy-synthesizing activities residing in the same protein, and indicate that adenine is also bound at the same site as adenosine. Thus adenosine binding is inhibited by both AdoHcy and adenine (Table 1), and adenine also inhibits AdoHcy synthesis competitively with adenosine (Table 2). The dissociation constant (K_D) of the lymphoblast enzyme for adenosine is about $5 \times 10^{-7} M$ (Fig. 3A) and its Michaelis constant (K_m) for adenosine in the synthesis of AdoHcy is about $1 \times 10^{-6} M$ (Fig. 3B). Similar values have been found with the placental protein which has recently been purified to apparent homogeneity (based on analytical ultracentrifugation and polyacrylamide gel electrophoresis under denaturing conditions) and has retained both AdoHcy hydrolase activity and high-affinity binding of adenosine in a constant ratio (*22*).

Adenosine-binding proteins have been purified extensively from rabbit erythrocytes (*23*) and mouse liver (*24*), but enzymatic activity has thus far not been associated with either, though AdoHcy hydrolase was not assayed. These proteins have subunit molecular weights similar to the value we find for homogeneous placental AdoHcy hydrolase [47,000 to 48,000 (*22*)], in addition to similar affinity for adenosine. Both the liver and erythrocyte proteins were reported to bind ^{3}H-labeled cyclic AMP, but in amounts less than the amount of adenosine bound. Adenosine blocked cyclic AMP binding, but the reverse did not occur. We find similar results with placental AdoHcy hydrolase. It seems reasonable to suggest that preparations of homogeneous mouse liver and rabbit erythrocyte adenosine-binding proteins be assayed for AdoHcy hydrolase activity.

Table 2. Inhibition of synthesis of AdoHcy by adenine. Partially purified lymphoblast extract was the source of enzyme activity. The AdoHcy synthesis was measured with assay 1 described in Fig. 2.

Substrate ([^{14}C]adenosine) concentration (μM)	Inhibitor (adenine) concentration (μM)	Inhibition of ^{14}C-labeled *S*-adenosylhomocysteine synthesis (%)
5	5	1.6
5	25	48.4
5	100	78.0
50	100	−3.3
50	500	59.2
50	1000	75.1

S-Adenosylhomocysteine is normally produced in a number of intracellular reactions in which the methyl group of *S*-adenosylmethionine is transferred to a variety of specific acceptor molecules, including residues in newly synthesized DNA and RNA, carbohydrates, and certain amino acid residues in proteins, and many small molecules. Many of the specific methylases which catalyze these reactions are subject to inhibition by AdoHcy with inhibition constants (K_i) in the range of 0.3 to 20 μM (*25–30*). To ensure that normal methylation occurs, it is

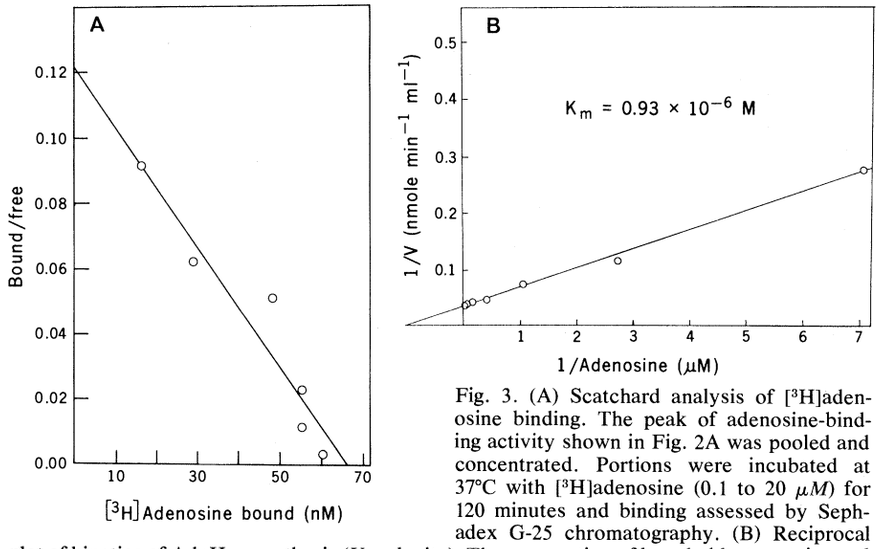

Fig. 3. (A) Scatchard analysis of [^{3}H]adenosine binding. The peak of adenosine-binding activity shown in Fig. 2A was pooled and concentrated. Portions were incubated at 37°C with [^{3}H]adenosine (0.1 to 20 μM) for 120 minutes and binding assessed by Sephadex G-25 chromatography. (B) Reciprocal plot of kinetics of AdoHcy synthesis (V, velocity). The preparation of lymphoblast protein used in (A) was assayed for AdoHcy synthesis activity according to the method for assay 1 (Fig. 2A) with 5 mM homocysteine. Incubation was for 1 to 5 minutes.

essential that AdoHcy be removed as it is formed, a function performed in mammalian cells by AdoHcy hydrolase. However, the equilibrium constant (K_{eq}) for hydrolysis greatly favors AdoHcy synthesis from adenosine and L-homocysteine, the products of AdoHcy cleavage (*31*). Under physiologic conditions AdoHcy hydrolysis proceeds because these products are efficiently removed, preventing the thermodynamically favored accumulation of AdoHcy.

Kredich and Martin (*7*) have shown that in ADA-inhibited mouse lymphoblasts, toxic concentrations of adenosine in fact caused both a marked accumulation of AdoHcy and inhibition of DNA methylation, findings we have extended in studies with human lymphoblasts (*32*). The toxicity of adenine may also be related to inhibition of AdoHcy cleavage. Studies showing that adenosine blocks ribosomal RNA maturation in a human plasma cell line also point to inhibition of methylation as a mechanism for its cytotoxicity (*33*). We believe that the affinity of AdoHcy hydrolase for adenosine may be an important factor in determining in which tissues AdoHcy might accumulate when other routes of adenosine metabolism are interrupted. In this regard it is interesting that the Michaelis constants reported for both the rat and beef liver enzymes for adenosine are from 0.4 to 1.5 m*M* (*34, 35*), in marked contrast to those of the human placental and lymphoblast enzymes, ~1 μM.

Whether or not accumulation of AdoHcy and inhibition of methylation contribute to the immune dysfunction in ADA-deficient individuals must await studies of affected individuals. Nevertheless, understanding of the regulation of nucleic acid and other methylase reactions is of considerable importance to several areas of investigation, including control of differentiation, gene expression, viral replication, and neoplastic transformation. Tight binding of adenosine to AdoHcy hydrolase in some tissues but not others may permit selective methods for modifying growth of tumor or virus-infected cells, and may provide a means for better defining the importance of various methylation reactions.

Note added in proof: We have recently found that adenosine-binding activity in extracts of mouse liver copurifies with AdoHcy hydrolase activity.

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References and Notes

1. L. Aranow. *Biochim. Biophys. Acta* **47**, 184 (1961).
2. E. R. Giblett, J. E. Anderson, F. Cohen, B. Pollara, H. J. Meuwissen, *Lancet* **1972-II**, 1067 (1972).
3. H. Hilz and E. Kaukel, *Mol. Cell. Biochem.* **1**, 229 (1973).
4. H. Green and T.-S. Chan, *Science* **182**, 836 (1973).
5. F. F. Snyder and J. E. Seegmiller, *FEBS Lett.* **66**, 102 (1976).
6. G. Wolberg, T. P. Zimmerman, K. Hiemstra, M. Winston, L.-C. Chu, *Science* **187**, 957 (1975).
7. N. M. Kredich and D. W. Martin, Jr., *Cell* **12**, 931 (1977).
8. F. F. Snyder, M. S. Hershfield, J. E. Seegmiller, *Cancer Res.* **38**, 2357 (1978).
9. H. Klenow, *Biochim. Biophys. Acta* **35**, 412 (1959).
10. E. C. Moore and R. B. Hurlbert, *J. Biol. Chem.* **241**, 4802 (1966).
11. D. A. Carson, J. Kaye, J. E. Seegmiller, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5677 (1977).
12. H. A. Simmonds, G. S. Panayi, V. Corrigall, *Lancet* **1978-I**, 60 (1978).
13. A. Cohen, R. Hirschhorn, S. D. Horowitz, A. Rubinstein, S. H. Polmar, R. Hong, D. W. Martin, Jr., *Proc. Natl. Acad. Sci. U.S.A.* **75**, 472 (1978).
14. M. S. Coleman, J. Donofrio, J. J. Hutton, L. Hahn, A. Daoud, B. Lampkin, J. Dyminski, *J. Biol. Chem.* **253**, 1619 (1978).
15. M. S. Hershfield and J. E. Seegmiller, *ibid.* **252**, 6002 (1977).
16. G. C. Mills, F. C. Schmalstieg, K. B. Trimmer, A. S. Goldman, R. M. Goldblum, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2867 (1976).
17. M. W. McBurney and G. Whitemore, *J. Cell. Physiol.* **85**, 87 (1974).
18. B. Ullman, A. Cohen, D. W. Martin, Jr., *Cell* **9**, 205 (1976).
19. S. H. Polmar, R. C. Stern, A. L. Schwartz, E. M. Wetzler, P. A. Chase, R. Hirschhorn, *N. Engl. J. Med.* **295**, 1337 (1976).
20. R. P. Agarwal, G. W. Crabtree, R. E. Parks, Jr., J. A. Nelson, R. Keightley, R. Parkman, F. S. Rosen, R. C. Stern, S. H. Polmar, *J. Clin. Invest.* **57**, 1025 (1976).
21. M. S. Hershfield, F. F. Snyder, J. E. Seegmiller, *Science* **197**, 1284 (1977).
22. M. S. Hershfield, *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 1466 (1978); in preparation.
23. K.-C. M. Yuh and M. Tao, *Biochemistry* **13**, 5220 (1974); R. A. Olsson, *ibid.* **17**, 367 (1978).
24. P. M. Ueland and S. O. Doskeland, *J. Biol. Chem.* **252**, 677 (1977); *ibid.* **253**, 1667 (1978).
25. J. Hurwitz, M. Gold, M. Anders, *ibid.* **239**, 3474 (1964).
26. V. Zappia, C. R. Zydek-Cwick, F. Schlenk, *ibid.* **244**, 4499 (1969).
27. A. E. Pegg, *FEBS Lett.* **16**, 13 (1971).
28. J. Hildeshein, R. Hildeshein, E. Lederer, *Biochimie* **54**, 989 (1972).
29. S. J. Kerr, *J. Biol. Chem.* **47**, 4284 (1972).
30. J. M. Glick, S. Ross, P. S. Leboy, *Nucleic Acids Res.* **2**, 1639 (1975).
31. G. De la Haba and G. L. Cantoni, *J. Biol. Chem.* **234**, 603 (1959).
32. N. M. Kredich and M. S. Hershfield, in preparation.
33. J. W. Bynum and E. Volkin, *J. Cell. Physiol.* **88**, 197 (1976).
34. R. D. Walker and J. A. Duerre, *Can. J. Biochem.* **53**, 312 (1975).
35. J. L. Palmer and R. H. Abeles, *J. Biol. Chem.* **251**, 5817 (1976); H. H. Richards, P. K. Chiang, G. L. Cantoni, *ibid.* **253**, 4476 (1978).
36. F. F. Snyder, J. Mendelsohn, J. E. Seegmiller, *J. Clin. Invest.* **58**, 654 (1976).
37. B. J. Davis, *Ann. N.Y. Acad. Sci.* **121**, 404 (1964).
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Entry of Insulin into Human Cultured Lymphocytes: Electron Microscope Autoradiographic Analysis

Abstract. *Electron microscope autoradiographs were prepared of IM-9 human cultured lymphocytes incubated with iodine-125-labeled insulin. With the use of [^{125}I]insulin and Ilford L-4 emulsion, the technique had a resolution half-distance of approximately 0.085 micrometer. Autoradiographs revealed a time-dependent entry of insulin into the cell interior that was maximal after 30 minutes of incubation. At this time point nearly 40 percent of the [^{125}I]insulin was in the interior of the cell at a distance 1 micrometer or greater from the plasma membrane. Grain distribution and volume density analyses revealed that the intracellular insulin was concentrated in the endoplasmic reticulum and nuclear membrane.*

Studies at this laboratory in which IM-9 human cultured lymphocytes were incubated with [^{125}I]insulin and then examined by either cell fractionation or light microscope autoradiography, have indicated that insulin can enter the interior of these cells and bind to the nucleus (*1*). These techniques, however, lack the sensitivity to define precisely where insulin is located in the cell. Because of their greater sensitivity, electron microscope autoradiographs have the potential of defining more precisely the intracellular organelles with which insulin or other hormones interact. Only recently have analytical techniques become available for quantitating electron microscope autoradiographs of labeled hormones (*2*). An important advance has been the development by Salpeter *et al.* of grain density histograms to determine the resolution of ^{125}I or other isotopes (*2*). Accordingly, we have prepared and analyzed electron microscope autoradiographs of human cultured lymphocytes incubated with [^{125}I]insulin.

Previously, we demonstrated that when IM-9 human cultured lymphocytes are incubated with [^{125}I]insulin in a complete culture medium supplemented with 10 percent fetal calf serum at 37°C the cellular uptake of insulin is maximal within 1 minute, and a steady state can be maintained for 90 minutes or longer (*1*). Under these conditions, cell viability remains greater than 97 percent of total,

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