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* A.J.D. is a visiting scientist on leave from the Universidad Catolica de Chile, Santiago, Chile.

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The Human Genes for *S*-Adenosylhomocysteine Hydrolase and Adenosine Deaminase Are Syntenic on Chromosome 20

Abstract. *Human–Chinese hamster cell hybrids and a monoclonal antibody to human* S-*adenosylhomocysteine hydrolase were used to identify chromosome 20 as the location of the human gene for this enzyme. The gene for adenosine deaminase had previously been mapped to this chromosome. The activity of* S-*adenosylhomocysteine hydrolase is dependent in vivo on that of adenosine deaminase, since the substrates for the deaminase, adenosine and deoxyadenosine, respectively, inhibit and inactivate* S-*adenosylhomocysteine hydrolase in genetic or drug-induced adenosine deaminase deficiency. This functional dependence and the likelihood that* S-*adenosylhomocysteine hydrolase, a eukaryotic enzyme, arose later than adenosine deaminase, which occurs in prokaryotes as well as eukaryotes, suggest that the occurrence of their genes on the same chromosome may have evolutionary significance. In addition, the unusual capacity of* S-*adenosylhomocysteine hydrolase to form stable complexes with adenosine and its cofactor, nicotinamide adenine dinucleotide, suggest that evolution of its gene may have involved recombination of a portion of the adenosine deaminase gene with an adenine nucleotide domain–coding sequence of another preexisting gene.*

In genetic deficiency of adenosine deaminase (ADA; E.C. 3.5.4.4) (*1*), accumulation of adenosine and 2′-deoxyadenosine results in profound depletion of lymphoid tissues and severe combined immunodeficiency disease. Investigation into the biochemical effects of these purine nucleosides has led to recognition that the activity of a second enzyme involved in the metabolism of adenosine, *S*-adenosylhomocysteine (AdoHcy) hydrolase (E.C. 3.3.1.1), is dependent in vivo on that of ADA. *S*-Adenosylhomocysteine hydrolase catalyzes the reversible hydrolysis of AdoHcy to adenosine and L-homocysteine (*2*). This reaction is important because AdoHcy is a potent inhibitor, as well as a product, of *S*-adenosylmethionine–dependent transmethylation, a step that occurs in the biosynthesis, modification, or regulation of the function of nucleic acids, proteins, phospholipids, and biogenic amines (*3*) (see Fig. 1). Cleavage of AdoHcy is the only source of homocysteine, which is used in the synthesis of the essential amino acid methionine in a reaction that also produces tetrahydrofolate, a cofactor for several enzymes participating in the synthesis of nucleic acid precursors.

Two mechanisms account for the dependence of the activity of AdoHcy hydrolase on that of ADA. First, because the equilibrium constant for AdoHcy hydrolysis is highly unfavorable (*2*), further metabolism of adenosine or homocysteine is required to promote AdoHcy cleavage and prevent its accumulation. In cultured lymphoid cells treated with ADA inhibitors, AdoHcy accumulation, causing inhibition of transmethylation reactions, is largely responsible for the toxic effects of adenosine in the concentration range 5 to 50 μ*M* (*4*, *5*). In addition to the inhibitory effect of adenosine, we have shown that deoxyadenosine is a "suicide-like" substrate analog that causes the inactivation of purified AdoHcy hydrolase (*6*), and of AdoHcy hydrolase in ADA-inhibited cultured cells (*7*). This effect of 2′-deoxyadenosine results in severe deficiency of AdoHcy hydrolase activity in the erythrocytes of ADA-deficient children (*8*), and in the erythrocytes and lymphoblasts of patients with lymphocytic leukemias when they are treated with the drug 2′-deoxycoformycin, a potent inhibitor of ADA that causes deoxyadenosine accumulation in vivo (*9*, *10*).

We now report that the genes for ADA and AdoHcy hydrolase are located on the same human chromosome. We mapped the location of AdoHcy hydrolase by using a mouse monoclonal antibody directed against human placental AdoHcy hydrolase (*11*) to develop an immunoprecipitation method for detecting human AdoHcy hydrolase in human-hamster hybrid cell lines that contain different subsets of human chromosomes. Somatic cell hybrids and monoclonal antibodies have recently been used to map the genes for human liver and muscle isozymes of 6-phosphofructokinase (*12*).

The monoclonal antibody designated

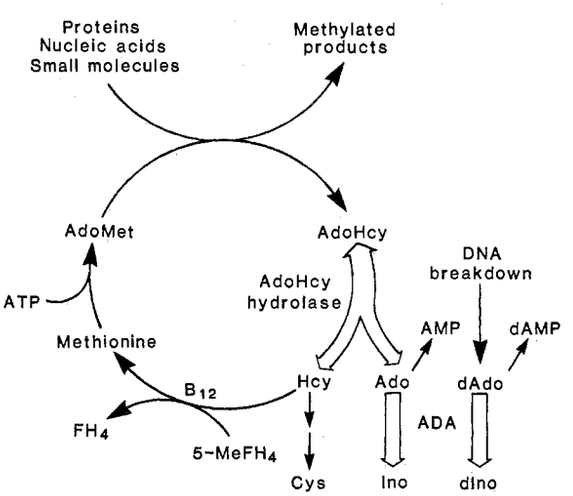

Fig. 1. Metabolic functions of AdoHcy hydrolase and ADA. Abbreviations: *AdoMet*, *S*-adenosylmethionine; FH_4, tetrahydrofolate; $5\text{-}MeFH_4$, 5-methyltetrahydrofolate; B_{12}, vitamin B_{12}; *Hcy*, homocysteine; *Cys*, cysteine; *Ado*, adenosine; *Ino*, inosine; *dAdo*, 2′-deoxyadenosine; *dIno*, 2′-deoxyinosine; *AMP*, adenosine monophosphate; *dAMP*, 2′-deoxyadenosine monophosphate; *ADA*, adenosine deaminase; and *AdoHcy*, *S*-adenosylhomocysteine.

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Table 1. Immunoprecipitation of AdoHcy hydrolase activity from Chinese hamster–human hybrid cell extracts by monoclonal antibody to human AdoHcy hydrolase. Pellets of Chinese hamster–human cell hybrids were lysed by freezing and thawing three times in 0.15 ml of 10 m*M* tris-HCl (*p*H 7.0) and 1 m*M* EDTA, and the lysates were centrifuged for 2 minutes in a Microfuge (Beckman). Portions (20 μl, 0.13 to 0.25 mg of protein) of each extract were incubated with 2.7 μg of B2A3 monoclonal antibody, the immune complexes were precipitated with staphylococcus and washed, and the staphylococcal pellet was assayed for AdoHcy hydrolase (assay tubes were incubated for 30 minutes). Controls were duplicate portions of the cell extracts treated similarly, but without anitbody; in no case was more than 0.5 percent of AdoHcy hydrolase activity precipitated. Human AdoHcy hydrolase was considered to be present if more than 5 percent of the total activity was recovered in the pellet. The presence or absence of human ADA in hydrid cell extracts was determined electrophoretically (*14*). Information regarding the human chromosome complement of the hydrid cell lines is given in Table 2; N.D., not done

Hybrid	AdoHcy hydrolase activity		Presence of human AdoHcy hydrolase	Presence of human ADA
	Total in incubation (nmole/hour)	In pellet (%)		
X-7A	62.2	29.8	+	+
XII-2D	82.0	0.1	−	−
XII-12B	143.7	0.3	−	−
XIII-1A	116.7	21.5	+	+
XIII-1B	76.1	27.8	+	+
XIII-3A	178.9	1.4	−	−
XIII-4D-1c	60.0	0.5	−	−
XV-15A-4a	70.8	16.2	+	+
XV-16B	114.3	20.7	+	N.D.
XV-16B aza	61.2	13.2	+	+
XV-18A-8b	61.9	30.3	+	N.D.
XV-18B-6a	49.3	39.6	+	+
XV-18B-7a	83.8	41.4	+	+
XXI-51B	95.3	8.9	+	+

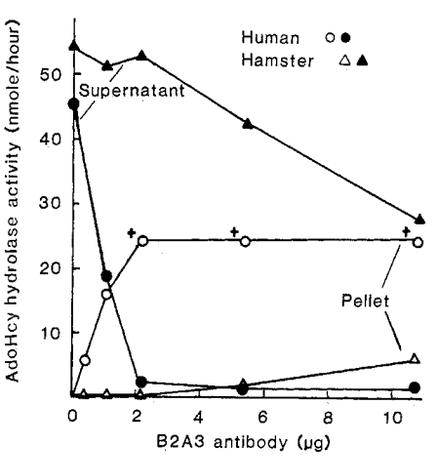

Fig. 2. Immunoprecipitation of human and Chinese hamster AdoHcy hydrolase. Mouse monoclonal antibodies directed against human AdoHcy hydrolase were produced by hydridizing spleen cells from an immune mouse with the P3X63Ag8 mouse myeloma cell line (*28*). Details of the immunization with purified human placental AdoHcy hydrolase, fusion and cloning techniques, screening assay, production of antibody-rich ascites fluid, and additional characterization of the antibodies obtained are described (*11*). Suspensions [2×10^8 cells per milliliter of 25 m*M* tris-HCl (*p*H 7.4), 1 m*M* EDTA, and 0.5 percent Nonidet P-40] of the human T-lymphoblastoid cell line CEM and the Chinese hamster lung fibroblast cell line 743 were frozen and thawed three times. After centrifugation (2 minutes in a Beckman Microfuge, 4°C), 20-μl portions of the supernatants were mixed with 0 to 50 μl of B2A3 monoclonal antibody to human placental AdoHcy hydrolase in phosphate-buffered saline (1:100 dilution), to give the amounts of antibody protein indicated on the abscissa. Phosphate-buffered saline was added to bring all volumes to 100 μl; tubes were incubated at 23°C for 90 minutes, after which 50 μl of a 10 percent suspension of heat-killed, Formalin-fixed staphylococcus (*13*) was added. After an additional 30 minutes, the tubes were centrifuged in a Microfuge (2 minutes) to precipitate the immune complexes bound to the staphylococcus. Portions (10 μl) of supernatants were used to assay AdoHcy hydrolase activity [in the direction of AdoHcy synthesis from [^{14}C]adenosine and L-homocysteine (*6, 26*)]. The remaining supernatants were aspirated and the pellets were washed three times in "staph buffer" (*29*). The washed pellets were then suspended in 42.5 μl of 29.4 m*M* potassium phosphate, *p*H 7.0; 1.2 m*M* EDTA; 1.2 m*M* dithiothreitol; 1.2 μ*M* 2′-deoxycoformycin; and 5.9 m*M* L-homocysteine. Then 7.5 μl of 1 m*M* [8-^{14}C]adenosine, 7600 cpm/nmole, was added and the tubes were incubated for 5 minutes at 37°C. Reactions were stopped by cooling on ice; the tubes were centrifuged; and 5 μl of supernatant was subjected to thin-layer chromatography to measure the [^{14}C]AdoHcy formed (*6, 26*). The symbol + indicates that the estimate of activity is approximate because the reaction had gone to completion.

Table 2. Assignment of gene for AdoHcy hydrolase to chromosome 20 by concordant segregation in a panel of Chinese hamster–human hybrids.

Hybrid*	Human AdoHcy hydrolase	Human chromosome (mean copy number per cell)																					
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
X-7A	+	1.0	0.5	1.5	1.0	0.9	1.4	0.4	0.9	0.9	0.7	1.2	1.4	1.3	0.5	1.0	0.4	0.3	1.1	0.5	0.9	0.8	0.9	0.7
XII-2D	−	0.8	0.4	1.5	1.0		1.1		1.0	0.5			0.8	1.0	0.9	1.3	0.9		1.2	0.9		1.0	1.0	1.0
XII-12B	−	0.8		1.0	1.0		1.0		0.8				0.8		0.9	0.9	0.9		1.6	1.0		0.9	0.8	
XIII-1A	+				1.0				0.7	0.9		1.0			0.2	0.9	0.6		0.5		1.0	0.9	0.8	1.0
XIII-1B	+													0.8	0.4†									0.4†
XIII-3A	−							0.5			0.1				0.8		0.5				0.6		0.3	0.9
XIII-4D-1c	−			0.5			0.9																	
XV-15A-4a	+	0.2‡	0.6	0.8	0.8		0.2‡	0.7	0.9		0.4	1.2	1.0	0.5	1.0		1.2	0.5	0.5		0.8	0.3	0.8	0.9
XV-16B	+	0.9§	0.7	1.0	1.0	0.9	0.9	1.0	0.8		0.8	1.0	0.8	1.0	1.0	0.9	1.0		1.7	0.9	0.7	1.0	1.0	1.0
XV-16B aza	+	1.0§	0.7	1.8		0.8	0.9		0.6					0.6	1.6		0.7		0.7	0.8	0.6	0.9	0.8	
XV-18A-8b	+	1.0§	0.7	0.9	0.9	1.0							0.9		1.6	0.9	1.0	0.7		0.8	0.5	1.4	0.7	
XV-18B-6a	+	0.8	0.9	1.4	0.9	0.9	0.9	1.0		0.9	0.7	1.0	1.9	0.7	0.9	1.5	1.0		0.6	0.9	1.6	1.1	1.5	1.0
XV-18B-7a	+	1.0§	0.7	1.1		0.9	1.0§		0.8	0.9			0.9		0.8	0.9	0.8	0.8	0.8	0.7	0.9	0.9	1.2	0.8
XXI-51B	+			0.9	0.7	0.6		1.3	0.8	0.2	0.6	0.7			0.7	0.8	0.8	0.2	0.7	0.7			0.9	
Ratio discordant‖		5/8	4/13	5/13	5/13	3/13	7/11	6/13	5/13	6/13	5/12	4/13	6/13	3/12	5/13	5/13	4/13	5/13	4/13	5/13	0/13	4/13	4/13	5/13
Percent discordant		63	31	38	38	23	64	46	38	46	42	31	46	46	25	38	31	38	31	38	0	31	31	38

*The derivation of the different series of hybrids has been summarized in (*30*). †der(X), t(X;14)(p22;q21). ‡der(1), t(1;6)(p32;p21). §der(6), t(1;6)(p32;p21). ‖Hybrids in which only parts of relevant chromosomes were present, or in which they were present in less than 15 percent of cells, were excluded from calculations of discordancy rates.

B2A3, in the presence of heat-killed, Formalin-fixed staphylococcus (*13*), was used to precipitate the AdoHcy hydrolase activity from an extract of human lymphoblastoid cells (Fig. 2). About 60 to 80 percent of the activity removed by centrifugation was recovered in the staphylococcal pellet. Approximately ten times more B2A3 antibody was required to precipitate an equivalent amount of AdoHcy hydrolase activity from an extract of Chinese hamster cells. However, a somewhat lower percentage of hamster activity was recovered in the pellet, probably reflecting release of the more weakly bound hamster AdoHcy hydrolase in the process of washing the pellet before assay. Neither the staphylococcal suspension nor the B2A3 antibody alone inhibited either human or hamster AdoHcy hydrolase activity in solution (not shown). With the proper concentration of antibody (indicated by the arrow in Fig. 2), the presence of the human enzyme is easily determined in mixtures containing various amounts of human and hamster activities by assaying for activity in washed staphylococcal pellets (not shown).

For mapping studies, we used a series of hybrid cell lines obtained from fusions of the Chinese hamster lung fibroblast cell line V-79 (similar to line 743) with either human fibroblasts, or human blood leukocytes. The 14 hybrid clones examined were derived from four different human donors. Each hybrid clone was karyotyped at a passage close to the one at which cells were harvested for enzyme studies. The frequency of occurrence of each human chromosome was determined by analysis of 10 to 35 trypsin-Giemsa–banded metaphase spreads. Extracts of the hybrid clones were analyzed for immunoprecipitable AdoHcy hydrolase with the B2A3 monoclonal antibody (Table 1), and the results were correlated with the human chromosome contents of the hybrids (Table 2).

Clone X-7A was used as a positive hybrid control, since it contains at least one copy of all human chromosomes. Roughly 30 percent of the AdoHcy hydrolase activity in the extract of this hybrid was precipitated by antibody to human AdoHcy hydrolase. In the 13 hybrids that were informative for mapping, human AdoHcy hydrolase expression segregated concordantly with chromosome 20. For all other autosomes and the X chromosome, the fraction of discordant clones was 23 percent or greater (Table 2). The assignment of the gene for AdoHcy hydrolase (for which we suggest the abbreviation *AHCY*) to chromosome 20 was further confirmed by demonstrating concordant segregation with *ADA* [cellulose acetate electrophoresis (*14*) being used to identify the presence of the human ADA isozyme in extracts of the hybrid clones] (Table 1). The gene for ADA has been mapped to the distal long arm of chromosome 20 (*15*).

Information on the distance between *ADA* and *AHCY* is not yet available. Their synteny could be due to chance alone. The probability of a gene occurring on chromosome 20 is 1 in 22 if only the haploid number of human autosomes is considered, but since chromosome 20 contains only about 2.4 percent of the total haploid autosomal length (*16*), the chance of any locus occurring on chromosome 20 is about 1 in 40.

There is abundant evidence of the conservation during evolution of amino acid sequences in the enzymes that catalyze the same metabolic reactions in prokaryotes and eukaryotes (*17*). However, the organization into linkage groups of the genes for enzymes that function sequentially in metabolic pathways, a frequent occurrence in prokaryotic chromosomes, is uncommon in eukaryotes. Apparently gene linkage has usually not conferred much additional selective advantage over conservation of the DNA coding sequences themselves. There are examples of probable gene linkages for the multifunctional proteins that contain the first three (*18–20*) and the last two enzyme activities of pyrimidine biosynthesis (*21, 22*). The genes coding for the third and sixth enzymes in the pathway of de novo purine nucleotide synthesis have been reported to map to human chromosome 21 (*23*). In the latter instance, there appears to be coordinate expression of these genes, which code for separate polypeptide chains. Two possibilities suggest themselves as factors that might be responsible for nonrandom occurrence of the genes for ADA and AdoHcy hydrolase on the same chromosome.

First, there is evidence that in the absence of ADA, its substrates, adenosine and deoxyadenosine, respectively, inhibit (*2, 4, 5*) and inactivate (*6–8*) intracellular AdoHcy hydrolase. Adenosine deaminase appears to have evolved before AdoHcy hydrolase, since ADA occurs in prokaryotes as well as eukaryotes, but AdoHcy hydrolase occurs only in the latter (*3*). Evolution of a catalytically efficient AdoHcy hydrolase, which presumably required a long time, may have first occurred in a cell in which the simultaneous presence of ADA activity was ensured by linkage of the ADA and evolving AdoHcy hydrolase genes.

The second, but not necessarily independent, possibility has to do with some unusual properties of AdoHcy hydrolase, which suggest that its evolution in eukaryotes may have involved the fusion of specialized domain–coding regions of preexisting genes for other proteins. *S*-Adenosylhomocysteine hydrolase is a tetramer with subunits of molecular weight 46,000 to 50,000 that contain one molecule of tightly, but not covalently, bound nicotinamide adenine dinucleotide (NAD^+) per subunit (*24–26*); no tissue-specific isozymes have been reported. In addition to its catalytic function, we have shown (*26*) that AdoHcy hydrolase can form a stable complex with adenosine (dissociation constant, $2 \times 10^{-7} M$ to $5 \times 10^{-7} M$), and have identified it as the cytoplasmic high-affinity "cyclic AMP–adenosine binding protein" (cyclic AMP is adenosine 3′, 5′-monophosphate) that had been isolated from various sources (*27*). The significance of this capacity of AdoHcy hydrolase to bind adenosine and cyclic AMP remains unclear. It is possible that a region of the AdoHcy hydrolase gene arose by tandem reduplication of, or recombination with, a portion of the ADA gene coding for an adenosine binding site, but lacking the nucleotide sequences that encode the catalytic (deamination) site of ADA. This region may have become combined with a DNA sequence encoding a primitive NAD^+-binding domain, and by further mutation and selection evolved into the AdoHcy hydrolase gene.

MICHAEL S. HERSHFIELD*
Departments of Medicine and
Biochemistry, Division of
Rheumatic and Genetic Diseases,
Duke University School of Medicine,
Durham, North Carolina 27710
UTA FRANCKE
Departments of Human Genetics and
Pediatrics, Yale University
School of Medicine,
New Haven, Connecticut 06510

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* To whom communications should be addressed.

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Adenosine Triphosphate Synthesis Coupled to K^+ Influx in Mitochondria

Abstract. *The influx of K^+ into swollen mitochondria in the presence of valinomycin results in the synthesis of adenosine triphosphate in which approximately one H^+ disappears per adenosine triphosphate synthesized. The synthesis is blocked by atractyloside but is insensitive to oligomycin and relatively insensitive to uncouplers.*

The reversibility of some cation transport pumps has been demonstrated in recent years (*1–6*). A net synthesis of adenosine triphosphate (ATP) from adenosine diphosphate (ADP) and inorganic phosphate (P_i) occurs when the systems are run in reverse. In mitochondria, ATP synthesis is coupled to K^+ efflux in the presence of valinomycin (*1, 2, 6*). Cockrell and Pressman (*6*) reported 1.8 ± 0.7 μmole of ATP and 3.2 ± 0.4 μmole of ATP synthesized per gram of protein, respectively, in the absence and in the presence of a glucose-hexokinase trap. Rossi and Azzone (*2*), using the glucose-hexokinase trap, report values as high as 15 to 20 μmole per gram of protein, probably because of their preloading of the mitochondria with K^+. An energy-dependent net efflux of K^+ has been demonstrated in swollen mitochondria (*7*).

In view of the reversibility of ion transport phenomena, we tested the possibility of synthesizing ATP in osmotically swollen mitochondria under conditions favoring a passive influx of K^+ in the absence of oxidative metabolism. Our results demonstrate the synthesis of 3 to 7 μmole of ATP per gram of protein per minute, and as much as 14.8 μmole per gram of protein per minute in the presence of a glucose-hexokinase trap and 150 m*M* KCl in the medium. The total ATP synthesized was as high as 16 μmole per gram of protein. The swollen mitochondria had been somewhat depleted of their internal K^+, which in two preparations corresponded to 27 ± 8 and 46 ± 5 μmole of K^+ per gram of protein, compared to 180 ± 40 found in fresh mitochondria (*8*). After valinomycin treatment, the internal K^+ increased in proportion to the external K^+ (not shown). In these experiments mitochondria were rapidly centrifuged through a silicon layer into a $HClO_4$ solution (*9*). The K^+ concentrations, determined by atomic absorption, were calculated after correction for the [*carboxyl*-^{14}C]carboxyldextran space (*10*). These experiments show that the ATP synthesis coincides with a K^+ influx. The ATP formed probably does not involve Ca^{2+} exchanges, since the same results were obtained after washing the mitochondria twice with 1 m*M* EGTA.

When mitochondria were suspended in a medium containing a high concentration of KCl and valinomycin at *p*H 7.8, P_i disappeared from the suspension (Table 1 and Fig. 1A) [the medium and the mitochondria were analyzed together (*10*)]. This disappearance approximately matched the ATP synthesized, as determined with the luciferin-luciferase reaction (Table 1). These results indicate that the observed P_i disappearance represents ATP production from ADP and P_i. This conclusion is supported by the inhibition of the P_i disappearance by atractyloside (Table 2), which blocks adenine nucleotide transport (*11*), and by the apparent stoichiometric uptake of H^+ accompanying the synthesis (see below). The effectiveness of the atractyloside also indicates that the orientation of the mitochondrial membrane (the sidedness) remained unchanged after the preparatory procedure.

In the presence of antimycin A and rotenone, no respiration was observed during the usual time course of the experiment (Table 3). In addition, the presence of 2 m*M* KCN had no effect on the phosphorylation in the presence of valinomycin and high KCl (experiment 2 in Table 3 and experiments 3b and 3c in Table 4). Therefore, the phosphorylation was not the result of residual oxidative phosphorylation.

The metabolically blocked preparation continuously produced H^+ (Fig. 1A), as previously observed (*12*). After the addition of valinomycin and a delay of about 1 minute, the disappearance of P_i took place and was matched by the disappearance of H^+, typically in approximately one-to-one stoichiometry (see legend to Fig. 1A). This stoichiometry is expected, at least approximately, from the reaction ADP + P_i + H^+ → ATP (*13*). General-

Table 1. Comparison of phosphate disappearance and ATP production. Mitochondria were isolated (*18*), then washed twice in cold 0.15*M* sucrose and 10 m*M* tris, *p*H 7.4. Disappearance of P_i from the whole suspension was measured colorimetrically (*19*) with an automated system (Autotechnicon) or conventionally. Adenosine triphosphate was estimated with luciferin-luciferase (DuPont) (3 mg in 0.1*M* $MgSO_4$ and 0.1*M* tris, *p*H 7.6) in a photometer (Aminco-Chem-Glow) with an integrator, after removal of the mitochondria. Background ATP was estimated with time in parallel on portions withdrawn just before addition of valinomycin. Oxygen was measured with a Clark electrode (Yellow Springs, model 53). The incubation medium was 0.15*M* KCl and 10 m*M* tris, *p*H 7.8, containing 0.33 m*M* ADP and 0.5 m*M* Na_2HPO_4, and maintained at 25°C. Rotenone and antimycin A were present at a concentration of 0.2 μg/ml. Phosphorylation and ATP levels were monitored in parallel. In experiments 1 and 2, 3 m*M* glycylglycine replaced the tris buffer. The mitochondria were present at 1.3 to 1.8 mg of protein per milliliter, and the concentration of valinomycin was 0.33 to 0.66 μg/ml. Generally, the optimal valinomycin concentration was determined at the onset of the experiment. In all tables and the figure, results are expressed as means ± standard deviation of at least four determinations, except where noted.

Experiment	P_i (nmole/min-mg)	ATP (nmole/min-mg)
1	5.0 ± 1.7	3.5 ± 2.8
2	3.6 ± 0.6	2.4 ± 1.6
3	7.0 ± 2.3	13.6 ± 1.8

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