brain cooling than we have observed. The ability to keep the brain cool during exercise provides these animals with an advantage over panting animals with no carotid rete. In those animals, such as the rabbit, brain temperature during exercise is higher than the temperature of blood in the body core (*3*). This may underlie, in part, the limited tolerance to heat and to exercise in rabbits and other panting mammals in which brain cooling does not occur.

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Hereditary Hemolytic Anemia with Increased Red Cell Adenosine Deaminase (45- to 70-fold) and Decreased Adenosine Triphosphate

Abstract. *Hereditary hemolytic anemia, a dominantly transmitted disorder, has affected 12 family members spanning three generations. The concentration of adenosine triphosphate in the red cells was about half that of comparably reticulocyte-rich blood. Since adenosine deaminase and adenosine kinase compete for a common substrate, the greatly increased activity of the former may interfere with nucleotide salvage via the latter.*

In 1970, we briefly reported a kindred in which dominantly transmitted hereditary hemolytic anemia associated with marked reduction in erythrocyte adenine nucleotides was present in several members (*1*). In the proband, the syndrome was characterized by nonspherocytic hemolytic anemia, splenomegaly, reticulocytosis up to 22 percent, a negative Coombs' test for autoimmune hemolytic anemia, and no evidence of hemoglobinopathy. The activities of erythrocyte enzymes of the Embden-Meyerhof pathway, of the dehydrogenases of the hexosemonophosphate shunt, and of adenylate kinase and glutathione (GSH) peroxidase were normal, as was the concentration of GSH. With the exception of nucleotide cofactors, glycolytic intermediates were present in normal concentration. In an expanded investigation of the syndrome to be presented in more detail elsewhere (*2*), we have observed 45- to 70-fold elevations of adenosine deaminase (ADA) activity in the red cells of affected family members.

The proband and 11 of 23 maternal relatives spanning three generations have an identical hemolytic syndrome. The disorder is transmitted as an autosomal dominant, with 4 out of 9 males and 8 out of 13 females at risk affected. The underlying abnormality is intrinsic to the erythrocyte. Labeled proband red cells have a very short ^{51}Cr half-life of 5 days when transfused into the normal father. Conversely, the half-survival of the father's red cells transfused into the proband was a normal 28 days. Anemia is mild and in some instances fully compensated. Reticulocytosis averages 6 percent (range 3.3 to 11.7 percent) and packed cell volumes 38.8 percent (range 35 to 41 percent). Table 1 records red cell nucleotide concentrations (*3*). The appropriate comparison is with comparably reticulocyte-rich blood, since young erythrocytes characteristically contain greater concentrations of nucleotides than do older cells. The adenine nucleotides average about 60 percent of the normal control mean and less than 50 percent of that of reticulocyte-rich blood. No affected subject has levels greater than 75 percent of the normal mean or greater than 50 percent of the mean of controls with reticulocytosis. In contrast, mean adenosine triphosphate (ATP) and total nucleotides in nine unaffected blood relatives are 96 and 103 percent, respectively, of control values.

Ten affected family members thus far studied have red cell ADA activities 45- to 70-fold greater than the normal mean (Table 2). This is confirmed both spectrophotometrically, measuring adenosine conversion to inosine at 265 nm, and by NH_3 released during deamination (*4*). Electrophoretically, proband ADA is of the common ADA 1-1 pattern (*5*), but to reduce gel staining for enzyme activity to normal intensity requires a 40- to 100-fold dilution of hemolyzate. In hemolyzates the apparent K_m (Michaelis constant) for adenosine is normal (20 μM by spectrophotometry, 18 μM by assay of NH_3 production) (*6*). The apparent K_m for adenosine was essentially identical with that of normal ADA 1-1, as was specific activity, heat stability, and K_i (inhibition constant) for the competitive ADA inhibitor guanylurea sulfate (*7*). By all criteria thus far employed, the greatly increased ADA activity represents overproduction of normal enzyme rather than a mutationally altered catalytic protein.

Methylmercaptopurine riboside is a substrate for adenosine kinase but not ADA, and is converted by human red cells to the nucleotide mono-, but not the di- or triphosphate (*8*). On incubation with methylmercaptopurine riboside in the presence of glucose at *p*H 8.0, proband erythrocytes formed large amounts of phosphorylated nucleoside, evidenced by precipitation as the barium salt in 80 percent ethanol at *p*H 8.0. Redissolved in HCl, the washed precipitate exhibited a strong absorbance peak at 296 nm, an indication of the presence of a major component of methylmercaptopurine-containing material. Treatment of the same incubated, neutralized, deproteinized red cell extracts with *Escherichia*

coli alkaline phosphatase (E.C. 3.1.3.1) prevented subsequent precipitation of barium salts of methylmercaptopurine-containing nucleotides. Thus, although difficult to quantitate precisely, abundant adenosine kinase activity appeared present in proband cells.

Other enzymes of nucleotide metabolism were also investigated in the proband (*9*). Purine nucleoside phosphorylase (E.C. 2.4.2.1) and adenosine monophosphate (AMP) deaminase (E.C. 3.5.4.6) activities are normal. In a limited number of affected family members thus far studied, ribosephosphate pyrophosphokinase (RPK, PRPP synthetase, E.C. 2.7.6.1) and adenine phosphoribosyltransferase (APRT, E.C. 2.4.2.7) activities were somewhat low. Since ATP-dependent production of 5-phosphoribose-1-pyrophosphate (PRPP) would be expected to be reduced in ATP-deficient cells, low values may be epiphenomena related to the reduced cellular content of adenine nucleotides. However, we have also observed a consistent two- to three-fold increase in the activity of pyrimidine 5′-nucleotidase (E.C. 3.1.3.5). The latter catalyzes the dephosphorylation of pyrimidine mononucleotides exclusively. To a lesser extent, then, abnormalities in certain enzymatic activities of nucleotide metabolism other than ADA are also present, suggesting that a constellation of abnormalities, of which increased ADA is most prominent, may be secondary to some thus far undefined underlying lesion.

There is evidence of substantial nucleotide turnover in mammalian red cells (*10*). In human red cells, deamination of AMP to inosine monophosphate is irreversible, and the replenishment of adenine nucleotides is dependent on two salvage pathways (*11*).

$$\text{ATP} + \text{ribose-5-phosphate} \xrightarrow{\text{RPK}} \text{PRPP} + \text{AMP} \quad (1)$$

$$\text{PRPP} + \text{adenine} \xrightarrow{\text{APRT}} \text{AMP} + \text{PP}$$

$$\text{Adenosine} + \text{ATP} \xrightarrow{\text{adenosine kinase}} \text{AMP} + \text{ADP} \quad (2)$$

The first is dependent upon PRPP synthesis and upon the availability of adenine. Although the latter is present in very small amounts in plasma, about 1½ mg is excreted daily in urine (*12*), and adenine from nonhematopoietic sources may play some role in maintaining red cell nucleotides (*13*).

The second is dependent upon adenosine, a toxic substance when present in more than minute concentrations, which is produced in brain and ischemic muscle and is measurable in plasma (*14*). It is the common substrate of adenosine kinase and ADA.

$$\text{Adenosine} + \text{H}_2\text{O} \xrightarrow{\text{ADA}} \text{inosine} + \text{NH}_3 \quad (3)$$

The fate of adenosine, therefore, is dependent on the proportion deaminated and on the proportion phosphorylated. Phosphorylation is favored at low substrate concentrations by the approximately 15- to 20-fold lower K_m of the kinase; however, in human red cells V_{max} of the deaminase is significantly greater than that of the kinase (*15*).

Adenosine has been suggested as a largely overlooked source of red cell adenine nucleotide replenishment, with the latter being governed in part by the balance of adenosine phosphorylating and deaminating activities (*16*). In hereditary ADA deficiency, a disorder associated with severe immunoincompetence (*17*), plasma, red cell, and lymphocyte adenine nucleotides are *increased*—in the lymphocyte severalfold (*18*). In this contrasting disorder in which ADA activity is greatly increased, erythrocyte nucleotides are markedly *decreased*. The salvage pathway mediated by adenosine kinase (Eq. 2) is at a disadvantage in the face of competition for the common substrate from the manyfold increased activity of ADA (Eq. 3). It is further compromised by low levels of ATP in the cell, since kinase activity is ATP-dependent. The associated hemolytic syndrome may result from the relative inability to salvage adequately adenine nucleotides upon which the nonnucleated erythrocyte, incapable of de novo nucleotide synthesis, is so dependent.

The 45- to 70-fold overproduction of apparently normal ADA 1-1 in a disorder dominantly inherited is not explained. A defect in feedback regulation concerned with induction and suppression of ADA synthesis in the nucleated red cell precursor is one possibility. Several mechanisms involving induction or repression of a single enzyme in familial hypercholesterolemia have been elucidated (*19*). In familial hypercholesterolemia, clinical manifestations are present in subjects inheriting a single copy of a defective gene. Among the etiological factors are mutations involving low-density-lipoprotein membrane receptor sites. The facilitated transport of adenosine is also mediated at specific membrane sites exhibiting both saturability and high affinity. Specific inhibitors of the transport mechanism also inhibit ADA activity when this is measured in intact cells (*20*). An abnormal regulator gene (if such exists in man) could also provide an intriguing but unsupported explanation.

Table 1. Red cell adenine nucleotides, expressed as nanomoles per 10^{10} erythrocytes (*3*).

Source	*N*	Mean	
		ATP	Total
Kindred L. (affected)	11	654	886
Kindred L. (unaffected)*	9	1091	1477
Shipped controls	5	1084	1406
Local controls	22	1140	1430
High reticulocyte controls†	15	1386	1931

*Blood relatives of proband only. †Reticulocytes, 2.3 to 22.3 percent; mean, 7.8 percent.

Table 2. Red cell adenosine deaminase activity in affected members of kindred L.

Family	Initials	ADA* (enzyme units)
Proband	J.L. Jr.	55.0
Sister	M.L.	51.6
Mother	M.L.	45.5
Son	E.L.	55.6
Maternal aunt	M.G.	59.9
Maternal uncle	F.B.	66.3
Maternal uncle	E.B.	73.5
Maternal cousin	M.B.	70.6
Maternal cousin	C.B.	66.2
Maternal cousin	P.B.	72.5
Controls (46)†		1.14 ± 0.52

*Expressed as micromoles of adenosine deaminated per minute per gram of hemoglobin at 37°C (*4*). †Mean and standard deviation for ADA activity in 46 control subjects, 36 with reticulocyte counts below 3 percent, and 10 with reticulocytosis of 3.8 to 37 percent. The mean ADA activity in the "high reticulocyte" group was 1.0.

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Albumin Phylogeny for Clawed Frogs (*Xenopus*)

Abstract. *Comparisons of albumin indicate that the frogs commonly used by North American molecular and developmental biologists under the name of* Xenopus muelleri *belong to another species,* X. borealis. *Phylogenetic analysis of the albumin data reveals two major groups of* Xenopus *species, one containing only* X. tropicalis *and the other, called the* X. laevis *group, containing the remaining species of the genus. The phylogenetic tree, in conjunction with evidence from chromosomes and DNA content, leads to the hypothesis that total genome duplication occurred in the common ancestor of the* X. laevis *group.*

Clawed frogs (genus *Xenopus*) are often used for research in molecular and developmental biology (*1*). Interpretation of the results of such research is sometimes dependent on accurate identification of the specimens used. Species of frogs which are very alike morphologically can differ greatly at the gene level (*2–4*) so that misidentification can be a serious problem for biologists working with them. Large genetic differences between morphologically similar frogs arise because frog anatomy has undergone slow evolutionary change while their genes and proteins have evolved at standard rates. By contrast, mammalian species that differ conspicuously in morphology can be extremely similar at the gene level (*5*).

The genus *Xenopus*, like several other frog genera, is old and morphologically conservative. This genus probably arose more than 90 million years ago at the same time as the common ancestor of all living placental mammals (*6*). As an aid to identifying and classifying the members of this genus, we have compared the serum albumins of a variety of species and subspecies by electrophoretic and immunological techniques. Serum albumin has already proved useful for distinguishing among other frog species and for elucidating their phylogenetic relationships (*2–4*).

Albumin, the major acidic protein in *Xenopus* plasma (*7*), was partially purified from plasma of six species by Sephadex G-200 gel filtration (*8, 9*) in Geneva and then shipped to Berkeley at ambient temperature in the presence of 1.5 percent phenoxyethanol as a preservative (*10*). The albumin was further purified by ammonium sulfate fractionation and polyacrylamide gel electrophoresis in Berkeley. Each purified protein was homogeneous by immunological criteria. Each was identified as albumin by several criteria, including solubility, electrophoretic mobility in the native state, strong fluorescence in the presence of 8-anilino-1-naphthalene sulfonate (*11*) and, in four cases, amino acid composition. The albumins analyzed showed significant similarity in amino acid composition to albumins of frogs of the genus *Rana* (*12, 13*). The average value of Metzger's index, a convenient measure of compositional difference (*14*), was 9 for the *Rana-Xenopus* comparison. This is indicative of significant homology in amino acid sequence between *Rana* and *Xenopus* albumins (*15*).

Antiserums were made by injecting each purified albumin into a group of three Dutch-Belted rabbits. After a 3-month period of immunization (*4*), antiserums were collected and pooled in inverse proportion to their titers in the microcomplement fixation test (*16*) and then tested for purity by several of the methods summarized elsewhere (*17*). Each antiserum pool was then reacted with the immunizing albumin as well as with the albumins of other frog species. The results of these comparisons are expressed as units of immunological distance. For albumin, there is indirect evidence for a correlation between immunological distance and the number of amino acid substitutions by which two albumins differ in sequence, one unit of immunological distance being roughly equivalent to one amino acid substitution (*3*). There is also a correlation between albumin immunological distance and genetic distance measured by the electrophoretic comparison of many enzymes of frogs (*18*).

The results obtained by testing the antiserum pool made against albumin from *X. laevis laevis* with albumin from other frogs are shown in Table 1. Each of these taxa was distinguishable from *X. l. laevis* on the basis of albumin reactivity in the microcomplement fixation test. The immunological distances of most taxa fell in the range from 5 to 19 units. The albumin of *X. tropicalis* was exceptional in differing by 57 units from that of *X. l. laevis* (*19*). *Hymenochirus* and *Pipa*, which belong with *Xenopus* in the family Pipidae, were extremely different from *Xenopus* with regard to the antigenic properties of albumin.

Many of the subspecies and species of *Xenopus* were distinguishable from one another on the basis of the electrophoretic mobility of their albumins, as in-