to ask why these women have antibodies to their own zona. One explanation is that repeated exposure of the immune system to zona for several years through egg atresia in the ovary and absorption of ovulated eggs in the reproductive tract may account for the production of antibodies to the zona. If this is true, then a higher frequency and titer of antibody might be expected in older (menopausal) women compared to younger ones, since the former would have had a longer exposure to the zona antigen. We are now testing serums from menopausal women for possible antibodies to the zona pellucida. Another possible explanation is that these women might have been sensitized to an antigen (from an unknown source) which cross-reacts with the zona; however, no such antigen has been identified. In fact, all studies to date point to the conclusion that the zona antigen is tissue-specific.

Should these autoantibodies prove to be responsible for infertility, the same system might be exploited in the future for contraceptive purposes. Utilization of zona antibodies as a means of contraception would appear to have several attractive features. Because only a single cell in the oviduct, or at most a few in the ovary, would be involved and because of the long time that the eggs would be available for exposure to the antibody in the ovary or oviduct, a low titer of antibody would, presumably, be required. Research on the mouse and hamster (*10*) has shown that a single injection of serum containing antibodies to the zona results in the antibody binding to the surface of ovarian zona-coated eggs but not to the immature, nonzona-coated ones. This binding to the zona prevented sperm-egg interaction. Infertility in these antibody-injected animals lasted for four estrous cycles (*12*), and thereafter fertility returned to normal levels, suggesting that the contraceptive effects of the antibody do not result in permanent sterility.

Much more work is required before immunocontraception by antibodies to the zona can be envisioned for humans, but all studies to date on animal species have been most encouraging. The identification in women of naturally occurring autoantibodies to the zona pellucida, the lack of any known side effects of these autoantibodies, and the ability of these antibodies to produce infertility make this method of contraception even more promising.

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Purine Nucleoside Phosphorylase Deficiency: Altered Kinetic Properties of a Mutant Enzyme

Abstract. *Erythrocyte purine nucleoside phosphorylase from two brothers had 0.5 percent of normal activity. It differed from the normal enzyme by a tenfold increase in the Michaelis constant for inosine, an inability of inosine to protect against thermal lability, and a more positive net charge. The altered kinetic properties may account for the milder disease in the patients compared to the previously described cases. The data provide evidence for a structural gene mutation and genetic heterogeneity in the new disease of purine nucleoside phosphorylase deficiency and T cell dysfunction.*

Purine nucleoside phosphorylase (E.C. 2.4.2.1) catalyzes the phosphorolysis of purine nucleosides to the corresponding purine base by the following reactions:

Inosine + $P_i \rightleftharpoons$
hypoxanthine + ribose-1-phosphate

Guanosine + $P_i \rightleftharpoons$
guanine + ribose-1-phosphate

This enzyme reaction is an essential component of the pathway by which purine nucleotides are degraded to uric acid in man. A genetic deficiency of this autosomally inherited enzyme is associated with a disorder of cellular immunity and a block of purine catabolism, characterized by hypouricemia, hypouricosuria, elevated plasma inosine, and increased urinary excretion of inosine, deoxyinosine, guanosine, and deoxyguanosine (*1*).

A milder form of T cell dysfunction with an almost normal serum and urinary uric acid and only a partial block of purine nucleoside degradation occurred in two brothers, ages 8 and 10, with purine nucleoside phorphorylase deficiency (*2*).

Table 1. Comparison of purine nucleoside phosphorylase in normal and enzyme-deficient hemolyzate. Diluted hemolyzate (50 μl) was incubated at 37°C with 0.2 m*M* [8-^{14}C]inosine (5 μc/μ*M*) and 50 m*M* sodium phosphate buffer, *p*H 7.4. Normal hemolyzate was diluted (1 to 1500) and was incubated for 10 minutes, and hemolyzate containing the deficient enzyme was diluted by 1 to 20 and incubated for 60 minutes. The reaction was stopped by heating to 85°C for 2 minutes. The tubes were centrifuged at 1500*g* for 5 minutes. A portion (20 μl) of supernatant was spotted on sheets of Whatman 3 MM chromatography paper, already spotted with nonisotopic hypoxanthine and inosine, each 1 mg/ml. The sheets were subjected to electrophoresis for 30 minutes in 50 m*M* sodium borate, *p*H 8.9, at 4000 volts and 250 ma. The hypoxanthine spots were located by ultraviolet light and cut out; radioactivity was counted in a Packard liquid scintillation spectrometer system. The reaction was linear with protein and with time up to 60 minutes. The mean enzyme activity in 65 patients was 2368 ± 614 nmole/mg per hour. With 2.0 m*M* inosine the same procedure was followed. The activity of the control enzyme increased by a factor of 3.4 upon increasing the inosine concentration tenfold. The mutant enzyme activity increased by factors of 4.1 and 3.8.

Inosine (m*M*)	Nucleoside phosphorylase as hypoxanthine (nmole/mg per hour)		
	Control	Patient 1	Patient 2
0.2	2955	9.9	9.9
2.0	9929	40.2	37.3

Activity of this enzyme in lysates of erythrocytes from these patients was 0.5 percent of normal when the concentration of inosine was 0.2 m*M* (Table 1). At a higher concentration of inosine (2 m*M*), the apparent enzyme activity increased 3.5-fold (Table 1). However, with similar studies of a normal erythrocyte enzyme, the same change was observed in accordance with the known substrate activating effect of inosine (*3*). When guanosine was used as substrate, the same degree of deficiency was observed in both brothers. The deficient and normal purine nucleoside phosphorylases appeared to have the same substrate specificity, since neither enzyme activity was inhibited by uridine, cytidine, or adenosine and both were substantially inhibited by guanosine and inosine.

Studies of the mutant erythrocyte purine nucleoside phosphorylase were designed to distinguish whether the increased activity of the enzyme upon the tenfold increase of inosine concentration was related to substrate activation or altered kinetic properties. The apparent K_m (Michaelis constant) of the normal and mutant enzymes was measured by means of initial velocity studies. Although both enzymes demonstrated hyperbolic kinetics when inosine varied from 3.3 to 200 μM, the apparent K_m values of the mutant enzymes were 370 and 343 μM, in contrast with the values for the normal enzyme of 25, 33, and 37 μM (Fig. 1). The latter observations agreed well with reported values (*3*, *4*). Kinetic experiments with guanosine or ribose-1-phosphate as substrate revealed 30- to 40- or 10- to 20-fold higher apparent K_m values, respectively, for the mutant enzyme, as compared to the normal enzyme (Table 2). Apparent K_m values for inorganic phosphate and hypoxanthine were similar for both types of enzymes (Table 2) and agreed with reported values (*4*). These studies indicated that the mutant enzyme had altered kinetic properties.

High concentrations of inosine failed to confer stability to the mutant enzyme during thermal inactivation at 70°C, whereas the normal erythrocyte purine nucleoside phosphorylase was partially protected by inosine (0.5 to 20.0 m*M*) (Fig. 2). The failure of high concentrations of inosine to protect the mutant enzyme indicates that either inosine was not significantly bound under experimental conditions or that the binding of inosine did not influence the rate of inactivation by heat. These observations indicate a major physical difference between mutant and normal enzymes apart from the kinetic studies. Altered structural properties of the mutant enzyme were also evident with isoelectric focusing (*5*). The normal enzyme had peaks of activity between *p*H 5.50 and 5.80, while the mutant enzyme had isoelectric *p*H values of 5.08 and 5.26. The normal isoelectric *p*H values agreed closely with reported values for the crystalline enzyme (*3*).

The purine nucleoside phosphorylase in extracts of cultured diploid fibroblasts derived from the affected children had

Table 2. Kinetic constants for purine nucleoside phosphorylase. The apparent K_m values were estimated by Eadie-Hofstee plots (Fig. 1) or by Lineweaver-Burk plots. Product formation was proportional to enzyme concentration and time under the conditions of the experiments for both the normal and mutant enzymes.

Compound	Apparent K_m values		
	Normal	Patient 1	Patient 2
Hemolysate			
Inosine (μM)	25, 33, 37	370	343
Guanosine (μM)	15	667	500
Ribose-1-phosphate (mM)	0.39, 0.26, 0.15	5.0	2.6
P_i (mM)	0.14, 0.16	0.45	0.28
Hypoxanthine (μM)	20	15	
Fibroblasts			
Inosine (μM)	22, 24	770	

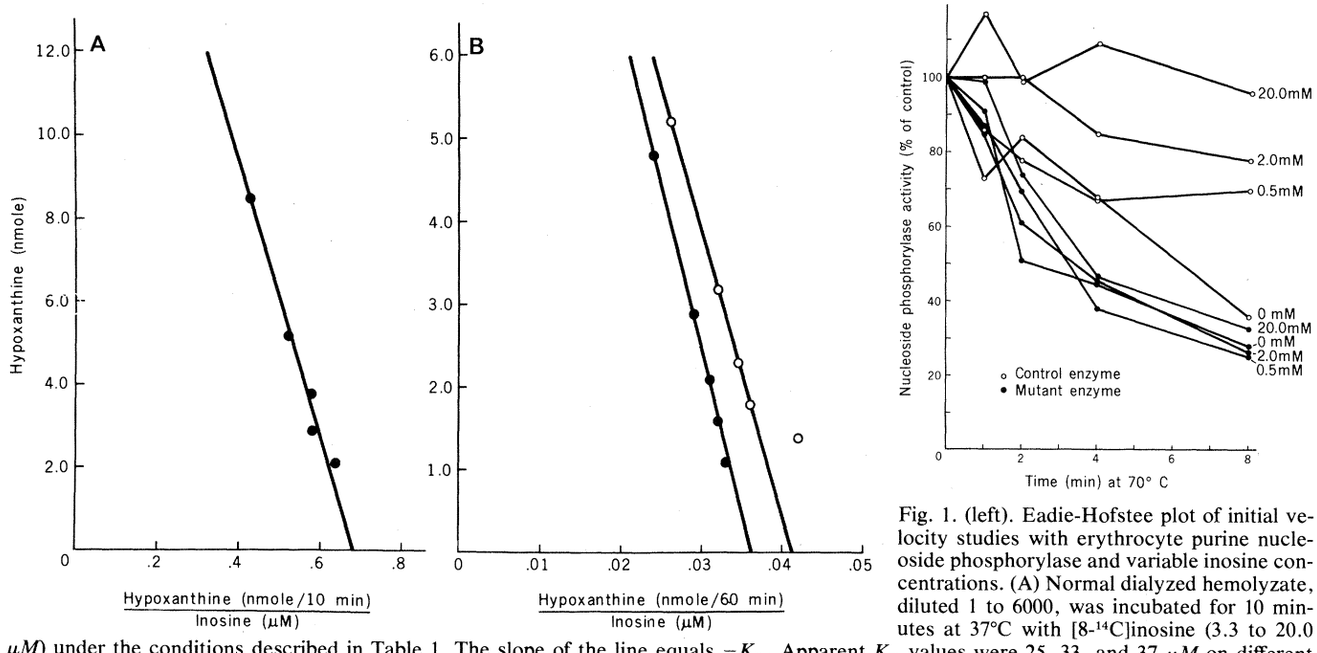

Fig. 1. (left). Eadie-Hofstee plot of initial velocity studies with erythrocyte purine nucleoside phosphorylase and variable inosine concentrations. (A) Normal dialyzed hemolyzate, diluted 1 to 6000, was incubated for 10 minutes at 37°C with [8-^{14}C]inosine (3.3 to 20.0 μM) under the conditions described in Table 1. The slope of the line equals $-K_m$. Apparent K_m values were 25, 33, and 37 μM on different occasions. (B) Similar studies of the mutant with a 1 to 20 dilution of hemolyzates and a 60-minute incubation gave indeterminate values with inosine (3.3 to 20 μM). Accordingly, [8-^{14}C]inosine from 33 to 200 μM was used and gave apparent K_m values of 370 and 343 μM, as shown above. Fig. 2. (right). Effect of inosine on inactivation of normal and mutant erythrocyte purine nucleoside phosphorylase at 70°C. Portions (50 μl) of dialyzed hemolyzate, diluted 1 to 4 with 50 mM sodium phosphate, *p*H 7.4, were incubated with 0 to 20.0 mM inosine at 70°C for 0 to 8 minutes. The samples were chilled rapidly to 4°C and further diluted and assayed as described (Table 1). The values are expressed as percentage of the activity of an unheated sample.

deficient enzyme activity with values of 0.4 as compared to 175 and 200 nmoles/mg per hour for two normal extracts. These extracts exhibited an increased apparent K_m for inosine of 770 μM as compared to the normal value of 22 μM. Therefore, the altered kinetic properties observed with the mutant enzyme were found in more than one cell type.

The discovery of a purine nucleoside phosphorylase with altered kinetic and physical properties in two patients with a disorder of cellular immunity is important for at least two reasons. First, an increase of the apparent K_m for inosine indicates that the enzyme will become more active if inosine concentrations increase. Such an elevation of inosine concentrations, by activating the mutant enzyme, could decrease the apparent block of nucleoside degradation and could account for the less severe clinical features in the two brothers studied (*2*). Elevated inosine in the concentration of plasma, presumably a reflection of intracellular inosine concentrations, has been observed in this disorder (*1, 2*). Second, this variant provides evidence for a structural alteration of the enzyme protein and implies that a structural gene mutation has resulted in purine nucleoside phosphorylase deficiency. These observations give the first direct evidence for genetic heterogeneity in this disease. Finally, this structural gene mutation supports the hypothesis that a block of purine nucleoside degradation is the cause of the immune disorder observed, rather than a consequence of a deletion of two closely linked genes. Such cause and effect relationship was initially suggested by the structural alterations of adenosine deaminase in severe, combined immunodeficiency (*6*). The two diseases are genetically distinct since adenosine deaminase and purine nucleoside phosphorylase are coded by different chromosomes (*7*). Thus the discovery of two inherited disorders of the immune system associated with enzyme deficiencies of purine catabolism provide important clues for a role of purine nucleoside degradation in the regulation of immune function.

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A Specific High-Affinity Binding Macromolecule for 1,25-Dihydroxyvitamin D_3 in Fetal Bone

Abstract. *Cytosol fractions were prepared from fetal rat or embryonic chick calvaria and analyzed for binding of vitamin D_3 metabolites on sucrose density gradients. Both cytosol fractions contain a 3.5*S *macromolecule which specifically binds 1,25-dihydroxy-[^3H]vitamin D_3 and in addition, a 5 to 6*S *macromolecule which binds 25-hydroxy-[^3H]vitamin D_3. In rat calvaria cytosol, 1,25-dihydroxy-[^3H]vitamin D_3 also binds to the 5 to 6*S *macromolecule but appears to have greater affinity for the 3.5*S *component.*

Several observations have suggested that 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$], the metabolically active form of vitamin D_3, can be considered a hormone. 1,25-$(OH)_2D_3$ is produced in the kidney but acts in intestine and bone, and its synthesis is regulated by the need for calcium (*1*). Accordingly, evidence has been collected to demonstrate that 1,25-$(OH)_2D_3$ may stimulate calcium absorption in the intestine by a receptor protein-mediated mechanism. A 3.0 to

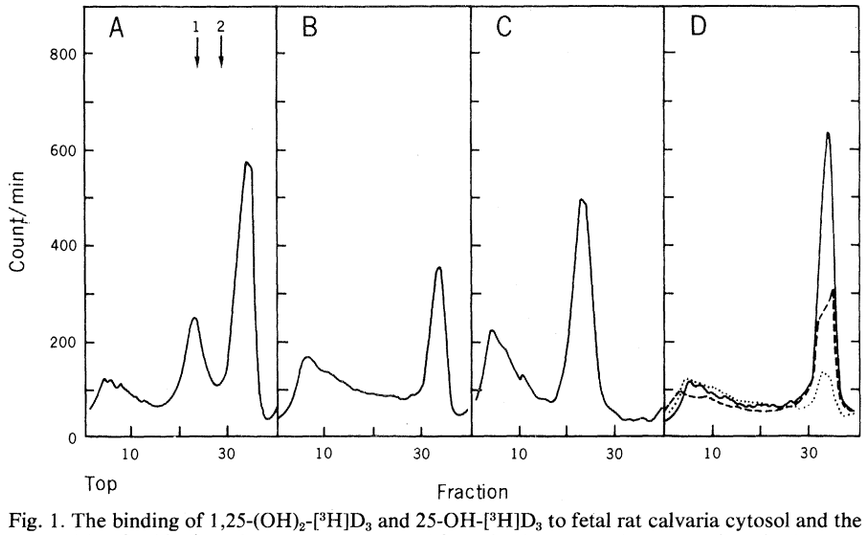

Fig. 1. The binding of 1,25-$(OH)_2$-[^{3}H]D_3 and 25-OH-[^{3}H]D_3 to fetal rat calvaria cytosol and the competition for binding. Cytosol was prepared from fetal rat calvaria as described in the text. Cytosol (2.7 mg protein) was incubated with vitamin D_3 metabolites and binding was analyzed on 4 to 20 percent sucrose gradients. (A) Binding of 0.25 n*M* 1,25-$(OH)_2$-[^{3}H]D_3 and competition by (B) 3 n*M* 1,25-$(OH)_2D_3$ and (C) 3 n*M* 25-OH-D_3. (D) Binding of 0.25 n*M* 25-OH-[^{3}H]D_3 (—) and competition by 3 n*M* 1,25-$(OH)_2D_3$ (---) or 3 n*M* 25-OH-D_3 (···). The arrows indicate the sedimentation of ovalbumin (1) and bovine serum albumin (2).