Erythrocyte Metabolism in Purine Nucleoside Phosphorylase Deficiency after Enzyme Replacement Therapy by Infusion of Erythrocytes

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ABSTRACT Purine nucleoside phosphorylase deficiency is associated with a severely defective T-cell immunity. A patient with purine nucleoside phosphorylase deficiency was treated with transfusions of irradiated erythrocytes and plasma. This resulted in a remarkable correction of the metabolic disturbances in the patient. The urinary excretion of inosine, deoxyinosine, guanosine, and deoxyguanosine decreased, whereas uric acid excretion as well as serum uric acid concentration increased. It could be shown that the enzyme activity of the circulating erythrocytes correlated inversely with the urinary excretion of nucleosides and directly with the excretion of uric acid. As a consequence of the therapy, several glycolytic intermediates of the erythrocytes were increased, especially 2,3-diphosphoglycerate. The high 2,3-diphosphoglycerate level caused a shift to the right of the oxygen dissociation curve (P_{50} = 32.9 mm Hg).

The immunological status of the patient showed definite improvement after the enzyme replacement therapy.

INTRODUCTION

Purine nucleoside phosphorylase (EC 2.4.2.1 purine orthophosphate ribosyltransferase) catalyzes the phosphorolysis of deoxyinosine and deoxyguanosine. Patients deficient in purine nucleoside phosphorylase (PNP)[1] show disturbances in thymus-dependent immunity and have normal or nearly normal humoral immunity (1–5).

Deoxyguanosine, one of the substrates of PNP, accumulates in the urine of PNP-deficient children (6). dGTP is present in erythrocytes from PNP-deficient patients, but not in normal erythrocytes (7). It was suggested that dGTP may be toxic for the T cells (7). Ullman et al. (8) could demonstrate in T-lymphoma cells, deficient in PNP, that deoxyguanosine is first phosphorylated by deoxycytidine kinase and accumulated as dGTP. By inhibiting ribonucleotide reductase, dGTP depletes the cell of dCTP, thus preventing the synthesis of DNA. Adenosine deaminase deficiency is associated with severe combined immunodeficiency. In these patients dATP accumulates in the erythrocytes (9). Polmar et al. (10) reported that transfusions of erythrocytes containing adenosine deaminase may provide a way to treat patients with adenosine deaminase deficiency. After a few transfusions of erythrocytes, restoration of immunological function (10) and diminished erythrocyte dATP levels were observed (9). Normal human erythrocytes are rich in PNP and we decided to treat our patient with PNP deficiency with erythrocyte transfusions. It is the aim of this paper to report the effects of the transfusion of normal erythrocytes on the metabolic state of a patient with a PNP deficiency. This treatment has led to a gradual and partial restoration of T-cell immunity that has been described in detail separately (11, 12).

METHODS

Venous blood was collected with heparin (30 U/ml). Immediately after collection of the blood a part of it was deproteinized for the determination of glycolytic intermediates. Deproteinization and determination of glycolytic intermediates except 2,3-diphosphoglycerate, were performed according to the methods of Minakami et al. (13). The 2,3-diphosphoglycerate content of the acid extract was determined with a Sigma

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[1] *Abbreviation used in this paper*: PNP, purine nucleoside phosphorylase.

test-kit (Sigma Technical Bulletin 35-UV [12–74]), Sigma Chemical Col., St. Louis, Mo.

Glycolytic enzymes were determined by the methods of Beutler (14). Substrates, coenzymes, and auxiliary enzymes for determination of glycolytic enzymes, purine enzymes, and intermediates, except for the determination of 2,3-diphosphoglycerate, were obtained from C. F. Boehringer and Sons, Mannheim, West Germany. All other chemicals were of analytical grade of purity.

PNP activity was determined according to Kalckar (15). A unit of activity is defined as the amount of enzyme required to catalyze the conversion of 1 μmol inosine to hypoxanthine per minute. The specific activity is expressed as units per gram Hb.

Determination of urinary ribonucleosides and deoxyribonucleosides was done on aliquots of 24-h urine collections each time before transfusion. During the entire period of enzyme replacement therapy the patient was on an ad lib. diet; earlier (16) it was shown that a purine-restricted diet had little or no influence on the total daily excretion of ribonucleosides and deoxyribonucleosides. Ribonucleosides and deoxyribonucleosides were determined with high pressure liquid chromatography as described in detail earlier (17). Uric acid was determined enzymatically with uricase.

Case summary and the effect of treatment with enzyme replacement on the immunological parameters. R.V., a female child, now 3.5 yr old, is the fourth child of healthy, not knowingly related parents. Of these parents, the first two children, both females, died early in life as a consequence of a selective impairment of T-cell function and an apparently normal B-cell function (18). The third child, a boy, is healthy and is now 5 yr old. The fourth child developed a selective impairment of T-cell function during the 1st yr of life, whereas B-cell function apparently developed quite normally (5). The immunodeficiency in the fourth child was associated with a PNP deficiency as could be concluded from the absence of enzyme activity in erythrocytes, lymphocytes, and fibroblasts of the patient (4, 5) as well as from the excretion pattern of purine metabolites: high urine and serum levels of inosine, deoxyinosine, guanosine, and deoxyguanosine were present, whereas levels of urinary and serum uric acid were extremely low (5, 17). The parents and healthy brother showed PNP activities encompassing the heterozygous state. Retrospective analysis of frozen-stored serum, urine samples, and lymphocytes of the first two siblings showed that they too had suffered from a PNP deficiency (5).

Clinically, the fourth child remained well until the age of 15 mo. From that time—which coincided with the development of extreme lymphopenia and almost complete absence of in vitro T-cell functions of peripheral blood lymphocytes (5) —a few hospitalizations were needed for recurrent ear and respiratory infections responding to conventional antibiotic therapy. Weight and height developed normally. X rays of the thorax showed no thymus shadow. Lymphoid tissue was not palpable and tonsils hardly developed. The patient showed spastic tetraparesis. On a few occasions erythrocytes were found in the urine.

Before treatment the patient showed a megaloblastic bone marrow and a microcytic blood picture. At the age of 15 mo the granulocyte number was abnormally low, i.e., <1,500 granulocyte/mm^3. Blood platelets were normal in number. Function of granulocytes and platelets unfortunately were not evaluated before treatment.

In the first instance, the patient was maintained on a purine-restricted diet in order to obtain base levels of excreted purines and pyrimidines. Afterward, adenine, uridine, hypoxanthine, and allopurinol were given, each during a short period. In summary it can be said that in vivo administration of adenine and uridine has some demonstrable effect on the percentage of E-rosetting T cells, but not on the lymphocyte count or the in vitro phytohemagglutinin response; also the granulocyte number and the megaloblastic bone marrow characteristics remained unchanged (12, 17).

After 25 November 1976, the patient was treated with irradiated erythrocyte and plasma transfusions according to Polmar et al. (10). Before each transfusion ~70 ml of blood was withdrawn to avoid hemosiderosis. Packed erythrocytes (15 ml/kg body wt) and plasma (20 ml/kg body wt) were given at a general frequency of once a month. Initially, erythrocytes and plasma were given together, subsequently (from the fifth transfusion) they were given alternately with an interval of 14 d. The immunological status of the patient showed definite improvement after the enzyme replacement therapy. The results have been published in detail separately (11, 12), and they are summarized below. After the second blood transfusion, the number of peripheral blood lymphocytes increased up to 1,200/mm^3. The increase was transient and was observed subsequently after each erythrocyte and plasma transfusion. After the third transfusion, the percentage of E-rosetting T lymphocytes gradually increased to normal levels; this was followed by an increase in the phytohemagglutinin responsiveness of the lymphocytes and soon thereafter by the appearance of immunoglobulin (Ig)M-binding T lymphocytes. It could be concluded that partial restoration of cellular immunity was gradually attained. During the follow-up period of 2.5 yr, the various immunological parameters varied widely; however, the extremely depressed starting levels were never seen again. Finally, it should be mentioned that during the enzyme replacement therapy the granulocytopenia disappeared and the megaloblastic bone marrow became normal.

RESULTS AND DISCUSSION

Before enzyme replacement, no PNP activity could be detected in the erythrocytes of the patient (3). Fig. 1 shows the result of erythrocyte transfusion on the PNP activity in the peripheral blood of the patient. This figure shows a period of 10 wk out of 2 yr of transfusion therapy; this period is given because the interval between the transfusions is 6 wk and thus demonstrates the greatest difference expected in PNP activity. The PNP activity in the patient's blood increased after each transfusion and was in the range of half-to-low normal values. The variation on PNP activity after each transfusion most probably reflects the PNP activity of the donor erythrocytes. After a transfusion the elevated PNP activity declined rapidly, and it could be shown that after 6 wk very little activity remained.

Before transfusion therapy urinary excretion of purines, i.e., inosine, deoxyinosine, guanosine, and deoxyguanosine was very high (17). The mean daily loss of nucleosides was increased (0.3 mmol/kg) (16). Normally in children the daily urinary excretion is ~0.15 mmol/kg (as uric acid). As a result of the transfusion the excretion of purine nucleosides decreased, and uric acid excretion as well as serum level of uric acid increased. From Table I one may conclude that there exists a relation between the PNP activity and the urinary levels of purine nucleosides as well as the uric acid. When little PNP activity was present, there was a

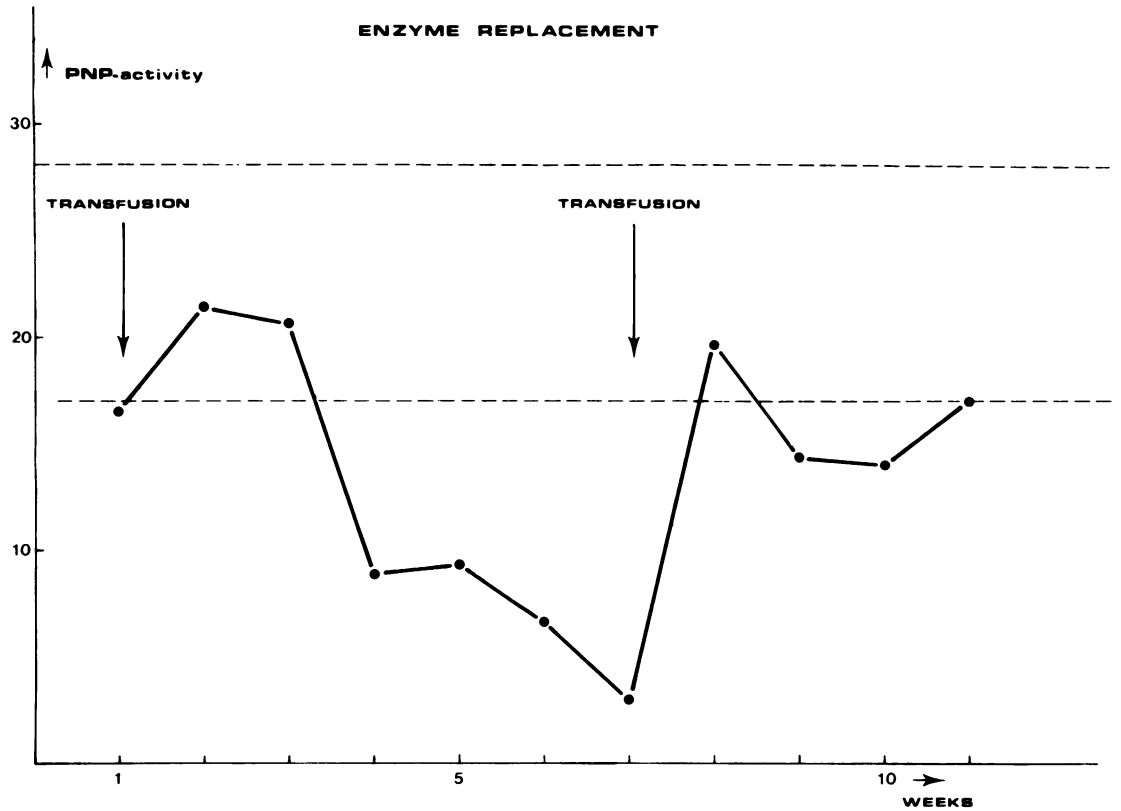

FIGURE 1 Effect of transfusion of irradiated erythrocytes on erythrocyte PNP activity. Enzyme activity is expressed as units per gram Hb.

reduced excretion of uric acid, whereas the urinary concentration of purine nucleosides was high. When PNP activity was relatively high, the reverse was found. Therefore it seems likely that the PNP activity is causally related to the concentrations of nucleosides and uric acid. As was already reported (7), abnormally high levels of dGTP (8 nmol/ml packed erythrocytes) were found in the erythrocytes of our patient. In contrast, normal controls do not have detectable amounts of dGTP (<0.5 nmol/ml packed erythrocytes). Therefore we monitored the dGTP during the transfusion. On 11 January 1979, 28 d after a transfusion, the PNP activity was estimated to be 13.8 U/g Hb, whereas dGTP levels in the erythrocytes were found to be 2.4 nmol/ml packed erythrocytes. This latter value is significantly less than that before transfusion but still much higher than the levels found in normal controls. No measurement of dGTP could be done in lymphocytes because of their paucity in the patient. Before and during the course of transfusion therapy measurements of PNP activity, 2,3-diphosphoglycerate concentrations and levels of erythrocyte glycolytic intermediates were performed.

The 2,3-diphosphoglycerate content was related to the PNP activity (Table II). When PNP activity was low, 2,3-diphosphoglycerate was normal (normal value, 5.2±0.4 μmol/ml packed erythrocytes). When PNP activity increased however, unusually high levels of 2,3-diphosphoglycerate content caused a shift in the oxygen dissociation curve to the right, resulting in an increase of oxygen release to the tissues. We determined

TABLE I
Urinary Excretion of Purine Compounds during Erythrocyte Transfusion Therapy

	Inosine	Guanosine	Deoxyinosine	Deoxyguanosine	Uric acid	PNP activity
6 January 1977	3.8	2.9	1.8	0.9	7.2	11.0
21 July 1977	9.7	6.0	4.8	2.5	1.8	4.0
1 September 1977	2.3	1.5	0.9	0.5	8.7	16.3

Purine values are expressed as millimoles per gram of urinary creatinine (per 24 h). PNP activity is expressed as units per gram Hb. PNP activity at 21 July correlated with the lowest PNP activity in Fig. 1. Dates of relevant transfusions are 16 December 1976, 23 June 1977, and 28 August 1977.

TABLE II
2,3-Diphosphoglycerate Content and PNP Activity during Transfusion on Different Dates

Date	2,3-Diphosphoglycerate	PNP activity
6 January 1977	9.3	11.0
21 July 1977	4.6	4.0
1 September 1977	9.5	16.3
12 December 1977	10.1	16.8

2,3-Diphosphoglycerate is expressed as micromoles per milliliter packed erythrocytes; PNP activity as units per gram Hb.

the oxygen dissociation curve at a 2,3-diphosphoglycerate concentration of 10.0 μmol/ml erythrocytes. The P_{50} (millimeters of mercury of oxygen at 50% saturation) was shifted to 32.9 mm Hg (normal range, 26–28 mm Hg). The effect of transfusion on 2,3-diphosphoglycerate was investigated in a subject who had to be transfused after surgery. In this control experiment no difference in 2,3-diphosphoglycerate level before and after transfusion was found.

The contents of some glycolytic intermediates of the erythrocytes before and after transfusion are shown in Table III. With the exception of glyceraldehyde-3-phosphate, the other glycolytic intermediates are much higher after transfusion than before transfusion. From Table III one can calculate the ratio of glucose-6-phosphate:fructose-6-phosphate, fructose-6-phosphate:fructose-1,6-diphosphate, and fructose-1,6-diphosphate:dihydroxyacetone-phosphate (Table IV). The ratios before and after transfusion are about the same, indicating that the enzymes phosphoglucose isomerase, phosphofructokinase, and aldolase are not inhibited by 2,3-diphosphoglycerate in vivo. One may expect such an inhibition because these enzymes under certain conditions in vitro are inhibited by 2,3-diphosphoglycerate (19).

What is the reason for an increase in the levels of 2,3-diphosphoglycerate and glycolytic intermediates? It is well known that incubation of erythrocytes with inosine, pyruvate, and inorganic phosphate leads to an increase in 2,3-diphosphoglycerate content. The effect of these substrates results from the conversion of inosine to hypoxanthine and ribose-1-phosphate by PNP. Ribose-1-phosphate is converted to ribose-5-phosphate by the enzyme phosphoribomutase and metabolized further via the pentose phosphate shunt and glycolysis. The sequence of reactions involved in the formation of 2,3-diphosphoglycerate from inosine is given in Fig. 2. Inosine can be substituted by other purine ribonucleosides, which are substrates of PNP. The increased 2,3-diphosphoglycerate content may be explained by the reaction sequence given in Fig. 2. By administration of normal erythrocytes and therefore PNP, the accumulated substrates inosine and guanosine will enter the donor erythrocytes and will be converted to the purine bases and ribose-1-phosphate. The lowered purine nucleoside excretion found in the course of the transfusions (Table I) and the increased content of the glycolytic intermediates (Table III) are in agreement with the assumption.

Evidence has been obtained that proliferating cells, such as bone marrow and spleen cells, use preformed purine compounds as supplementary nutrients (20). They are probably provided by the liver and carried to other organs by the erythrocytes. Normal erythrocytes are rich in PNP activity. As a result of the blood transfusion the accumulated purine nucleosides will enter the donor erythrocytes. The infused normal erythrocytes convert the accumulated purine nucleosides in their purine bases. This leads to a decreased level of deoxyguanosine.

One may also expect a decrease in dGTP level. The

TABLE III
Erythrocyte Glycolytic Intermediates before and during Erythrocyte Transfusion (27 January 1977)

	Before transfusion	During transfusion	Normal controls (mean±SD, n = 20)
Glucose-6-phosphate	45	255	36±6.6
Fructose-6-phosphate	15	83	14±2.5
Fructose-1,6-diphosphate	20	82	10±3.4
Dihydroxyacetone phosphate	11	60	12±5.0
Glyceraldehyde-3-phosphate	5	7	5±1.6
ATP	1,700	2,200	1,300±250

Concentrations are expressed as nanomoles per milliliter packed erythrocytes.

TABLE IV
Ratios of Glycolytic Intermediates

	Before transfusion	During transfusion	Normal controls (mean±SD, n = 20)
Glucose-6-phosphate / Fructose-6-phosphate	3.0	3.0	2.56±0.53
Fructose-6-phosphate / Fructose-1,6-diphosphate	0.75	1.0	1.25±0.31
Fructose-1,6-diphosphate / Dihydroxyacetone phosphate	1.8	1.4	0.69±0.24

Values of glycolytic intermediates are calculated from Table III.

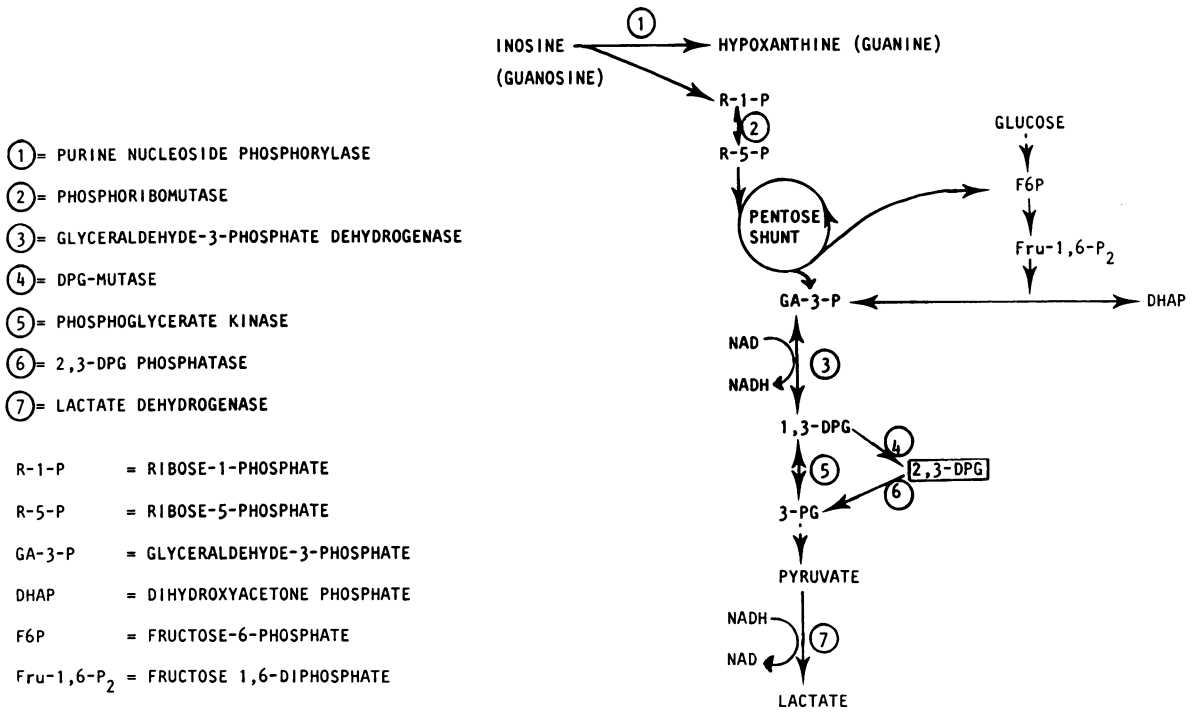

FIGURE 2 Sequence of reactions involved in the formation of 2,3-diphosphoglycerate from inosine and guanosine.

value reported here is lower than before transfusion and still much higher than in normal controls. Unfortunately no dGTP values are available from the lymphocytes of the patient. The immunological status of the patient showed partial improvement after the enzyme replacement therapy. It may be possible that the residual dGTP precludes complete restoration of immunological function.

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REFERENCES

1. Carapella-De Luca, E., F. Aiuti, P. Lucarelli, L. Bruni, C. D. Baroni, C. Imperato, D. Roos, and A. Astaldi. 1978. A patient with nucleoside phosphorylase deficiency, selective T-cell deficiency and autoimmune hemolytic anemia. *J. Pediatr.* **93:** 1000–1003.
2. Gelfand, E. W., H. M. Dosch, W. D. Bigger, and I. H. Fox. 1978. Partial purine nucleoside phosphorylase deficiency. Studies of lymphocyte function. *J. Clin. Invest.* **61:** 1071–1080.
3. Giblett, E. L., A. J. Ammann, D. W. Wara, R. Sandman, and L. K. Diamond. 1975. Nucleoside phosphorylase deficiency in a child with severely defective T-cell immunity and normal B-cell immunity. *Lancet* **I:** 1010–1013.
4. Siegenbeek van Heukelom, L. H., G. E. J. Staal, J. W. Stoop, and B. J. M. Zegers. 1976. An abnormal form of purine nucleoside phosphorylase in a family with a child with severe defective T-cell and normal B-cell immunity. *Clin. Chim. Acta.* **72:** 117–124.
5. Stoop, J. W., B. J. M. Zegers, G. F. M. Hendrickx, L. H. Siegenbeek van Heukelom, G. E. J. Staal, P. K. de Bree, S. K. Wadman, and R. E. Ballieux. 1977. Purine nucleoside phosphorylase deficiency associated with selective cellular immunodeficiency. *N. Engl. J. Med.* **296:** 651–655.
6. Cohen, A., D. Doyle, D. W. Martin, Jr., and A. J. Ammann. 1976. Abnormal purine metabolism and purine overproduction in a patient deficient in purine nucleoside phosphorylase. *N. Engl. J. Med.* **295:** 1449–1454.
7. Cohen, A., L. J. Gudas, A. J. Ammann, G. E. J. Staal, and D. W. Martin, Jr. 1978. Deoxyadenosine triphosphate as a potentially toxic metabolite in the immunodeficiency associated with purine nucleoside phosphorylase deficiency. *J. Clin. Invest.* **61:** 1405–1409.
8. Ullman, B., L. J. Gudas, S. M. Clift, and D. W. Martin. 1979. Isolation and characterization of purine-nucleoside phosphorylase deficient T-lymphoma cells and secondary mutants with altered ribonucleotide reductase: genetic model for immunodeficiency disease. *Proc. Natl. Acad. Sci. U. S. A.* **76:** 1074–1078.
9. Cohen, A., R. Hirschhorn, S. O. Horowitz, A. Rubenstein, S. H. Polmar, R. Hong, and D. W. Martin. 1978. Deoxyadenosine triphosphate as a potentially toxic metabolite in adenosine deaminase deficiency. *Proc Natl. Acad. Sci. U. S. A.* **75:** 472–476.
10. Polmar, S. H., R. C. Stern, A. L. Schwartz, E. M. Wetzler, P. A. Chase, and R. Hirschhorn. 1976. Enzyme replacement therapy for adenosine deaminase deficiency and severe combined immunodeficiency. *N. Engl. J. Med.* **195:** 1337–1343.
11. Zegers, B. J. M., J. W. Stoop, G. F. M. Hendrickx, S. K.

Wadman, and G. E. J. Staal. 1979. Purine nucleoside phosphorylase deficiency associated with cellular immunodeficiency: immunological studies during treatment. *In* Inborn Errors of Immunity and Phagocytosis. F. Güttler, J. W. T. Seakins, and R. A. Harkness, editors. MTP Press, Ltd. Lancaster, England. 137–145.

12. Zegers, B. J. M., J. W. Stoop, G. E. J. Staal, and S. K. Wadman. 1979. An approach to the restoration of T cell function in a purine nucleoside phosphorylase deficient patient. *Ciba Found. Symp.* In press.
13. Minakami, W., C. Suzuki, T. Saito, and W. Yoshikawa. 1965. Studies on erythrocyte glycolysis. I. Determination of the glycolytic intermediates in human erythrocytes. *J. Biochem. (Tokyo).* **85:** 543–550.
14. Beutler, E. 1975. *In* Red Cell Metabolism: a Manual of Biochemical Methods. Grune & Stratton, Inc., New York. 2nd edition. 38–70.
15. Kalckar, H. M. 1947. Differential spectrophotometry of purine compounds by means of specific enzymes. *J. Biol. Chem.* **167:** 429–475.
16. Wadman, S. K., P. K. de Bree, A. H. van Gennip, J. W. Stoop, B. J. M. Zegers, G. E. J. Staal, and L. H. Siegenbeek van Heukelom. 1977. Urinary purines in a patient with a severely defective T cell immunity and a purine nucleoside phosphorylase deficiency. *In* Advances in Experimental Medicine and Biology. M. M. Müller, E. Kaiser, and J. E. Seegmiller, editors, Plenum Publishing Corporation New York. **76A:** 471–476.
17. Wadman, S. K., P. K. de Bree, G. F. M. Hendrickx, B. J. M. Zegers, and J. W. Stoop. 1979. Purine nucleoside phosphorylase deficiency associated with cellular immunodeficiency: metabolic studies during treatment. *In* Inborn errors of immunity and phagocytosis. F. Güttler, J. W. T. Seaking and R. A. Harkness, editors. MTP Press, Ltd. Lancaster, England. 147–154.
18. Stoop, J. W., V. P. Eijsvoogel, B. J. M. Zegers, B. Blok-Schut, D. W. Van Bekkum, and R. E. Ballieux. 1976. Selective cellular immunodeficiency: effect of thymus transplantation and transfer factor administration. *Clin. Immunol. Immunopathol.* **6:** 289–298.
19. Beutler, E. 1979. 2,3-Diphosphoglycerate affects enzymes of glucose metabolism in red blood cells. *Nat. New Biol.* **232:** 20–21.
20. Pritchard, J. B., F. Chave-Peon, and R. D. Berlin. 1970. Purines: supply by liver to tissues. *Am. J. Physiol.* **219:** 1263–1267.