Hypoxanthine-Guanine Phosphoribosyltransferase: Characteristics of the Mutant Enzyme in Erythrocytes from Patients with the Lesch-Nyhan Syndrome

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ABSTRACT The Lesch-Nyhan syndrome is characterized clinically by choreoathetosis, spasticity, self-mutilation, and mental and growth retardation. Biochemically, there is a striking reduction of hypoxanthine-guanine phosphoribosyltransferase (HGPRT) activity in affected individuals. We have examined erythrocytes from 14 patients with the Lesch-Nyhan syndrome for the presence of hypoxanthine-guanine phosphoribosyltransferase activity and enzyme protein. In contrast to the usual finding of no detectable hypoxanthine-guanine phosphoribosyltransferase activity, we have found low levels (0.002–0.79 nmoles/mg protein per hr) of hypoxanthine-guanine phosphoribosyltransferase activity in erythrocyte lysates from five of these patients. In three of the five patients, hypoxanthine-guanine phosphoribosyltransferase activity appeared to be substantially more labile in vivo than normal using erythrocytes which had been separated according to their density (age).

Immunochemical studies using a monospecific antiserum prepared from a homogeneous preparation of normal human erythrocyte hypoxanthine-guanine phosphoribosyltransferase revealed immunoreactive protein (CRM) in hemolysate from all 14 patients with the Lesch-Nyhan syndrome. The immunoreactive protein from each patient gave a reaction of complete identity with normal erythrocyte hypoxanthine-guanine phosphoribosyltransferase and was present in quantities equal to those observed in normal erythrocytes. In addition, a constant amount of CRM was found in erythrocytes of increasing density (age) from patients with the Lesch-Nyhan syndrome despite the decreasing hypoxanthine-guanine phosphoribosyltransferase activity.

These studies confirm previous data which indicate that the mutations leading to the Lesch-Nyhan syndrome are usually, if not always on the structural gene coding for hypoxanthine-guanine phosphoribosyltransferase. In addition, although the mutant proteins appear to be present in normal amounts, they are often very labile in vivo with respect to enzymatic activity. These observations suggest that therapy directed at stabilization or activation of enzyme activity in vivo may be of potential benefit.

Received for publication 22 November 1971 and in revised form 4 February 1972.

INTRODUCTION

The Lesch-Nyhan syndrome is a bizarre, X-linked disease characterized by spasticity, choreoathetosis, self-mutilation, mental and growth retardation as well as hyperuricemia and hyperuricaciduria (1) which is due to a striking reduction of hypoxanthine-guanine phosphoribosyltransferase (HGPRT)[1] activity (2). The discovery of a patient with the classical clinical syndrome in whom the reduced hypoxanthine-guanine phosphoribosyltransferase activity was due to an altered affinity for both substrates provided the first evidence for genetic heterogeneity in the Lesch-Nyhan syndrome and demonstrated that the mutation at least in this individual was on the structural gene coding for this enzyme (3). Further studies of the mutant hypoxanthine-guanine phosphoribosyltransferase in cultured skin fibroblasts derived from affected patients provided further evidence for the existence of substantial genetic heterogeneity among patients with the syndrome (4).

In the present study, we have demonstrated the presence of a low level of hypoxanthine-guanine phosphoribosyltransferase activity in circulating erythrocytes from

[1] *Abbreviations used in this paper:* CRM, cross-reactive material; GMP, guanosine monophosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; PP-ribose-P, tetrasodium phosphoribosylpyrophosphate.

some patients with the Lesch-Nyhan syndrome. The studies to be presented not only confirm the heterogeneity of the mutations on the structural gene coding for hypoxanthine-guanine phosphoribosyltransferase in patients with the Lesch-Nyhan syndrome but also describe an additional manifestation of the mutation in some patients: an accelerated half-life of enzyme activity in circulating erythrocytes. Despite the rapid loss of enzyme activity the mutant protein itself appears to exhibit normal stability.

METHODS

Hypoxanthine-8-^{14}C (3.07 mCi/mmole) and guanine-8-^{14}C (6.05 mCi/mmole and 60 mCi/mmole) were purchased from New England Nuclear Corp., Boston, Mass. Tetrasodium phosphoribosylpyrophosphate (PP-ribose-P) was purchased from Sigma Chemical Co., St. Louis, Mo. Purified Noble Agar and Freund's complete adjuvant were purchased from Difco Laboratories, Detroit, Mich. Coomassie blue was purchased from Mann Research Labs, Inc., New York. Methyl phthalate and di-*n*-butyl phthalate came from Eastman Chemical Products, Inc., Kingsport, Tenn. All other reagents were of the highest quality commercially available.

Hypoxanthine-guanine phosphoribosyltransferase was assayed by a previously described radiochemical technique (5). All hemolysates were dialyzed at 4°C against 1 mM Tris buffer pH 7.4 for 2 hr before being assayed. Under the usual assay conditions the final reaction mixture contained 50 mM Tris, 5 mM $MgCl_2$, 1 mM PP-ribose-P, 0.1 mM purine base, and enzyme protein at pH 7.4 in a final volume of 100 μl. The reaction was allowed to proceed for 20 min at 37°C. Hypoxanthine-guanine phosphoribosyltransferase in hemolysate from 13 patients with the Lesch-Nyhan syndrome was assayed under the same conditions except guanine-8-^{14}C of high specific activity (60 mCi/mmole) was used and the incubation time was prolonged to 120 min. Hypoxanthine-guanine phosphoribosyltransferase from patient E. S. unless otherwise indicated was assayed at a final concentration of 10 mM PP-ribose-P for 120 min. Identification of the product of the reaction as guanosine monophosphate (GMP) after incubation of mutant hemolysate with guanine-^{14}C, Mg, and PP-ribose-P was confirmed by ascending chromatography in 0.2 M ammonium formate pH 5.0 on Whatman DE81 (R_f 0.80), descending chromatography in butanol: acetic: water, 2:1:1 on Whatman 1 (R_f 0.19), and high voltage electrophoresis in 0.2 M citrate buffer, pH 3.2 and 0.05 M borate buffer pH 8.5 on Whatman 3 MM. The latter method was employed routinely for separating the products of the reaction. Background (150–250 cpm) was defined by the radioactivity migrating with carrier GMP after incubation of a reaction mixture which was complete except for the absence of PP-ribose-P.

Hypoxanthine-guanine phosphoribosyltransferase was purified to homogeneity from normal human (male) erythrocytes as described previously (6). After DEAE-cellulose chromatography and heat treatment, preparative isoelectric focusing resulted in the isolation of three electrophoretic variants (pKi 5.6, 5.8, 6.0). Each possessed only a single precipitin line on immunoelectrophoresis and was homogeneous when subjected to electrophoresis on analytical polyacrylamide gels at three different cross-linkages. Comparative immunoelectrophoresis and immunodiffusion demonstrated that all were immunologically identical. Undiluted normal human hemolysate is estimated to contain 140 μg/ml of catalytically active hypoxanthine-guanine phosphoribosyltransferase protein based on the recovery of hypoxanthine-guanine phosphoribosyltransferase activity and protein from this purification.

Antiserum was produced in fully grown white male rabbits against each of the highly purified hypoxanthine-guanine phosphoribosyltransferase isoenzymes as well as a partially purified (491 ×) preparation of hypoxanthine-guanine phosphoribosyltransferase. Initially separate rabbits received 0.23, 0.45, and 0.53 mg of electrophoretic variant I, II, and III, respectively, which had been emulsified in Freund's complete adjuvant and injected intradermally in the foot pads and neck. 1 month later the rabbits received a similar injection. Those receiving the partially purified preparation initially were injected in similar sites with 0.9 mg of protein emulsified in Freund's complete adjuvant. Blood was obtained by laceration of the marginal ear vein and was allowed to sit at 4°C for 24 hr. After centrifugation at 10,000 *g* for 15 min at 4°C the antiserum was removed, divided into 1 ml portions, and stored at −70°C.

Direct inhibition of hypoxanthine-guanine phosphoribosyltransferase by antiserum was demonstrated by the addition of a constant amount of heterospecific antiserum (50–250 μl) to an equal volume of serial dilutions of dialyzed hemolysate followed by incubation at 37°C for 30 min and then at 4°C for 16 hr. After centrifugation at 5000 *g* for 20 min at 4°C, the supernatant was assayed for hypoxanthine-guanine phosphoribosyltransferase activity. Incubation of hemolysate with serum obtained from the same rabbits before immunization and processed in a fashion identical to the antiserum did not inhibit hypoxanthine-guanine phosphoribosyltransferase activity. A low level of hypoxanthine-guanine phosphoribosyltransferase activity present in unimmunized and immunized sera was found to be related to the amount of hemolysis occurring during collection of the sample and was subtracted from the final incubation results.

A similar procedure was used to demonstrate the removal of the antibody to hypoxanthine-guanine phosphoribosyltransferase after incubation of mutant hemolysate with antiserum. Undiluted dialyzed mutant hemolysate (50 μl) was added to 50 μl of antiserum and incubated at 37°C for 30 min and at 4°C for 16 hr. After centrifugation at 5000 *g* for 20 min at 4°C, a 50 μl portion of the supernatant was removed and assayed for its ability to inhibit hypoxanthine-guanine phosphoribosyltransferase as described above using 50 μl of a 1:32 dilution of normal hemolysate.

Immunodiffusion and immunoelectrophoresis were performed as previously described (6). The location of hypoxanthine-guanine phosphoribosyltransferase activity in the agar after immunoelectrophoresis was determined by cutting a companion slide (not exposed to antiserum) into 2 mm sections. Each section was placed in 200 μl of 50 mM Tris buffer pH 7.4 at 4°C. After 18 hr a 50 μl portion of the supernatant was assayed as previously described. A modification of the technique of quantitative immunodiffusion was employed to obtain an estimate of hypoxanthine-guanine phosphoribosyltransferase protein in preparations of normal and mutant hemolysates (7). Serial dilutions of the sample containing hypoxanthine-guanine phosphoribosyltransferase protein were made in 50 mM Tris buffer at pH 7.4. Antigen and antiserum (monospecific for hypoxanthine-guanine phosphoribosyltransferase) wells were filled once. Precipitin patterns were allowed to develop for 24 hr at 4°C. Unprecipitated protein was then removed by washing with 0.15 N NaCl for 72 hr at 4°C. Slides were stained with 0.3% Coomassie blue and destained in 45% methanol and 10%

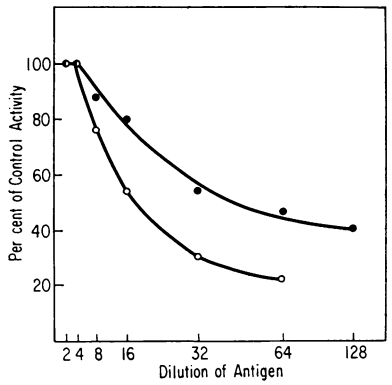

FIGURE 1 Direct inhibition of catalytic activity of normal hypoxanthine-guanine phosphoribosyltransferase (●——●) and hypoxanthine-guanine phosphoribosyltransferase from patient E. S. (○——○) by rabbit antiserum against a partially purified (491 ×) preparation of hypoxanthine-guanine phosphoribosyltransferase. (Antigen hypoxanthine-guanine phosphoribosyltransferase in hemolysate.)

acetic acid. The amount of antigen present in a sample was calculated from the greatest dilution at which a precipitin line was seen.

Erythrocyte fractionation studies were performed while the patients were on the Clinical Research Unit of Duke University Medical Center. All patients were on a diet essentially free of purines and were receiving allopurinol (300 mg/day) for at least 1 wk before the studies were performed. Fractionation of circulating erythrocytes was performed by a slight modification of the method of Danon and Marikovsky (8).[2] After the final centrifugation, all erythrocyte fractions were washed three times with an equal volume of cold 154 mM NaCl, hemolyzed by freeze-thawing, dialyzed for 2 hr at 4°C against 1 mM Tris HCl, pH 7.4, and assayed as described above.

Protein was estimated by the method of Lowry, Rosenborough, Farr, and Randall using crystalline bovine serum albumin as a standard (9).

RESULTS

Low levels of hypoxanthine-guanine phosphoribosyltransferase activity were reproducibly detected in hemolysates from five patients with the Lesch-Nyhan syndrome assayed with guanine-^{14}C as substrate (Table I). In one of these five hemolysates a similar level of hypoxanthine-guanine phosphoribosyltransferase activity was detectable using hypoxanthine-^{14}C of relatively low specific activity as substrate. In the other four hemolysates hypoxanthine-guanine phosphoribosyltransferase activity was substantially less (D. C.) or not detectable (W. E., J. K., and D. G.) using hypoxanthine-^{14}C. Hemolysate from nine other patients with the Lesch-Nyhan syndrome which had been stored from 10 days to over 1 yr had no detectable activity with either guanine or hypoxanthine as substrate.

Antisera produced against both an impure and a homogeneous preparation of normal erythrocyte hypoxanthine-guanine phosphoribosyltransferase were capable of inhibiting both the normal hypoxanthine-guanine phosphoribosyltransferase and the hypoxanthine-guanine phosphoribosyltransferase obtained from patient E. S. (Fig. 1). Although not shown here, 100% inhibition of the catalytic activity of normal hypoxanthine-guanine

TABLE I

Hypoxanthine-Guanine Phosphoribosyltransferase Activity and Enzyme Protein in Hemolysates from Patients with the Lesch-Nyhan Syndrome

Patient	Specific activity*		Duration of storage at −20°C	Inhibition reversal‡
	Guanine	Hypoxanthine		
	nmoles/mg protein/hr		*days*	*% of control*
Normal	98±14	97±19		
Lesch-Nyhan syndrome				
W. E.	0.011	<0.01	0	95
J. K.	0.023	<0.01	0	94
E. S.	0.61	0.79	0	98
D. C.	0.071	0.021	0	90
D. G.	0.038	<0.01	0	100
T. Styer	<0.001	<0.01	>365	91
J. S.	<0.001	<0.01	>365	100
M. W.	<0.001	<0.01	>365	100
S. M.	<0.001	<0.01	>365	100
M. J.	<0.001	<0.01	>365	100
M. Z.	<0.001	<0.01	10	90
C. R.	<0.001	0.013	16	99
T. S.	0.002	<0.01	16	100
M. I.	<0.001	<0.01	10	96

* Results shown with guanine as substrate are the average of two to three determinations for hemolysates from all patients except M. Z., C. R., T. S., and M. I. which were assayed once. The normal value is the mean ±SD in 119 subjects. Subject E. S. is the "Km mutant" reported previously (3); the value reported here for this patient represents specific activity at 1 mM PP-ribose-P. A portion of hemolysate containing 30–40 μg of protein was routinely used for assays of normal hypoxanthine-guanine phosphoribosyltransferase activity while from 300–400 μg of protein was used for assays of mutant hypoxanthine-guanine phosphoribosyltransferase activity in hemolysate.

‡ The values given represent the hypoxanthine-guanine phosphoribosyltransferase activity observed when an equal volume of antiserum and the patient's hemolysate were mixed before being added to a 1:32 dilution of normal hemolysate. Unabsorbed antiserum reduced hypoxanthine-guanine phosphoribosyltransferase activity to 58% of control (uninhibited) activity.

[2] Beardmore, T. D., J. S. Cashman, and W. N. Kelley. 1972. Mechanism of allopurinol-mediated increase in enzyme activity in man. *J. Clin. Invest.* **51**: 1823.

phosphoribosyltransferase could be achieved with the monospecific antiserum. The hypoxanthine-guanine phosphoribosyltransferase activity of all other mutant hemolysates were too low to allow a study of antibody mediated inhibition of enzyme activity. However, hemolysates from all 14 patients with the Lesch-Nyhan syndrome contained cross-reactive material (CRM) which absorbed antibody to the normal hypoxanthine-guanine phosphoribosyltransferase present in the heterospecific antiserum and thereby reversed antibody-mediated inhibition of the normal enzyme (Table I). Similar studies were performed on hemolysate from three patients with the Lesch-Nyhan syndrome using the monospecific antiserum. The CRM in hemolysates from patients E. S., D. C., and D. G. also removed the antibody to normal hypoxanthine-guanine phosphoribosyltransferase present in this monospecific antiserum. Cross-reactive material could also be demonstrated by gel precipitation techniques using a monospecific antiserum for normal hypoxanthine-guanine phosphoribosyltransferase. As shown in Fig. 2, both a normal and mutant hemolysate possessed only a single precipitin arc on immunoelectrophoresis. Assays of a companion slide demonstrated that normal hypoxanthine-guanine phosphoribosyltransferase activity was present only where the precipitin arc subsequently formed. Immunodiffusion of the 14 mutant hemolysates against the monospecific antiserum revealed only a single precipitin line for each mutant hemolysate which in each instance showed a reaction of complete identity with each other and normal hypoxanthine-guanine phosphoribosyltransferase (Fig. 3).

Hypoxanthine-guanine phosphoribosyltransferase activity is relatively similar in erythrocytes of increasing density (age) obtained from normal subjects suggesting that the normal erythrocyte enzyme is stable in vivo. However, the mutant hypoxanthine-guanine phosphoribosyltransferase enzyme in circulating erythrocytes from at least three of the five patients studied with the Lesch-Nyhan syndrome (J. K., D. C., and W. E.) appeared to be unstable when compared with the normal (Fig. 4). In one other patient (D. G.) the level of hypoxanthine-guanine phosphoribosyltransferase activity may also have declined in the densest (oldest) cells. In one patient (E. S.), there was no decrease in activity in the more dense cells suggesting that hypoxanthine-guanine phosphoribosyltransferase activity was relatively stable in this subject.

Quantitative immunodiffusion of a homogeneous preparation of hypoxanthine-guanine phosphoribosyltransferase showed that the technique could detect as little as 0.15 μg of hypoxanthine-guanine phosphoribosyltransferase protein and could reproducibly distinquish changes in concentration of hypoxanthine-guanine phosphoribosyltransferase protein as small as 10%. Using this technique the amount of immunoreactive hypoxanthine-guanine phosphoribosyltransferase protein in normal and mutant

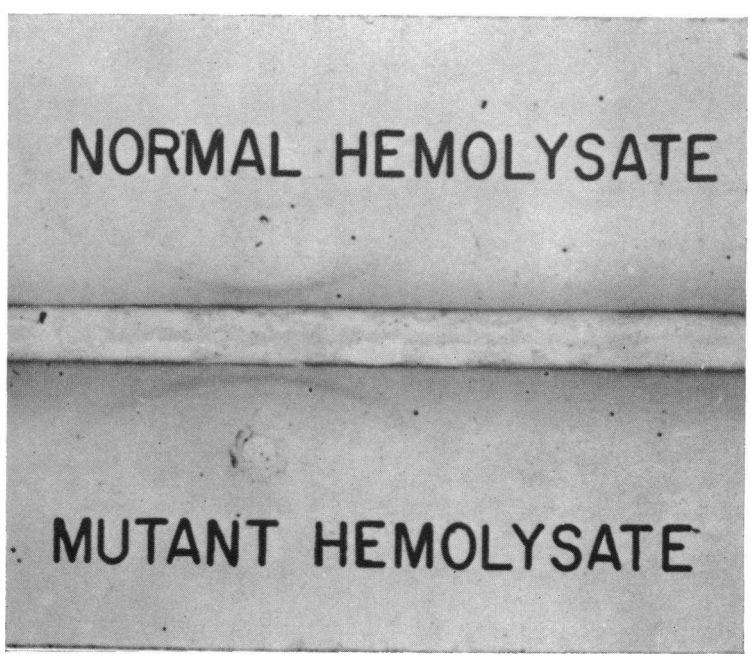

FIGURE 2 Immunoelectrophoresis of normal hemolysate (2 μl; upper well) and hemolysate from a patient with the Lesch-Nyhan syndrome (2 μl; lower well) using antiserum (100–150 μl) specific for normal hypoxanthine-guanine phosphoribosyltransferase. The anode is to the left.

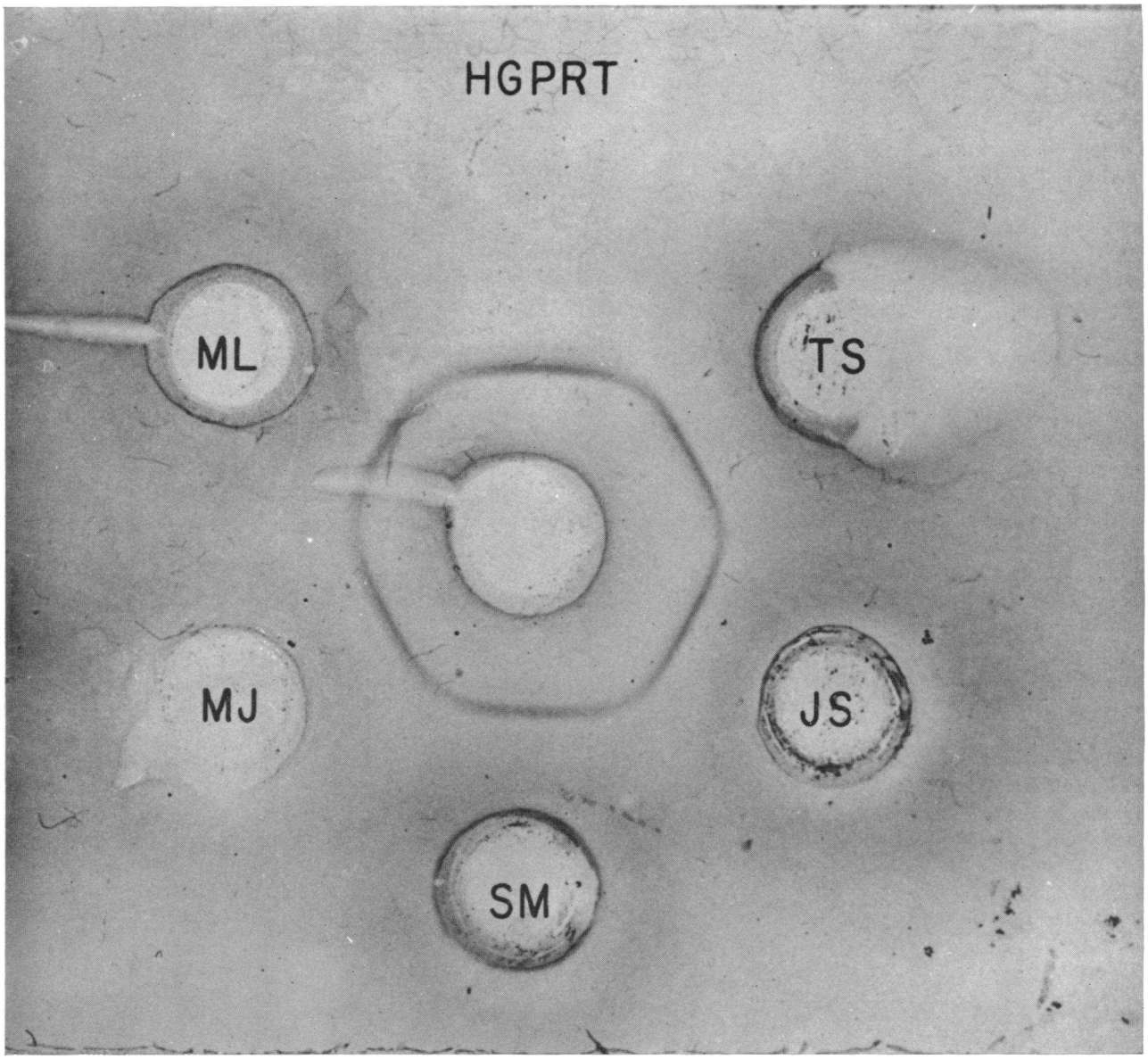

FIGURE 3 Immunodiffusion of normal highly-purified hypoxanthine-guanine phosphoribosyltransferase (HGPRT) (1.0 μg) and hemolysate from five patients with the Lesch-Nyhan syndrome (10 μl) using antiserum specific for normal hypoxanthine-guanine phosphoribosyltransferase. Identical patterns were obtained with hemolysate from nine other patients with the Lesch-Nyhan syndrome.

erythrocytes was the same as that calculated from the purification results (approximately 140 μg hypoxanthine-guanine phosphoribosyltransferase per milliliter hemolysate). Despite the decreased hypoxanthine-guanine phosphoribosyltransferase activity in the most dense erythrocytes (specific gravity > 1.094) as compared with the least dense (specific gravity < 1.086) erythrocytes from four of the five patients with the Lesch-Nyhan syndrome, the amount of CRM was essentially the same in these two groups of cells in all five patients (Table II). In addition, the amount of cross-reactive material did not change with prolonged storage of mutant hemolysate despite a marked decrease in hypoxanthine-guanine phosphoribosyltransferase activity.

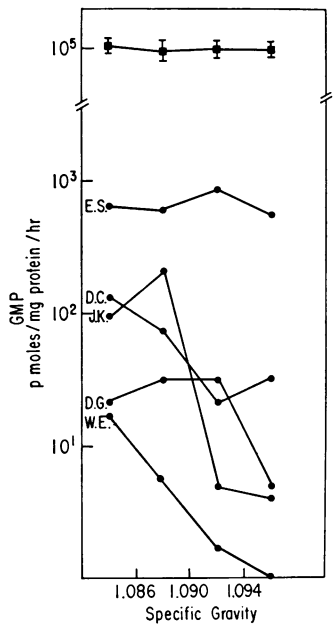

FIGURE 4 Density fractionation of intact circulating erythrocytes from six normal subjects (■———■) and from five patients with the Lesch-Nyhan syndrome (●———●). The results shown for patients with the Lesch-Nyhan syndrome are the average of two separate fractionations. The values shown for the normal subjects represent the mean ±1 SD.

DISCUSSION

The characteristic clinical features of the Lesch-Nyhan syndrome were first recognized in 1964 (1) while the striking deficiency of hypoxanthine-guanine phosphoribosyltransferase in this syndrome was described in 1967 (2). Since that time, many cases have been reported and in most studies essentially no hypoxanthine-guanine phosphoribosyltransferase activity has been found in erythrocyte lysates (10–13). This virtually complete absence of hypoxanthine-guanine phosphoribosyltransferase in patients with the Lesch-Nyhan syndrome has distinguished them from a group of subjects with a partial deficiency of hypoxanthine-guanine phosphoribosyltransferase (0.03 to 30% of normal) who have gout and hyperuricemia but are without serious neurologic disease (5, 14). In the present study, we have found very low but reproducibly detectable levels of hypoxanthine-guanine phosphoribosyltransferase activity in erythrocytes of patients with the Lesch-Nyhan syndrome as have other investigators (15, 16). However, the levels of hypoxanthine-guanine phosphoribosyltransferase activity in hemolysates from patients with the Lesch-Nyhan syndrome are still much lower than observed in most patients with gout who have the "partial" enzyme defect. The low levels of hypoxanthine-guanine phosphoribosyltransferase activity found in some hemolysates from patients with the Lesch-Nyhan syndrome are consistent with the low levels of hypoxanthine-guanine phosphoribosyltransferase activity found in cultured fibroblasts from affected individuals (17, 4).

The stability of the hypoxanthine-guanine phosphoribosyltransferase activity in vivo was examined by density fractionation of intact circulating erythrocytes from normal subjects and patients with the Lesch-Nyhan syndrome. The observation that the hypoxanthine-guanine phosphoribosyltransferase activity in erythrocytes from normal subjects remains constant despite increasing cell age has also been reported by Fox, Wood, and O'Sullivan who used a technique nearly identical to the one used in the present study (18). Also, Rubin, Balis, Piomelli, Berman, and Dancis using a different technique have noted a long half-life for normal hypoxanthine-guanine phosphoribosyltransferase activity (19). The marked reduction of the hypoxanthine-guanine phosphoribosyltransferase activity in the most dense (oldest) erythrocytes from three of five patients with the Lesch-Nyhan syndrome as compared with the least dense (youngest) erythrocytes suggests that the mutant enzyme has an altered half-life in vivo in these three patients (J. K., D. C., and W. E.). This finding is perhaps comparable to previous studies in cultured fibroblasts from patients with the Lesch-Nyhan syndrome in which the hypoxanthine-guanine phosphoribosyltransferase enzyme from seven of eight mutant strains (including cell strains derived from J. K. and W. E.) studied was found to be abnormally labile to heat treatment (4). Significantly, the patient E. S., whose hypoxanthine-guanine phos-

TABLE II

Comparison of Hypoxanthine-Guanine Phosphoribosyltransferase Activity and Enzyme Protein in Erythrocytes of Different Density from Patients with the Lesch-Nyhan Syndrome

Patient	Hypoxanthine-guanine phosphoribosyltransferase activity (Erythrocytes of density >1.094/erythrocytes of density <1.086) × 100	Cross-reactive material (Erythrocytes of density >1.094/erythrocytes of density <1.086) × 100
	%	%
Normal	93	100
Lesch-Nyhan syndrome		
E. S.	93	100
D. C.	23	100
D. G.	25	100
W. E.	6	90
J. K.	4	90

phoribosyltransferase activity in cultured fibroblasts exhibited normal heat stability (4), also was found to have a normal half-life in vivo by density fractionation of erythrocytes. In addition, after 60 days of storage at −70°, undiluted hemolysate from patient D. C. had only 20% of control hypoxanthine-guanine phosphoribosyltransferase activity while hemolysate from patient E.S. and a normal retained essentially 100% of control activity. Several structural variants of glucose-6-phosphate dehydrogenase (A^-, Gd^{A-}, $Gd^{Mediterranean}$) have also been shown to have an abnormal half-life in vivo by fractionation of circulating erythrocytes (20, 21).

Material which cross-reacts with an antibody to normal hypoxanthine-guanine phosphoribosyltransferase was demonstrated in hemolysates from patients with the Lesch-Nyhan syndrome by three different techniques. Direct antibody-mediated inhibition of the catalytic activity of hypoxanthine-guanine phosphoribosyltransferase was shown for both the normal enzyme and the enzyme from patient E. S. which was the only mutant form of the enzyme with sufficient activity to allow this type of study. Preincubation of this antiserum with hemolysates from 14 different patients with the Lesch-Nyhan syndrome decreased the antibody-mediated inhibition of the normal hypoxanthine-guanine phosphoribosyltransferase. This indicates that in each of these hemolysates material was present which was capable of reacting with the hypoxanthine-guanine phosphoribosyltransferase antibody such that it was no longer free to inhibit the normal enzyme. Rubin, Dancis, Yip, Nowinski, and Balis have recently reported a similar finding in four patients with the Lesch-Nyhan syndrome (22). In addition, immunodiffusion and immunoelectrophoresis using antiserum monospecific for normal hypoxanthine-guanine phosphoribosyltransferase provided independent confirmation of the presence of CRM in hemolysates from all 14 patients and indicated that each possessed a reaction of complete identity with the normal enzyme. From these studies it is apparent that the low levels of catalytic activity for hypoxanthine-guanine phosphoribosyltransferase in erythrocytes from patients with the Lesch-Nyhan syndrome are not due to a comparable deficiency of hypoxanthine-guanine phosphoribosyltransferase protein.

The relatively short half-life of hypoxanthine-guanine phosphoribosyltransferase activity in at least three patients with the Lesch-Nyhan syndrome does not appear to represent an accelerated degradation of enzyme protein but rather an accelerated loss of enzyme function. This is suggested by the finding of normal quantities of CRM in hemolysate from all 14 patients with the Lesch-Nyhan syndrome. More direct evidence for this hypothesis is provided by the finding of a constant amount of CRM in young and old erythrocytes from patients with the Lesch-Nyhan syndrome as well as from normal subjects. To our knowledge this phenomenon has not been shown previously for a mammalian protein. However, it is similar in some respects to the studies of Gershon and Gershon who used immunochemical techniques to identify two different pools of isocitrate lyase protein in nematodes; one enzymatically active, the other inactive (23). With increasing age the latter pool grew larger at the expense of the former. The authors concluded that this represented a postgenetic modification leading to the inactivation of a large proportion of the enzyme molecules in the older organism.

The finding of hypoxanthine-guanine phosphoribosyltransferase protein with relatively labile activity in the Lesch-Nyhan syndrome may be of therapeutic importance. The possibility exists that the defective hypoxanthine-guanine phosphoribosyltransferase protein could be activated or that the active enzyme could be stabilized. This approach to therapy could be assessed serially by direct assay of hypoxanthine-guanine phosphoribosyltransferase in circulating erythrocytes separated by their density.

ACKNOWLEDGMENTS

The authors acknowledge the assistance of Dr. H. Sage with the immunological techniques and Doctors J. E. Seegmiller and P. J. Benke for their generous gift of hemolysates from several patients with the Lesch-Nyhan syndrome.

This work was supported by U. S. Public Health Service Grants AM14362 and AM05620, and Cerebral Palsy Grant R-247-71.

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