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Research Article

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Inosinic Acid Dehydrogenase Activity in the Lesch-Nyhan Syndrome

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ABSTRACT Inosinic acid dehydrogenase was evaluated in normal subjects and in patients with the Lesch-Nyhan syndrome. A significant difference in activity was found between erythrocytes derived from normal controls (1.21±0.47 pmoles/hr per mg protein) and from 15 patients with the Lesch-Nyhan syndrome (6.72 ±6.23 pmoles/hr per mg protein). However, no difference in activity was demonstrable in muscle or leukocytes derived from normal and Lesch-Nyhan patients. The increased activity of inosinic acid dehydrogenase in erythrocytes from patients with the Lesch-Nyhan syndrome is due to stabilization of the enzyme in vivo as well as the absence of an inhibitor which is present in erythrocytes from normal subjects.

INTRODUCTION

Individuals with the Lesch-Nyhan syndrome exhibit a functional deficiency of hypoxanthine-guanine phosphoribosyltransferase (HGPRT)[1] which is necessary for the phosphoribosylpyrophosphate (PP-ribose-P)-dependent formation of inosine-5′-monophosphate (IMP) and guanosine-5′-monophosphate (GMP) from the purine bases hypoxanthine and guanine, respectively (1). Clinically, the defect is manifested by hyperuricemia and hyperuricaciduria, self-mutilation, choreoathetosis, spasticity, and mental retardation (2).

In addition to a deficiency of hypoxanthine-guanine phosphoribosyltransferase, several other biochemical abnormalities have been consistently described in patients with the Lesch-Nyhan syndrome. These include an accelerated rate of purine biosynthesis (2), increased intracellular levels of PP-ribose-P in both erythrocytes (3) and cultured skin fibroblasts (4), and increased activity of the enzyme adenine phosphoribosyltransferase in erythrocytes (1, 5). All of these effects may be interpreted as secondary manifestations of the deficiency of hypoxanthine-guanine phosphoribosyltransferase (5).

In this paper we describe a significant and consistent elevation of activity of the enzyme inosinic acid dehydrogenase in erythrocyte lysates from patients with the Lesch-Nyhan syndrome. Inosinic acid dehydrogenase (IMP: NAD^+ oxidoreductase, E.C. 1.2.1.14) catalyzes the nicotine adenine dinucleotide (NAD^+)-dependent conversion of IMP to xanthosine-5′-monophosphate (XMP) and represents the first reaction unique to the synthesis *de novo* of GMP from IMP. This enzyme, which had not previously been assayed in human tissues, should play a critical role in the Lesch-Nyhan syndrome. In the absence of hypoxanthine-guanine phosphoribosyltransferase activity, the pathway initiated by IMP dehydrogenase represents the only known mechanism for the production of guanine nucleotides. Several different factors appear to be responsible for the observed increase in enzyme activity.

METHODS

Hypoxanthine-8-^{14}C (4.2, 52.0, and 60.1 mCi/mmole) was obtained from New England Nuclear Corp., Boston, Mass., Schwarz Bio Research Inc., Orangeburg, N. Y., and Amersham/Searle Corp., Arlington Heights, Ill., respectively. Nicotine adenine dinucleotide (NAD^+) was purchased from Sigma Chemical Company, St. Louis, Mo. The dimagnesium salt of PP-ribose-P and the tetra sodium salt of PP-ribose-P were obtained from P-L Biochemicals, Inc., Milwaukee,

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[1] *Abbreviations used in this paper:* GMP, guanosine-5′-monophosphate; HGPRT, hypoxanthine-guanine phosphoribosyltransferase; IMP, inosine-5′-monophosphate; NAD^+, nicotine adenine dinucleotide; PP-ribose-P, phosphoribosylpyrophosphate; XMP, xanthosine-5′-monophosphate.

Wis., and Sigma, respectively. All other reagents were of the highest purity commercially available.

Five patients (E. S., J. K., W. E., D. C., and D. G.) with the Lesch-Nyhan syndrome were admitted to the Clinical Research Center at Duke Hospital where they were maintained on a diet essentially free of purines containing 1800 k-cal and 70 g protein. The only medications were allopurinol and diazepam which were found to have no effect on inosinic acid dehydrogenase levels. Hemolysates were also obtained from 10 other patients with the Lesch-Nyhan syndrome. These samples were stored at −20°C for varying periods of time before assay.

Blood obtained by venipuncture was twice washed with normal saline and centrifuged at 1200 *g* for 10 min. The washed, packed erythrocytes were frozen at −20°C, thawed, and the resulting hemolysate dialysed against 0.05 M sodium phosphate buffer, pH 7.4, for 2 hr at 4°C. All hemolysates obtained from normal volunteers were prepared in a similar manner and assayed within 2 wk unless otherwise specified.

Muscle tissue from patients with the Lesch-Nyhan syndrome was obtained by biopsy while muscle tissue from subjects with normal HGPRT activity was obtained either by biopsy or at autopsy. Enzyme activity was comparable in muscle obtained by either technique. Muscle tissue was homogenized in 10 vol of 0.05 M sodium phosphate buffer, pH 7.4. The homogenate was centrifuged at 13,000 *g* for 30 min and the supernate dialyzed in 0.05 M sodium phosphate buffer for 4 hr at 4°C.

Leukocytes were prepared by a modification of the method of Chodirker, Bock, and Vaughn (6). Venous blood (25 ml) was drawn and immediately mixed with 475 ml of 0.85% NaCl with 0.1% bovine serum albumin. After centrifugation at 800 *g* for 30 min, the supernate was discarded and the pellet mixed with 100 ml of 0.2% NaCl for 20 sec and then restored to isotonicity by the addition of 100 ml of 1.61% NaCl. The cells were centrifuged at 400 *g* for 10 min and the lysis procedure was repeated once. The cells obtained from this procedure were washed twice in cold isotonic saline. After the final wash the white cell pellet was dissolved in 0.01 M Tris buffer pH 7.4 (80 mg wet weight per ml). The cells were lysed by freezing and thawing twice in a dry ice and acetone bath. All leukocyte preparations were assayed for inosinic acid dehydrogenase within 24 hr of venipuncture.

Erythrocytes were fractionated according to their density after the method of Danon and Marikovsky (7). These workers have demonstrated a linear relation between red cell density and age in vivo. Additionally, their findings with ^{59}Fe-labeling in vivo suggest very little cross-contamination of old with young cells using this procedure. Methyl phthalate and di-*n*-butyl phthalate were mixed in different proportions to yield fluids with specific gravities of 1.090, 1.086, and 1.094. Freshly drawn, heparinized blood was placed in a Nalgene centrifuge tube (Nalge Co., Rochester, N. Y.) and 2 ml of the phthalate mixture of specific gravity 1.090 was layered on top of the whole blood. This was then centrifuged at 12,000 *g* for 90 min at 15°C effecting the primary separation. After removal of the plasma and buffy coat, the top and bottom layers were transferred to two separate centrifuge tubes. To the tube containing the top layer, 1 ml of the phthalate mixture of specific gravity 1.086 was added; 1 ml of phthalate of specific gravity 1.094 was added to the tube containing the bottom layer. These were again centrifuged at 12,000 *g* for 90 min at 15°C. The four layers which resulted from this procedure were washed three times with normal saline and the cells lysed by freezing and thawing twice. The hemolysates were then dialyzed in 0.05 M sodium phosphate, pH 7.4, for 2 hr and assayed.

Inosinic acid dehydrogenase activity was determined by quantitating the production of xanthosine monophosphate (XMP)-^{14}C utilizing modifications of the assays developed by Salser and Balis (8) and Saccocia and Miech (9). The assay was optimized with regard to substrate concentration and cation requirement and contained 0.01 μmoles IMP-^{14}C, 0.1 μmoles NAD^+, 10 μmoles KCl, 4 μmoles sodium phosphate buffer, pH 7.4, and the specified amount of the enzyme preparation to be assayed in a total volume of 100 μl. The reaction was initiated by placing the tubes containing the reaction mixture in a water bath at 37°C. After incubation for 2 hr at 37°C the reaction was stopped by the addition of 100 μl of cold 95% ethanol. Protein was removed by centrifugation at 1200 *g* for 15 min. 50 μl of the supernate was spotted on Whatman DE-81 paper with 0.1 μmoles of carrier XMP and developed in an ascending system for 4 hr in 0.2 M ammonium formate buffer, pH 5.0 (R_f: XMP, 0.32; IMP, 0.72). After development, the spot containing XMP was detected with an ultraviolet light source, cut out, and counted at 63% efficiency in a Packard Tri-Carb liquid scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Guanosine monophosphate (GMP) was separated from IMP and XMP by high voltage electrophoresis in 0.05 M sodium citrate buffer, pH 3.53. Identity of XMP was confirmed by ascending chromatography in ethanol: 1.0 M ammonium acetate, pH 5.0 70:30; (R_f: 0.11) and high voltage electrophoresis on Whatman 3MM paper in both 0.05 M sodium borate, pH 8.8, and 0.05 M sodium citrate, pH 3.53. XMP production, under these assay conditions, was linear with respect to incubation time from 15 min to 3 hr and protein concentration from 1.25 to 12.5 mg/0.10 ml using crude hemolysate.

Adenine phosphoribosyltransferase and HGPRT activity were assayed by the method of Kelley, Rosenbloom, Henderson, and Seegmiller (10).

IMP-8-^{14}C of sufficient purity and specific radioactivity for this study was not commercially available. Therefore, IMP-8-^{14}C was enzymatically synthesized from hypoxanthine-8-^{14}C using a partially purified preparation of HGPRT prepared as described below. The reaction mixture contained 1.0 μmoles of hypoxanthine-8-^{14}C, 2.0 μmoles of PP-ribose-P, 5 μmoles of Mg^{++}, 10 μmoles of Cl^-, 0.08 μmoles of Tris HCl, pH 7.4, and 1.6 mg of partially purified human HGPRT with a specific activity of 9000 nmoles/mg per hr in a final volume of 0.650 ml. The reaction mixture was incubated for 90 min at 37°C, and the reaction was stopped by the addition of 500 μl of 95% ethanol and 25 μl of 0.1 M Na_2 EDTA. Protein was removed by heating for 90 sec in a boiling water bath and centrifugation for 30 min at 10,000 rpm. The IMP thus formed was stored at −20°C and used within 2 wk. The radioactive product formed was 97% IMP and 3% inosine. Identity of the major product as inosinic acid was confirmed by co-chromatography with authentic IMP in two ascending chromatography systems (1.0 M ammonium acetate, pH 5.0: 95% ethyl alcohol, 70:30 on Whatman 3 MM [R_f: 0.24]; and 0.2 M ammonium formate pH 5.0 on Whatman DE81, [R_f: 0.72]); and by high voltage electrophoresis in 0.05 M sodium borate pH 8.8. The ultraviolet absorption spectrum of the IMP-^{14}C synthesized in this manner was identical to that of a commercial sample of IMP in H_2O adjusted to pH 1.0 by addition of HCl. The final concentration of IMP produced was calculated using the molar extinction coefficient. Additionally, the concentration of IMP-^{14}C was calculated from

the specific radioactivity assuming no change from that of the hypoxanthine-8-^{14}C used as substrate. Both methods gave comparable values.

Inosinic acid dehydrogenase and HGPRT were partially purified from human erythrocytes as follows. Crude hemolysate (300 ml) was absorbed to 72 g of DEAE-cellulose at pH 7.0 after the method of Hennessey, Waltersdorph, Heunnekens, and Gabrio (11). The DEAE-cellulose was washed twice with 1500 ml of 0.003 M sodium phosphate buffer, pH 7.0, followed by two washes with 100 ml of 0.05 M sodium phosphate buffer pH 7.4 which removed the hemoglobin. The DEAE-cellulose with remaining adsorbed protein was poured into a 10 × 40 cm column, and eluted with a 2000 ml linear gradient of 0–0.3 M KCl in 0.05 M sodium phosphate buffer, pH 7.4. Fractions (10 ml) were collected and assayed for IMP dehydrogenase and HGPRT activity. Fractions containing approximately 75% of the total enzyme activity were pooled and concentrated above an Amicon ultrafilter (Amicon Corp., Lexington, Mass., UM10).

Protein was estimated by the method of Lowry, Rosenbrough, Farr, and Randall (12).

Multivariate analysis of variance was performed on the Xerox Sigma 5 Computer after the method of Starmer and Grizzle (13).

RESULTS

Inosinic acid dehydrogenase and HGPRT activities in hemolysates from normal subjects and from patients with the Lesch-Nyhan syndrome are compared in Table I. Hemolysates from 23 normal subjects had a mean inosinic acid dehydrogenase activity of 1.21±0.47 (SD) pmoles/hr per mg protein. Hemolysates from patients with the Lesch-Nyhan syndrome yielded a mean activity of 6.72±6.23 (SD). This difference was significant at the 0.001 level (Student's two-tailed *t* test). In 14 of the 15 Lesch-Nyhan patients, the values observed were greater than 2 standard deviations above the mean of the normal values even though some of these samples had been stored for more than 2 yr.

TABLE I

Specific Activity of Inosinic Acid Dehydrogenase in Dialyzed Erythrocyte Lysates from Patients Deficient in Hypoxanthine-Guanine Phosphoribosyltransferase

Subjects	Duration of storage (−20°C) of dialyzed hemolysate	HGPRT	Inosinic acid dehydrogenase
	days	*nmoles/hr per mg protein*	*pmoles/hr per mg protein*
Normal	0–14	98±14 (SD)*	1.21±0.47 (SD)‡ (range 0.31–2.08)
Lesch-Nyhan (15)			6.72±6.23 (SD)
E. S.	12	0.05	4.40
J. K.	4	<0.001	2.57
W. E.	96	<0.001	7.64
T. H.	21	<0.001	6.40
R. C.	118	<0.001	4.63
Ma. I.	112	<0.001	4.12
Mi. I.	112	<0.001	1.30
T. S.	118	<0.001	5.10
M. W.	>2 yr	<0.001	2.78
M. J.	—	<0.001	7.21
S. M.	>2 yr	<0.001	9.80
D. C.	4	<0.001	10.40
J. S.	>2 yr	<0.001	3.30
T. S.	>2 yr	<0.001	3.14
D. G.	40	0.004	28.0

* 119 subjects.

‡ 23 subjects.

Activity of those Lesch-Nyhan hemolysates assayed soon after phlebotomy (E. S., and J. K., and D. C.) compare well with those that had been stored in the frozen state for long periods. Reassay of these hemolysates after periods of storage at −20°C up to 2 months gave results consistent with the initial assay. Normal hemolysate reassayed after 6 months of storage at −20° showed 73% of initial activity. No increase in enzyme activity was noted with storage of hemolysate from normal or Lesch-Nyhan subjects.

Inosinic acid dehydrogenase activity was determined in muscle and in leukocytes in both normal and Lesch-Nyhan subjects. Results are detailed in Table II. The mean value in muscle obtained from four Lesch-Nyhan patients was 268 pmoles/hr per mg protein, whereas the mean value for four normal subjects was 324 pmoles/hr per mg protein. Evaluation of enzyme activity in leukocytes revealed a mean value of 747 pmoles/hr per mg protein in 10 normal subjects. Two Lesch-Nyhan subjects exhibited a mean value of 598 pmoles/hr per mg protein. There was no statistically significant difference in inosinic acid dehydrogenase activity in leukocytes or muscle tissue between the two groups.

TABLE II

Comparison of Inosinic Acid Dehydrogenase Activity in Leukocytes and Muscle in Normal Subjects and Patients with the Lesch-Nyhan Syndrome

	Striated muscle	Leukocytes
	pmoles/hr per mg protein ±SD	
Normal	324±224 (4)*	747±243 (10)
Lesch-Nyhan syndrome	268±43 (4)	598±322 (2)

* Number of subjects indicated in parentheses.

The possible mechanisms underlying the difference in inosinic acid dehydrogenase activity of erythrocytes from normal and Lesch-Nyhan individuals were investigated. In all assays, radiolabeled IMP remaining at the completion of the incubation was counted to determine the extent of substrate utilization by alternate pathways and no difference was noted in hemolysates from normal and Lesch-Nyhan subjects. To evaluate the possibility that the product, XMP, was being con-

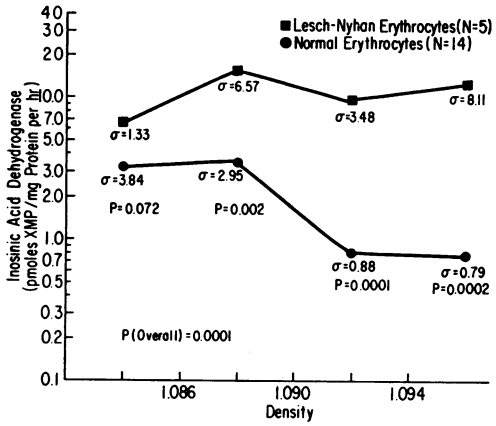

FIGURE 1 Inosinic acid dehydrogenase activity in erythrocytes fractionated by density (age). Normal, ●———●; Lesch-Nyhan syndrome, ■———■; *P* values as determined by multivariate analysis of variance are given for the differences between normal and Lesch-Nyhan subjects at each density, and for the curves overall. σ, standard deviation at each point.

verted to GMP, the formation of GMP-^{14}C was determined. Under standard assay conditions, no GMP-^{14}C was formed in hemolysates from either normal or Lesch-Nyhan subjects.

Michaelis constants for both substrates of inosinic acid dehydrogenase were determined for the enzyme derived from patients with the Lesch-Nyhan syndrome and for the enzyme derived from normal hemolysate. A partially purified preparation of the enzyme present in erythrocytes was used in both cases with the nonvariable substrate at saturating concentrations. The Michaelis constant was estimated from double reciprocal plots. For the normal enzyme the K_m (IMP) was 5.0×10^{-5} M and the K_m (NAD^+) was 2.0×10^{-4} M. For the Lesch-Nyhan enzyme the K_m (IMP) was 7.7×10^{-5} M while the K_m (NAD^+) was 3.4×10^{-4} M. No significance was attached to these differences.

Fresh erythrocytes from normal subjects and from patients with the Lesch-Nyhan syndrome were fractionated into four groups of increasing density (age) as described in methods. The relationship between erythrocyte age in vivo and inosinic acid dehydrogenase activity is presented in Fig. 1. Inosinic acid dehydrogenase activity was higher in each cell fraction in patients with the Lesch-Nyhan syndrome as compared to normal. Multivariate analysis of variance was performed to assess the significance of the observed difference between normal and Lesch-Nyhan patients at each point and of the curves as a whole. Differences yielded significant *P* values for all points except for the lowest density fraction (youngest cells) where no significant difference between the means was observed. The overall difference between the curves was significant at the 0.0001 level. These results suggest that inosinic acid dehydrogenase is stabilized in vivo in erythrocytes from patients with the Lesch-Nyhan syndrome. Alternatively, these findings could be explained by accumulation of an "inhibitor" in the normal erythrocytes with age.

Further evidence for stabilization of inosinic acid dehydrogenase in erythrocytes from subjects with the Lesch-Nyhan syndrome was obtained from a study of the heat stability of the enzyme in vitro. Enzyme activity present after heating at 60°C for 4 min was compared with the unheated control. In hemolysates derived from four patients with the Lesch-Nyhan syndrome, 64.5±6.1% (SD) of the original activity remained after heating. Only 38.8±9.6% (SD) of the control activity was present after heating hemolysates from four normal subjects.

To further assess possible mechanisms responsible for this stabilization in vivo, protection of inosinic acid dehydrogenase against thermal denaturation was studied using a preparation of the enzyme partially purified from a normal subject. The effect of substrates for the enzyme as well as PP-ribose-P in concentrations found in normal individuals (5×10^{-6} M) and in patients with the Lesch-Nyhan syndrome (5×10^{-5} M) (3) was investigated. PP-ribose-P at concentrations exceeding those found in vivo did not stabilize the enzyme. IMP and NAD^+ provided moderate protection of the enzyme against heat inactivation at the concentrations used in the enzyme assay (Fig. 2). However, it is not known if the concentration of these compounds is altered in patients with the Lesch-Nyhan syndrome as compared to normal subjects, or if the stabilization observed in vitro can be extrapolated to the situation in vivo. Preliminary attempts to measure the intracellular concentration of IMP both in cultured fibroblasts (4) as well as in circulating erythrocytes were not helpful since the levels of IMP in both cell

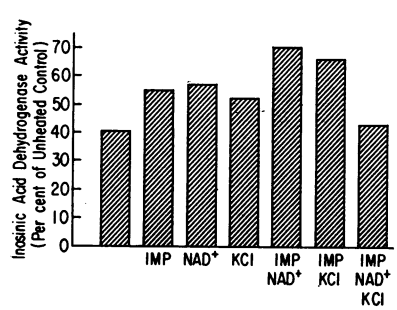

FIGURE 2 Effect of substrates for inosinic acid dehydrogenase on inactivation of the partially purified enzyme at 70°C for 8 min; IMP, 1.5×10^{-4} M; NAD^+, 1.0×10^{-3} M; and KCl, 1×10^{-1} M.

types were below the level of detectability in our assay. Accordingly, the exact factors responsible for the stabilization in vivo and in vitro remain unclear.

Another mechanism for the difference in inosinic acid dehydrogenase activity in erythrocytes between normal and Lesch-Nyhan subjects was suggested when it was noted that during purification of the normal enzyme, substantially more total activity was recovered in the purified form than had been assayable in the hemolysate dialyzed for 2 hr. This phenomenon appeared much less striking when the enzyme from Lesch-Nyhan hemolysate was treated in a similar fashion. For example, enzyme activity from normal erythrocytes was accentuated by DEAE-cellulose chromatography whereas the enzyme from Lesch-Nyhan hemolysate exhibited no activation (Table III). Similar results were observed with hemolysate from other normal and Lesch-Nyhan subjects.

Prolonged dialysis against 0.05 M sodium phosphate buffer pH 7.4 was found to be another method of "activating" the enzyme present in normal hemolysate. Fig. 3 demonstrates the results of dialyzing hemolysates at 4°C from normal and from Lesch-Nyhan subjects in the same bath for periods up to 72 hr. While the normal enzyme is activated to the same extent as was observed during partial purification, the Lesch-Nyhan-derived enzyme exhibited only minimally increased activity.

These results suggested that an "inhibitor" of inosinic acid dehydrogenase activity is present in normal hemolysate but not in hemolysate from patients with the Lesch-Nyhan syndrome. To further test this hypothesis, normal and Lesch-Nyhan hemolysates were dialyzed for 2 hr against 0.05 M sodium phosphate buffer, pH 7.4, in the same bath, and then assayed, alone and in combination. Activity observed in the mixture of the two enzyme preparations was 44% of that expected when the two were assayed independently. Assay of inosinic acid dehydrogenase activity in the presence of partially purified HGPRT did not suppress enzyme activity. Accordingly, the lower inosinic acid dehydrogenase activity in erythrocytes from normal individuals could not be attributed to inhibition by HGPRT which is defective in individuals with the Lesch-Nyhan syndrome.

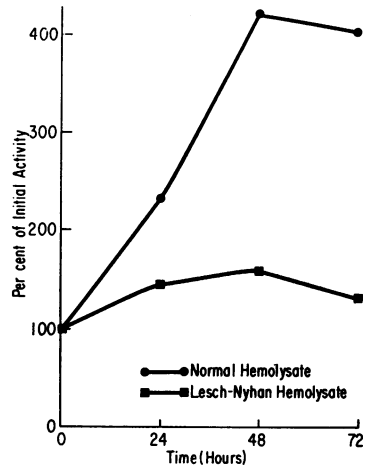

FIGURE 3 Effect of prolonged dialysis in 0.05 M sodium phosphate, pH 7.4, on inosinic acid dehydrogenase activity. Activity is compared to initial sample dialyzed for 2 hr. Normal, ●———●; Lesch-Nyhan syndrome, ■———■. Samples were dialyzed in the same bath which was changed every 24 hr.

Hemolysate from three normal patients and one Lesch-Nyhan patient was incubated for 30 min at 4°C in 0.05 M sodium phosphate buffer, pH 7.4, which contained either 2×10^{-3} M reduced glutathione (GSH), 1×10^{-3} M dithiothreitol (DTT), or no sulfhydryl reducing agent. The activity of inosinic acid dehydrogenase in the hemolysates from normal and Lesch-Nyhan patients was not altered by the sulfhydryl reducing agents. This suggests that the difference in inosinic acid dehydrogenase activity cannot be attributed to a difference in the state of oxidation of the sulfhydryl groups of the enzyme in these two groups of subjects.

TABLE III

Comparison of DEAE-Cellulose Chromatography on Inosinic Acid Dehydrogenase Activity in Erythrocytes from Normal Subjects and Patients with the Lesch-Nyhan Syndrome

	Hemolysate	DEAE-cellulose chromatography	Per cent of initial activity
Normal	579*	1,960	338
Lesch-Nyhan syndrome	10,600	7,080	67

* Activity expressed as total recovery in picomoles per hour.

DISCUSSION

Inosinic acid dehydrogenase catalyzes the NAD^+-dependent conversion of IMP to XMP and represents the first reaction unique to the synthesis of GMP *de novo* from IMP (14). The enzyme derived from nonhuman sources has been shown to be subject to feedback inhibition by GMP (15) and can be repressed in bacterial systems (16). However, no previous studies have been performed on the human enzyme. In the present study we have described a radiochemical assay for inosinic acid dehydrogenase which for the first time has permitted direct assay of this enzyme in human tissue. In addition, we have demonstrated that individuals with the

Lesch-Nyhan syndrome and a functional deficiency of HGPRT have significantly elevated levels of inosinic acid dehydrogenase in circulating erythrocytes.

At least two mechanisms are apparently responsible for this observed elevation of inosinic acid dehydrogenase activity. One mechanism contributing to the increased inosinic acid dehydrogenase activity in the Lesch-Nyhan syndrome is the absence of an inhibitor which is present in normal hemolysate. The exact nature of this inhibitor remains unestablished. Preliminary studies indicate that the inhibitor is not HGPRT. It can be removed by anion exchange chromatography or by prolonged dialysis of the untreated hemolysate. Although the mechanism of action of the inhibitor is unknown, it does not appear to inhibit inosinic acid dehydrogenase by altering the oxidation state of the sulfhydryl groups on the enzyme.

A second mechanism appears to be stabilization of the enzyme in circulating erythrocytes. This is similar to the mechanism thought to be responsible for the increased activity of adenine phosphoribosyltransferase in erythrocytes from patients with the Lesch-Nyhan syndrome (17, 18). However, stabilization does not appear to be due to the increased concentration of PP-ribose-P which is postulated to be responsible for the increased adenine phosphoribosyltransferase activity (18). At the present time, the exact basis for enzyme stabilization remains unclear.

The observation that inosinic acid dehydrogenase activity is elevated in erythrocytes from patients with the Lesch-Nyhan syndrome could be potentially important. Inosinic acid dehydrogenase catalyzes the first of two sequential steps leading to the synthesis of GMP (Fig. 4). In the absence of HGPRT activity this pathway represents the only known mechanism for GMP synthesis. Should the activity of inosinic acid dehydrogenase also be increased in tissues other than erythrocytes in patients with the Lesch-Nyhan syndrome, as for example in the central nervous system, this could perhaps provide the Lesch-Nyhan patient with an enhanced capacity for synthesizing GMP *de novo*. This adaptation could help in overcoming a deleterious cellular deficiency of GMP and could account for the wide variability in clinical manifestations observed in patients with this syndrome (5). This interpretation would focus attention on the second enzyme in the pathway, XMP aminase, which under these conditions could become the rate-limiting step. This would be consistent with the recent observation that glutamine, an essential substrate for XMP aminase, increases the conversion of adenine to GMP in fibroblasts cultured from patients with the Lesch-Nyhan syndrome (19). Glutamate, which is presumably converted to glutamine, has also been reported to improve the

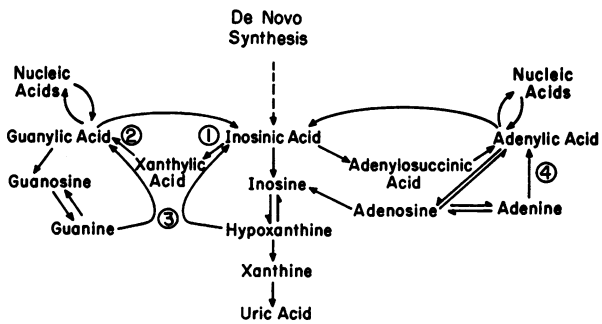

FIGURE 4 Interconversion of purine nucleotides. 1. Inosinic acid dehydrogenase; 2. XMP aminase; 3. Hypoxanthine-guanine phosphoribosyltransferase (HGPRT); and 4. Adenine phosphoribosyltransferase.

neurological and behavioral abnormalities in the Lesch-Nyhan syndrome (20). Our finding that levels of inosinic acid dehydrogenase activity in nucleated cells such as leukocytes and muscle do not appear to be significantly elevated in the Lesch-Nyhan syndrome suggests that the elevated activity may be limited to the erythrocyte. Though this would tend to detract from the overall significance of the finding, the increased activity in the red cell pool could still contribute substantially to the synthesis of GMP in the Lesch-Nyhan patient.

Finally, the finding of a second enzyme with increased activity in the Lesch-Nyhan syndrome, which is presumably due in some manner to an indirect effect of a primary deficiency of HGPRT, should instill caution in the interpretation of increased enzyme activity in diseases of unknown etiology. For example, in a disease such as acute intermittent porphyria, great significance has been attached to the finding of increased activity of the enzyme delta-aminolevulinic acid synthetase (21). Indeed, data are now being presented which suggest that the increased enzyme activity observed in this illness is not the primary genetic aberration but more likely represents a secondary manifestation of the basic enzyme defect (22, 23).

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