JCI The Journal of Clinical Investigation

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J Clin Invest. 1968;47(10):2281-2289. https://doi.org/10.1172/JCI105913.

Research Article

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Adenine Phosphoribosyltransferase Deficiency: A Previously Undescribed Genetic Defect in Man

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ABSTRACT A deficiency of adenine phosphoribosyltransferase (A-PRTase) is described in four members in three generations of one family. A-PRTase is coded by an autosome and the mutants described in this report are heterozygotes for this enzyme defect. The level of enzyme activity in these heterozygotes was inappropriately low, ranging from 21 to 37% of normal rather than the expected 50% of normal. Examination of various physical and chemical properties of the A-PRTase obtained from the mutant heterozygotes failed to reveal differences from the normal enzyme. These patients have no discernable abnormality in uric acid production despite the finding that patients with a deficiency of a closely related enzyme, hypoxanthine-guanine phosphoribosyltransferase, invariably produce excessive quantities of uric acid. A relationship of the A-PRTase deficiency to the disturbance in lipoprotein metabolism observed in the propositus has not been firmly established. Possible manifestations of the homozygous form of this enzyme deficiency will require identification of such individuals in the future.

This study was presented in part at the 60th Annual Meeting of the American Society for Clinical Investigation, Atlantic City, N. J., 6 May 1968. (*J. Clin. Invest.* 47: 53 a. 1968.)

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Received for publication 19 April 1968.

INTRODUCTION

A partial deficiency of an enzyme of purine metabolism, hypoxanthine-guanine phosphoribosyltransferase (HG-PRTase) (Fig. 1), has recently been described in five patients with gout who produce excessive quantities of uric acid (1). Subjects with a complete deficiency of this enzyme also produce uric acid in excess but in addition have a bizarre neurological and behavioral syndrome characterized by self-mutilation, choreoathetosis, spasticity, and mental deficiency (2). These findings have suggested that the enzyme HG-PRTase is somehow concerned with the normal regulation of purine biosynthesis in man. Genetic data based on biochemical (3) as well as clinical studies (4–7) have established that the gene for this enzyme is located on the X-chromosome.

In the present study a previously undescribed deficiency of a closely related enzyme, adenine phosphoribosyltransferase (A-PRTase) (Fig. 1), is reported in four members in three generations of one family. Despite apparently close functional similarity of this enzyme to HG-PRTase, its partial deficiency in these subjects is not associated with excessive uric acid excretion. The propositus, however, does have Type II hyperbetalipoproteinemia (8). From the limited genetic data available in this kindred, the gene for A-PRTase appears to be located on an autosome rather than being X-linked and affected individuals may be heterozygous for the defect, even though their

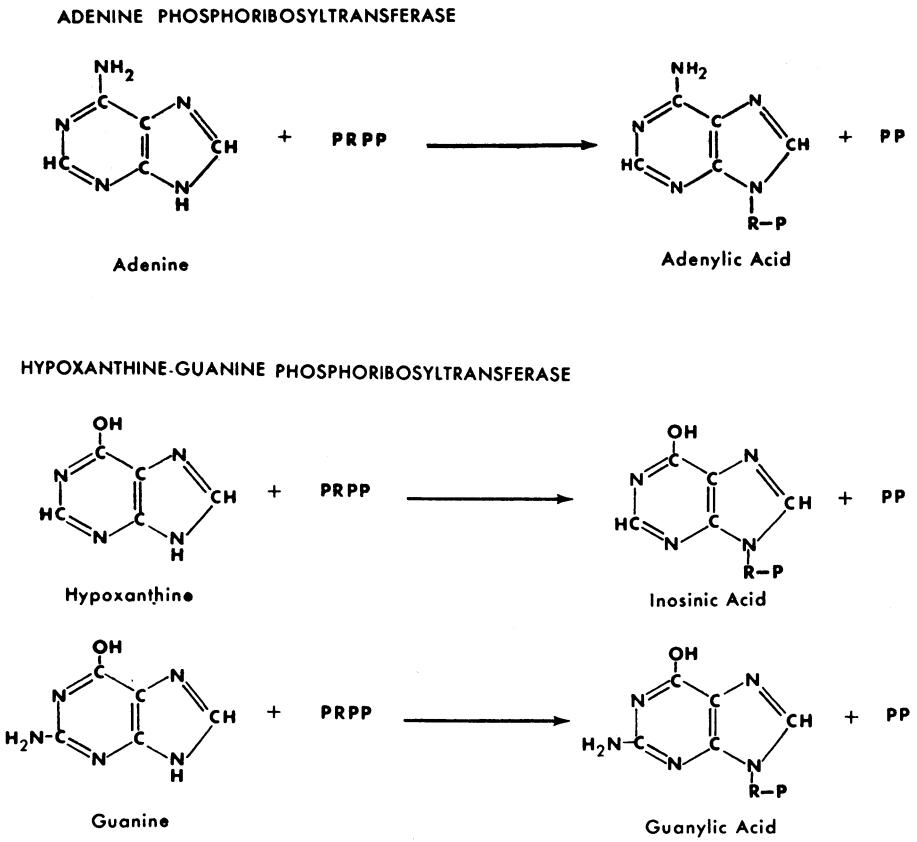

FIGURE 1 Reactions catalyzed by the two enzymes, adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase.

enzyme activity is substantially less than 50% of normal.

Case report. The propositus, E. H., a 45 yr old scientist (Ph.D.) and administrator, who has continued to enjoy excellent health, first developed arcus juvenilis at age 35. Serum cholesterol at that time was said to be elevated. In 1962 he began to develop nodules on both external pinnae. In 1967 a colleague felt that the lesions on his ears might represent tophaceous deposits and a serum urate was reported to be 7.2 mg/100 ml. Because of this finding, he was referred to our service at the Clinical Center for further evaluation. The patient had no history suggestive of gouty arthritis or nephrolithiasis, but a disorder of lipoprotein metabolism was suspected on the initial examination because of the presence of arcus and the history of a high cholesterol in the past. Erythrocytes were obtained for determination of HG-PRTase and A-PRTase as part of a control series being obtained in our out-patient department. His erythrocyte A-PRTase activity was quite low despite normal HG-PRTase activity, and he was admitted to the Clinical Center for further study.

Clinical history revealed an asymptomatic proteinuria until age 18 and an episode of orchitis in 1961 at the age of 39. There was no history of xanthomata, diabetes, or early death in the family. His mother had a history a recurrent mild monoarticular arthritis associated with mild hyperuricemia which had been diagnosed as gout. The patient has three daughters (Fig. 2) two of whom (III-4 and III-5) are exceedingly bright, alert, and healthy young ladies. A third daughter (III-6), equally cooperative, has a mild cerebellar disorder attributed to an episode of encephalitis which occurred at the age of 8 months.

Physical examination including detailed neurological and ophthalmological evaluation was negative except for the presence of bilateral arcus juvenilis. There were no xanthelasmata nor tendon or tuberous xanthomata. Hemogram, including

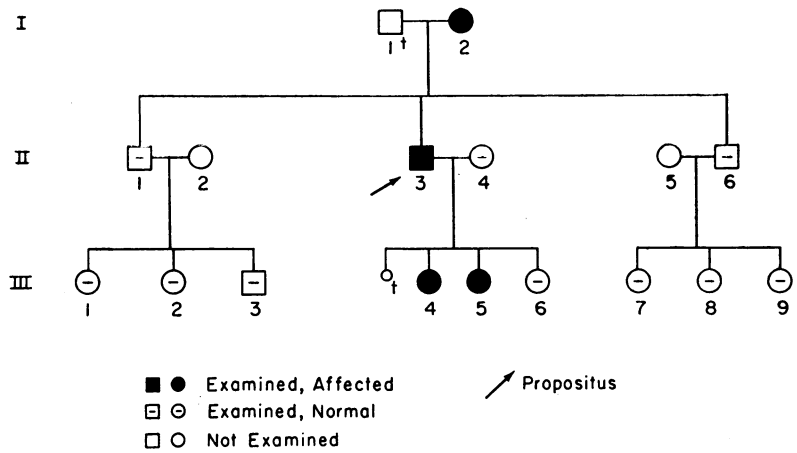

FIGURE 2 "H" Family Pedigree, showing inheritance of adenine phosphoribosyltransferase deficiency.

hemoglobin, hematocrit, white blood cell count and differential, erythrocyte sedimentation rate, red blood cell indexes, platelet count, and reticulocytes, was normal. Renal function assessed by urinalysis, timed phenolsulfonphthalein excretion, blood urea nitrogen, and creatinine clearance was normal. There was no proteinuria. Protein-bound iodine, 24 hr ^{131}I uptake, thyroid scan, oral glucose tolerance test, basal excretion of 17-hydroxycorticoids and 17-ketosteroids, and serum calcium and phosphorus concentrations revealed no evidence of endocrine dysfunction. Liver function tests including alkaline phosphatase, serum glutamic oxalacetic transaminase, serum glutamic pyruvic transaminase, lactate dehydrogenase, thymol turbidity, and bilirubin were normal. There was, however, 10% retention of Bromsulfalein after 45 min. Additional studies including protein electrophoresis, electrolytes (Na^+, K^+, Cl^-, HCO_3^- and Mg^{++}), acid phosphatase, lipase, amylase, ceruloplasmin, copper, total serum iron, and VDRL were normal, as were ECG and EEG. Audiometry revealed bilateral high tone hearing loss of the sensorineural type. X-rays of the chest, axial skeleton, and large joints were unrevealing. The lipoprotein values obtained in this patient are indicated in Table I. The values remained essentially unchanged on repeated determinations over a span of 5 months, including a period in the hospital when his dietary intake of cholesterol was controlled.

METHODS

A-PRTase and HG-PRTase activities were assayed in dialysed lysates of washed erythrocytes by a radiochemical method described previously (1). The samples for enzyme assay obtained from the propositus, E. H., as well as his wife and three daughters, were frozen immediately without loss of enzyme activity. The samples on the remainder of the family were airmailed in ice from California, with 2 to 3 days intervening before the samples could be frozen. This delay could have resulted in a loss of not more than 20% of the HG-PRTase and A-PRTase activities. Orotate phosphoribosyltransferase (orotidylic pyrophosphorylase) was assayed in a similar manner using 0.6 mM orotic-6-^{14}C acid hydrate (3.5 mc/mmole) (New England Nuclear Corp. Boston, Mass.); 1 mM 5-phosphoribosyl-1-pyrophosphate (PRPP) (Pabst Research Laboratories Milwaukee, Wis.); 55 mM Tris buffer, pH 7.4; 5 mM $MgCl_2$; and 3–6 mg of hemolysate protein in a final volume of 100 μl. After incubation for 3 hr at 38°C, the reactions were terminated by the addition of 2 μmoles of ethylenediaminetetraacetate (EDTA) and immediately frozen in a dry ice acetone bath. 20 μl of the reaction mixture was placed on 3MM Whatman paper with 0.06 μmole of orotidylic (a gift of Dr. Herbert Windmueller) and uridylic acid, and the reaction products were separated from the substrate by high voltage electrophoresis in 0.05 M borate buffer, pH 9.0, containing 0.001 M EDTA at 4000 v for 30 min. The areas of the paper containing orotidylic and uridylic acid were located by inspection of the paper under ultraviolet light or by radioautography and cut out and counted in a liquid scintillation counting system at 60% efficiency. The total counts present in orotidylic and uridylic acid were taken as a measure of phosphoribosyltransferase activity. The values so obtained compare closely to the values reported in erythrocytes by Smith, Huguley, and Bain using a different assay system (9).

Cholesterol (10) and triglyceride (11) concentrations

TABLE I

Erythrocyte Adenine Phosphoribosyltransferase Activity and Plasma Lipids in Members of the "H" Family

Patient	Relationship	Age	Adenine phosphoribosyltransferase activity	Total cholesterol	Triglyceride	VLDL* cholesterol	Beta cholesterol	Alpha cholesterol
			mμmoles/mg protein per hr	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
E. H.	Propositus II-3	45	6.6‡	318§	160	36	248§	34
P. H.	Daughter III-4	12	9.0	140	54	8	90	42
D. H.	Daughter III-5	10	9.6	162	28	4	113	45
V. H. G.	Mother I-2		6.0	298	164	58	204	36
Er. H.	Daughter III-6	8	21.7	170	30	6	110	54
J. H.	Wife II-4	40	26.0	142	25	2	80	60
G. H.	Brother II-6	44	28.8	180	63	10	120	50
L. H.	Niece III-7	16	27.1	178	100	20	116	42
Ev. H.	Niece III-8	18	27.1	168	122	28	103	35
Je. H.	Niece III-9	14	27.0	146	80	16	79	51
O. H.	Brother II-1	47	22.8	260	262§	52	180	28
Ba. H.	Niece III-1	17	25.8	160	24			
Ga. H.	Nephew III-2	11	29.2	188	40			
S. H.	Niece III-3	14	27.5	164	53			
G. P.	Cousin	20	37.6	146	64	14	93	39

* Very low density lipoprotein (d > 1.006 g/ml).
‡ Normal value — 31 ± 6 (mean ± 1 SD in 32 normal control subjects).
§ Abnormal value (8).

were determined by methods previously described. The methodology of lipoprotein quantification as well as age and sex-corrected normal limits for these values has been described elsewhere (8). The blood samples in each case were drawn with the patient in the fasting state.

We are indebted to Mrs. Mary McGinniss and Mrs. Mary Ann Campbell of the Clinical Center Blood Bank Department for determination of the red cell phenotypes in members of the "H" family.

RESULTS

The specific activity of the A-PRTase assayed in dialysed erythrocyte hemolysates obtained from 15 members of the "H" family is indicated in Table I. The A-PRTase activity was distinctly reduced in four members of this family, the propositus (E. H. II-3), his mother (V. H. G. I-2) and two of his three daughters (P. H. III-4, D. H. III-5). The values obtained in these four subjects (6.0–9.6 mμmoles/mg protein per hr) ranged from 21 to 31% of normal when compared with those of other family members. A comparison of A-PRTase and HG-PRTase activity in 13 of the family members is illustrated in Fig. 3. For each member of the family tested, including those with reduced A-PRTase activity, the HG-PRTase activity,

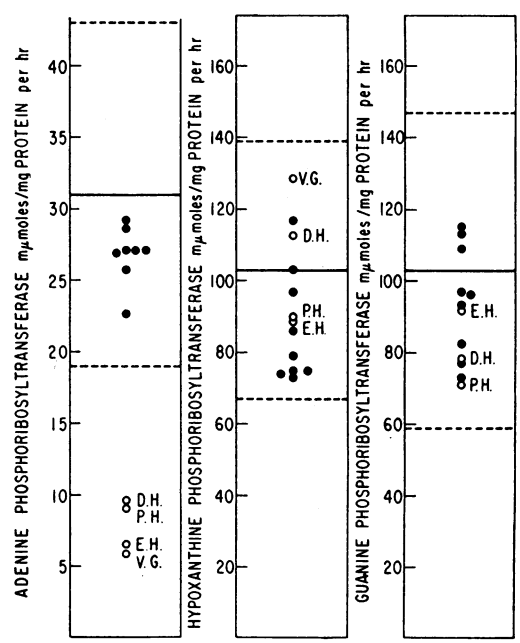

FIGURE 3 Specific activity of adenine phosphoribosyltransferase and hypoxanthine-guanine phosphoribosyltransferase in members of the "H" family. The solid and broken lines indicate the mean ± 2 SD in 32 normal control subjects. The subjects with reduced A-PRTase activity are indicated by the open circles.

TABLE II

Adenine Phosphoribosyltransferase Activity and Plasma Lipids in Patients with Familial Hyperbetalipoproteinemia

Patient	Age	Adenine phosphoribosyltransferase activity	Total cholesterol	Triglyceride	VLDL* cholesterol	Beta cholesterol	Alpha cholesterol
		mμmoles/mg protein per hr	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*	*mg/100 ml*
J. N.	48	20.5‡	312§	128	10	264§	48
E. B.	43	21.2	314§	224§	46	235§	33
J. B.	30	22.8	338§	214§	58	261§	27
H. J.	34	33.8	382§	179§	44	306§	32
M. B.	24	32.2	418§	64	6	370§	42
M. H.	43	32.2	462§	70	22	388§	52

* Very low density lipoproteins (d > 1.006 g/ml).
‡ Normal value — 31 ± 6 (mean ± 1 SD in 32 normal control subjects).
§ Abnormal values (8).

when assayed with either hypoxanthine or guanine as substrate, was within two standard deviations of the mean. Mixtures of the hemolysate obtained from the propositus, E. H., with a hemolysate known to have normal A-PRTase activity, gave the value expected from such a combination, indicating that the decreased A-PRTase activity observed in patient E. H. was not due to the presence of a reversible inhibitor.

The electrophoretic migration of the deficient enzyme in starch gel, as well as its heat stability, has been normal on repeated testing. In addition, preliminary studies involving gel filtration through Sephadex, electrophoretic migration in Geon-Pevikon, determination of its Michaelis constants,[1] and pH optimum have also failed to reveal properties unique to this variant. Therefore, no evidence to suggest a structural alteration in the deficient enzyme has yet been obtained.

In addition to a partial deficiency of A-PRTase, the propositus, E. H., also had an abnormal lipoprotein pattern consistent with type II hyperlipoproteinemia (8) characterized by an increased concentration of cholesterol and betalipoprotein in his plasma. Investigation of 14 other members of the family, including the two daughters and mother, who had a reduction in A-PRTase activity failed to reveal a similar lipoprotein abnormality (Table I). His brother, O. H. (II-1), had an abnormal lipoprotein pattern quite unlike that found in the propositus, a pattern characterized by endogenous hypertriglyceridemia (Type IV

[1] Henderson, J. F., W. N. Kelley, F. M. Rosenbloom, and J. M. Seegmiller. The kinetics of human adenine phosphoribosyltransferase. Manuscript in preparation.

TABLE III

Red Cell Antigenic Phenotype in Members of the "H" Family

		Rh factors							MNS					P	Kell				Duffy		Kidd		Lewis		
Patient	ABO	C	C^w	D	D^u	E	c	e	M	N	S	s	U	P_1	K	k	Kp^a	Kp^b	Fy^a	Fy^b	Jk^a	Jk^b	Le^a	Le^b	Xg^a
E. H. II-3	O	+	−	+		−	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	−
P. H. III-4	O	+	−	+		−	−	+	+	−	+	+	+	+	+	+	−	+	−	+	−	+	−	+	+
D. H. III-5	O	+	−	+		−	−	+	+	+	+	+	+	+	+	+	−	+	+	+	−	+	−	+	−
Er. H. III-6	O	+	−	+		−	−	+	−	+	−	+	+	+	−	+	−	+	+	+	+	+	−	+	+
J. H. II-4	A	+	−	+		−	−	+	+	+	−	+	+	+	−	+	−	+	+	+	−	+	−	+	+
G. H. II-6	O	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	−	−
L. H. III-7	A	+	−	+		−	+	+	+	+	+	+	+	−	+	+	−	+	−	+	+	+	−	+	−
Ev. H. III-8	O	+	−	+		−	+	+	+	+	+	+	+	+	−	+	−	+	−	+	−	+	−	+	−
Je. H. III-9	O	+	−	+		−	+	+	+	−	+	−	+	+	+	+	−	+	−	+	−	+	−	+	−
O. H. II-1	O	−	−	−	−	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+	+	+	−	−
Ba. H. III-1	O	+	−	+		−	+	+	+	−	+	+	+	+	−	+	−	+	−	+	+	+	+	−	−
Ga. H. III-2	O	−	−	−	−	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+	−	+	−	−
S. H. III-3	O	−	−	−	−	−	+	+	+	−	+	−	+	+	−	+	−	+	−	+	+	−	−	+	+

hyperlipoproteinemia). A repeat sample of blood 2 months later showed no abnormality.

A-PRTase activity was assessed in six patients previously demonstrated to have familial type II hyperlipoproteinemia (hyperbetalipoproteinemia) (8). In each of these subjects, the activity of this enzyme was normal (Table II).

None of the affected members of the "H" family were hyperuricemic. The propositus excreted 514 mg of uric acid in his daily urine after 4 days of dietary purine restriction (normal < 600 mg/day). Serum urate at that time was 5.2 mg/100 ml.

The red cell antigenic phenotypes obtained on each member of the "H" family are illustrated in Table III. The data obtained on each subject are compatible with the pedigree proposed in Fig. 2. The small number of affected individuals prevents an accurate estimate of the linkage of this enzyme with any of these blood groups.

DISCUSSION

In the present study a previously unreported deficiency of an enzyme of purine metabolism, adenine phosphoribosyltransferase (A-PRTase), has been described. The reduced A-PRTase activity in the propositus, E. H., has been constant with repeated assay over a period of 8 months, indicating that this defect was not due to some unknown transient effect on his circulating erythrocytes. The observation of reduced activity in his mother and in two of his three children also provides evidence that the deficiency of this enzyme represents a genetic and not an acquired defect.

The genetic data available suggest that the propositus, his mother, and his two affected daughters are probably heterozygotes for this enzyme defect and that the defective allele is most likely located on an autosome. If the propositus were a mutant homozygote carrying a double dose of the defective gene, all three of his children would be heterozygotes and comparable values for A-PRTase would have been predicted in all three progeny. However, one had normal A-PRTase activity, whereas the other two had distinctly reduced activity. Similarly, if the defective gene were located on the X-chromosome and the propositus were a mutant hemizygote, all three daughters would also have been obligate heterozygotes. We are not able to exclude completely the possibility of X-linkage of A-PRTase based on the study of this family, since only one cell type was examined and male to male transmission of the enzyme defect was not observed. One could argue that the enzyme is X-linked and that patient Er. H. (III-6) is heterozygous for the enzyme defect despite normal erythrocyte A-PRTase activity if it is assumed on the basis of the Lyon hypothesis that there was early inactivation of the abnormal X-chromosome in the hematopoietic system in this individual. However, recent studies in other families of a thermostable A-PRTase isoenzyme which has normal enzyme activity revealed that male to male transmission of this allele did occur,[2] providing strong evidence that A-PRTase is not X-linked and therefore must be coded for by autosomal DNA. The proposed mode of inheritance is also based on the assumption of full penetrance, the absence of a "neutralizing" or "suppressor" factor in the apparently unaffected daughter, and the proposed parenthood which is in agreement with the erythrocyte antigenic phenotypes.

The failure to detect evidence of a structural abnormality in the A-PRTase obtained from the propositus is compatible with the suggestion that he is a heterozygote and that the enzyme present is the product of only the normal allele. The normal allele would be expected to code for a normal A-PRTase, whereas the mutant allele might code for a protein with no enzyme activity by our assay. It must be emphasized, however, that many structural gene mutations are possible which might affect enzyme activity and not alter the properties which we have examined and that indisputable evidence for a structurally normal A-PRTase in this kindred would require knowledge of its full amino acid sequence.

In most autosomal disorders in which adequate studies of enzyme activity in heterozygotes have been conducted, the enzyme activity in question is found to be reduced to values close to 50% of normal, as would be expected. A striking exception is found in orotic aciduria. Heterozygotes for this disorder have approximately 20–35% or normal activity for the enzyme(s) orotate phosphoribosyltransferase (orotidylic pyrophosphorylase) and orotidylic decarboxylase which are virtually

[2] Henderson, J. F., W. N. Kelley, F. M. Rosenbloom, and J. M. Seegmiller. Inheritance of purine phosphoribosyltransferases in man. Manuscript in preparation.

absent in the mutant homozygotes (9). This unexpected finding in the heterozygotes for orotic aciduria has been attributed to the presence of an abnormal regulator gene product produced at the mutant locus (12). Preliminary data reported by Krooth, using cultured fibroblasts derived from a mutant homozygote with this disorder, led him also to conclude that the mutation may be at a regulator site, rather than on a structural gene (13, 14).

The A-PRTase activities observed in the four subjects heterozygous for this enzyme defect ranged from 21 to 37% of normal. Because these values are inappropriately low and similar in range to the enzyme values reported in the heterozygotes for orotic aciduria, a regulator gene mutation could be proposed by analogy with the latter case. The inappropriately low enzyme activity, however, could also result from a structural gene mutation and this finding in itself does not necessarily require that a regulator mutation be postulated. One possible model for such a reduction in activity due to a structural gene mutation would require the enzyme to be a dimer formed by random aggregation of identical subunits from each allele. If this protein had enzymatic activity only when it existed as a dimer composed of two normal subunits, then a structural gene mutation involving one allele in the heterozygote would lead to a reduction in enzyme activity to values approximately 25% of normal, as is observed in both A-PRTase deficiency and orotic aciduria. There is, in fact, some evidence which suggests a subunit composition for A-PRTase.[3] It is apparent that what seems by our present techniques to be a structurally normal A-PRTase in the mutant heterozygotes is compatible not only with a mutation on a structural gene as discussed earlier, but also with an alteration at a regulator site. In conclusion, it is not possible at this time to determine whether the mutation in the "H" family involves a site on a structural or on a regulator gene.

In addition to the similarities noted in the heterozygotes deficient for these enzymes, orotate phosphoribosyltransferase and A-PRTase also have quite similar enzymatic functions since they catalyze the transfer of the ribose-5-phosphate moiety of 5'-phosphoribosyl-1-pyrophosphate to a pyrimidine and purine base, respectively, to form the appropriate 5'-mononucleotides. The possibility that the four subjects in this report were actually heterozygotes for orotic aciduria and that the reduced A-PRTase activity observed reflected either a deficiency of yet a third enzyme in orotic aciduria or the reduced orotate phosphoribosyltransferase activity itself, has been excluded, since orotate phosphoribosyltransferase activty was normal in the propositus.

The subjects in this report had no detectable abnormality of purine metabolism despite the finding that the deficiency of a closely related enzyme, HG-PRTase, is always associated with excessive purine synthesis.

The relationship of the A-PRTase deficiency and the hyperbetalipoproteinemia observed in the propositus is not known. This abnormal lipoprotein pattern is often familial, being inherited as an autosomal dominant (8). The common causes of acquired hyperbetalipoproteinemia including exaggerated dietary intake of cholesterol, hypothyroidism, nephrosis, myeloma, macroglobulinemia, and obstructive liver disease were excluded in this patient. The failure to find evidence of hyperbetalipoproteinemia in the remainder of the family studied was somewhat surprising, but far from inconceivable on genetic grounds. However, since no other family members had hyperbetalipoproteinemia, evidence of complete genetic segregation of these two biochemical disorders could not be obtained in this kinship. Evaluation of the father of the propositus could have provided definitive evidence of such genetic segregation, but he died in an accident in middle age without a clinical history of cardiovascular disease.

There are many apparent interrelationships between purine and lipoprotein metabolism which are unanswered. An elevated serum urate, the ultimate end product of purine metabolism in man, frequently occurs in patients with hypertriglyceridemia (Type III, IV, and V hyperlipoproteinemia) (15, 16, 8) although this association is not usually noted in patients such as the propositus, with hyperbetalipoproteinemia (Type II) (17, 8). Beta lipoprotein release from the liver in rats can be strongly inhibited by the administration of orotic acid, an effect which is completely reversed by exogenous adenine, suggesting that purines and

[3] Gadd, R. E. A., and J. F. Henderson. Unpublished data.

pyrimidines may be involved in the regulation of betalipoprotein metabolism (18). Patients with familial Type II hyperlipoproteinemia have an increased quantity of a lipoprotein which is structurally normal (8), suggesting that the mutation responsible for this abnormality acts by producing a defect in a normal control mechanism. High normal beta lipoprotein levels in the mother and normal concentrations of beta lipoprotein in the two young daughters of the propositus, who also had reduced A-PRTase activity, suggest that the association of these two metabolic disorders may have been fortuitous in the propositus. It is possible that the two girls with the enzyme defect will ultimately manifest this disorder of lipoprotein metabolism, but the failure of the mother of the propositus to demonstrate this lipoprotein abnormality argues strongly against a causal relationship of these two disorders. Certainly the apparent high incidence of hyperbetalipoproteinemia in the American population would not mitigate against a chance association. The possibility still remains that hyperbetalipoproteinemia is expressed in association with A-PRTase deficiency only in the male. A-PRTase activity, however, was normal in six unrelated adult patients with familial Type II hyperlipoproteinemia, suggesting that the deficiency of this enzyme, if causally related at all, is not the basic defect in most patients with this disorder. Further studies are being conducted in the propositus in an attempt to delineate this issue.

No attempt has been made to estimate frequency of this mutation but the propositus was discovered in the course of assay of blood samples from about 150 subjects. The possible manifestations of this enzyme defect in the homozygote are difficult to predict. However, it would seem unlikely to us at this time that this would be a lethal mutation since the product of this reaction, adenylic acid, can be formed in most tissues by alternative biochemical pathways. Furthermore, normal growth of mutant mammalian cells (AMK) completely deficient in this enzyme, has been reported (19). On the other hand, mature human erythrocytes which lack a *de novo* pathway for synthesis of purine ribonucleotides might be adversely affected by a very low A-PRTase activity, particularly since the alternative pathways for the synthesis of adenylic acid from adenosine and inosinic acid are lacking in these cells (20). The possibility of finding a more severe deficiency of A-PRTase among patients with unexplained congenital hemolytic anemia remains to be explored.

ACKNOWLEDGMENTS

We wish to thank the propositus, E. H., for his cooperation and active participation in obtaining the historical information and appropriate blood samples on members of his family. We wish to thank Dr. Charles J. Epstein, previously of the National Institute of Arthritis and Metabolic Diseases, for his suggestions and specifically wish to acknowledge his role in proposing the model by which a structural gene mutation could lead to a reduction in enzyme activity to 25% of normal in a heterozygote.

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