

# Phosphoribosyl transferase activity in normal subjects, gout patients, and children with mental retardation

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**SUMMARY** Activity of hypoxanthine-guanine and adenine phosphoribosyl transferase enzymes has been assayed in erythrocytes from 10 normal adults, 37 subjects with gout, and 21 mentally retarded children with high and normal urinary uric acid:creatinine ratios. These were compared with one case of known HGPRTase deficiency. Apart from the last subject, no cases of HGPRTase deficiency were found.

The Lesch-Nyhan syndrome is a rare sex-linked disorder consisting of choreoathetosis, spasticity, mental retardation, aggressive behaviour, and compulsive self-mutilation of the lips and fingers (Lesch and Nyhan, 1964). It is associated with gross excess of uric acid synthesis and the development of gouty arthritis. The demonstration in this condition of a complete deficiency of hypoxanthine-guanine phosphoribosyl transferase (HGPRTase), an enzyme involved in the normal regulation of purine biosynthesis, was followed by reports of a partial deficiency of the same enzyme in affected members of families with gout (Seegmiller, Rosenbloom, and Kelley, 1967; Kelley, Rosenbloom, Henderson, and Seegmiller, 1967). Complete or partial deficiency was found in seven of 11 patients with gout and excessive urate production by Kelley *et al.* (1968) and, although this was clearly a selected group of patients, the question arises how commonly such a deficit may be found in patients with gout and in children with mental retardation. It has generally been observed that a deficiency of HGPRTase is accompanied by an increase in activity of adenine phosphoribosyl transferase.

Hyperuricaemia and neurological abnormality have also been associated with normal HGPRTase, but with increased APRTase activity (Nyhan, James, Teberg, Sweetman, and Nelson, 1969). The ratio of uric acid to creatinine (UA/C) in morning samples of urine has been shown to provide a screening test for the detection of HGPRTase deficiency

(Kaufman, Greene, and Seegmiller, 1968). In the present study, HGPRTase and APRTase activity has been assayed in 10 normal adults, 37 subjects with gout, and in 21 mentally retarded children with high and normal UA/C ratios; together with one known case of HGPRTase deficiency.

## METHODS

Hypoxanthine guanine phosphoribosyl transferase and adenine phosphoribosyl transferase activity were assayed by a radiochemical method (Kelley *et al.* (1967). Erythrocytes used as a source of enzymes were washed twice with two volumes of isotonic saline. They were haemolysed by rapid freezing and thawing in a dry-ice acetone bath and then dialysed for two hours in 0.001 M tris buffer, pH 7.4, at 4°C. Solutions for incubation were made up containing 55 mM tris buffer, pH 7.4, 5 mM MgCl<sub>2</sub>, 1mM-5-phosphoribosyl-1-pyrophosphate (PRPP), radioactive base, and protein from dialysed erythrocytes. The concentrations of the purine bases in the incubation mixture were as follows: hypoxanthine 0.59 mM (4.2 mCi/m-mole); guanine 0.13 mM (13.5 mCi/m-mole); adenine 0.56 mM (4.5 mCi/m-mole). The quantity of protein from dialysed erythrocytes added to the incubation solutions was 0.5 to 0.6 mg for solutions containing hypoxanthine or adenine, and 0.17 to 0.22 mg for solutions containing guanine. Each solution was incubated for 20 minutes at 38°C. The reaction was then terminated by the addition of 2 μ-mole neutralized ethylenediamine tetraacetate, followed by freezing in a dry-ice acetone bath.

Twenty μl. of the reaction mixture was placed on 3 mm Whatman paper with 0.06 μ-mole of the appropriate carrier nucleotide and the reaction products were separated from the substrates by high voltage electro-

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phoresis in 0.05M borate buffer, pH 9.0 containing 0.001 M EDTA, at 4,000 V for 20 minutes. The area of the paper containing the nucleotide product was located by inspection of the paper under ultraviolet light, cut out and counted in a toluene scintillation fluid.

#### SUBJECTS STUDIED

The ten normal adults were healthy subjects of both sexes. The 37 patients with primary gout were divided into those excreting 600 mg daily of urinary uric acid or less on a standard low purine diet containing 300 mg or less of purine daily (20 normal uric acid producers) and those excreting larger quantities (17 overproducers). The 21 children, aged 3 to 15 years, were hospitalized with varying combinations of severe mental retardation, behaviour disorders, epilepsy, hyperkinesia, and psychosis: none had the typical features of the Lesch-Nyhan syndrome.

Twelve of the children had a urinary UA/C ratio which was normal for their age, using data on 171 children given by Kaufman *et al.* (1968), while nine had values of more than 2 SD above the mean for their age, the highest being 3.5 in boy of 5 years of age (normal  $0.9 \pm 2$  SD 0.4). The known case of partial HGPRTase deficiency was that reported by Bluestone (1968), to whom we are indebted.

#### RESULTS

Results in the Table show that figures for normal subjects agree reasonably closely with other published reports—for example, Kelley *et al.* (1968). There was no difference in HGPRTase activity between the gouty subjects and normals, nor, among the gouty subjects, between normal producers and overproducers. In none of the individual gout patients did values depart significantly from the

TABLE

SPECIFIC ACTIVITY OF PHOSPHORIBOSYL TRANSFERASE OF ERYTHROCYTE HAEMOLYSATES FROM NORMAL SUBJECTS, GOUT PATIENTS, AND CHILDREN WITH MENTAL RETARDATION

Subjects	Phosphoribosyl transferase activity ( $\mu$ -mole/mg protein/hr)		
	Hypoxanthine (mean $\pm$ SD)	Guanine (mean $\pm$ SD)	Adenine (mean $\pm$ SD)
Normal adults (10)	82.3 $\pm$ 15.5	104.5 $\pm$ 21.1	35.7 $\pm$ 3.0
Gout			
normal uric acid producers (20)	81.3 $\pm$ 22.6	99.0 $\pm$ 18.8	31.0 $\pm$ 4.8
overproducers of uric acid (17)	82.1 $\pm$ 17.6	105.6 $\pm$ 18.2	33.7 $\pm$ 4.7
Known case of HGPRTase deficiency (1)	1.8	1.38	81.2
Mentally retarded children			
normal UA/C ratio (12)	not measured	122.5 $\pm$ 20.4	35.2 $\pm$ 8.3
high UA/C ratio (9)	not measured	113.9 $\pm$ 19.1	35.4 $\pm$ 5.5

mean. In contrast, low figures from the known case of partial deficiency of HGPRTase were confirmed.

In the 21 children with mental retardation there was no significant difference in enzyme values between those with high and normal UA/C ratios. None of the former were shown to have a deficiency of HGPRTase. The children were not investigated further by determination of the ratio on a low purine diet or by serum urate estimations.

#### DISCUSSION

From these results it is evident that complete or partial deficiency of HGPRTase must be a rare aetiological factor in gout as it is usually encountered. No deficiency was found among the 37 patients with gout, including 17 overproducers of uric acid, some of whom might have been expected possibly to show such a deficiency.

Similarly, no cases of enzyme deficiency were found among the mentally retarded children. Some of them had a high urinary UA/C ratio, which is therefore not to be regarded as indicating the likelihood of HGPRTase deficiency. Nevertheless, as Kaufman *et al.* (1968) point out, it is a useful indication of uric acid overproduction and serves as a screening test for activity of enzymes concerned in purine biosynthesis.

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#### REFERENCES

- Bluestone, R. (1968). Nyhan-Lesch syndrome and juvenile gout (2 cases). *Proc. roy. Soc. Med.*, **61**, 1119-29.
- Kaufman, J. M., Greene, M. L., and Seegmiller, J. E. (1968). Urine uric acid to creatinine ratio—a screening test for inherited disorders of purine metabolism. *J. Pediat.*, **73**, 583-592.
- Kelley, W. N., Rosenbloom, F. M., Henderson, J. F., and Seegmiller, J. E. (1967). A specific enzyme defect in gout associated with overproduction of uric acid. *Proc. nat. Acad. Sci. (Wash.)*, **57**, 1735-39.
- Kelley, W. N., Rosenbloom, F. M., Miller, J., and Seegmiller, J. E. (1968). An enzymatic basis for variation in response to allopurinol. *New Engl. J. Med.*, **278**, 287-293.
- Lesch, M., and Nyhan, W. L. (1964). A familial disorder of uric acid metabolism and central nervous system function. *Amer. J. Med.*, **36**, 561-570.
- Nyhan, W. L., James, J. A., Teberg, A. J., Sweetman, L., and Nelson, L. G. (1969). A new disorder of purine metabolism with behavioural manifestations. *J. Pediat.*, **74**, 20-27.
- Seegmiller, J. E., Rosenbloom, F. M., and Kelley, W. N. (1967). Enzyme defect associated with a sex-linked human neurological disorder and excessive purine synthesis. *Science*, **155**, 1682-83.