Synthesis and concentration of 5-phosphoribosyl-1-pyrophosphate in erythrocytes from patients with Down's syndrome

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SUMMARY Plasma urate levels and erythrocyte glucose-6-phosphate dehydrogenase activity were not significantly different in patients with Down's syndrome when compared with control subjects with mental retardation. No significant difference was found in 5-phosphoribosyl-1-pyrophosphate (PRPP) concentration and synthesis of PRPP in erythrocytes from glucose between subjects with Down's syndrome and controls. The purine metabolism of Down's syndrome is discussed.

Raised serum urate levels in patients with Down's syndrome have been confirmed by many investigators including Fuller *et al.* (1962) but the pathogenesis of the hyperuricaemia is not yet fully understood. Recently the intracellular concentration of 5-phosphoribosyl-1-pyrophosphate (PRPP) has been emphasized as an important factor in the regulation of *de novo* purine synthesis (Fox and Kelley, 1971). There has been no study of the concentration of PRPP in the erythrocytes of patients with Down's syndrome. The activity of glucose-6-phosphate dehydrogenase (G-6-PD, EC 1.1.1.49) has been reported to be increased in erythrocytes of patients with Down's syndrome (Phillips *et al.*, 1967), and the enzyme G-6-PD is considered to be the key enzyme in the hexose monophosphate shunt (Johnson *et al.*, 1973). Therefore, increased activity of this enzyme may result in an increased rate of synthesis of PRPP from glucose. We estimated the synthesis of PRPP from glucose and the PRPP content in the erythrocytes of the patients with Down's syndrome, and discuss their purine metabolism.

Methods and materials

Blood samples were collected from 20 institutionalized Down's syndrome patients at Moro Hospital and from 16 mentally retarded children

matched for age and sex at the same institution. Diagnosis of Down's syndrome was confirmed by karyotyping. 12 male and 8 female patients with a mean age of 23.1 years, range 8 to 43 years, were studied. The controls were 10 males and 6 females with a mean age of 25.4 years, range 11 to 41 years, with cerebral palsy and no known metabolic disorders. All children were receiving the same diet and other environmental factors were similar.

PREPARATION OF HAEMOLYSATES

All blood specimens were collected on the same day by venepuncture into heparinized tubes after an overnight fast. Samples were centrifuged at $1900 \times g$ for 15 minutes in the cold and the supernatant plasma transferred to another tube to determine urate levels. After washing twice with 4 volumes of cold physiological saline, 50 μ l of the packed washed erythrocytes were haemolysed with 100 μ l of cold distilled water and used for measurement of G-6-PD activity and PRPP content.

ASSAY OF PRPP CONTENTS

PRPP levels in erythrocytes were determined by the simplified method previously described by Sperling *et al.* (1972). A 100 μ l sample of the haemolysate was incubated for 20 minutes at 37°C in 110 mmol/l TRIS buffer, pH 8.5 containing 10 mmol/l $MgCl_2$, 20 mmol/l AMP, and 1 mmol/l 8-¹⁴C-hypoxanthine (10 mCi/mmol) in a total volume of 200 μ l. The reaction was stopped by the addition of 40 μ l of 15% perchloric acid. After centrifugation for 10 minutes at 3000 rpm, 25 μ l of the supernatant

Accepted for publication September 27, 1976

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Ann Rheum Dis 1991;51:262-265
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of the reaction mixture was spotted on Eastman (Kodak) thin layer chromatography and developed with butanol:methanol:water:25% NH_4OH (60:20:20:1 v/v). The purines were located on the chromatogram under ultraviolet light, inosinic acid spots were scraped off, suspended in Bray solution, and counted in a Packard Tri-carb liquid scintillation counter. The results were expressed in nmol PRPP/ml packed cells. Recovery experiments in which PRPP was added to haemolysates gave a satisfactory yield (92%) and a linear relationship was seen between the added PRPP and formed inosinic acid concentration up to 200 nmol.

ASSAY OF PRPP SYNTHESIS

50 μl of washed packed erythrocytes were suspended in 1.8 ml phosphate-glucose buffer, pH 7.4, containing 4.33 mmol/l glucose, at 37°C for intervals up to 120 minutes. The cells were spun down, the supernatant discarded, and the packed cells haemolysed with 150 μl distilled water. PRPP contents were assayed with a 100 μl sample of the haemolysate.

The activity of G-6-PD was determined by the method of Bruns and Werners (1962). Plasma urate levels were measured by the enzymatic methods of Liddle *et al.* (1959).

Results

The Table gives the mean ($\pm$ SD) values. The average plasma urate level in 20 patients with Down's syndrome was 5.02 ± 1.43 mg/100 ml (0.3 ± 0.09 mmol/l) which was higher than the average level of 4.15 ± 1.41 (0.2 ± 0.08 mmol/l) in the 16 matched controls, but the difference was not significant. Hyperuricaemia (>6.0 mg/100 ml; 0.36 mmol/l) was present in 5 of the patients with Down's syndrome, and in one control. The mean erythrocyte G-6-PD activity in the patients with Down's

syndrome and the controls was 174.4 ± 56.6 mU/ml packed cells and 139.4 ± 42.0 mU/ml packed cells respectively. This difference was not statistically significant.

The average value of synthesized PRPP from glucose in one hour was 71.00 ± 24.05 nmol/ml packed cells/h in the patients with Down's syndrome, while in the controls the mean value of PRPP formation was 66.44 ± 21.48 nmol/ml packed cells/h, with no significant difference. A relationship between G-6-PD activity and synthesized PRPP level showed no significant positive correlation between the patients with Down's syndrome and the controls. In patients with Down's syndrome, average erythrocyte PRPP level was 6.23 ± 3.20 nmol/ml packed cells which was slightly lower than the average level of 8.05 ± 3.02 nmol/ml packed cells in the controls, again not significantly different. Erythrocyte PRPP level showed no significant correlation with the serum urate level.

Discussion

Since Fuller *et al.* (1962) have found significant increases in serum urate levels in patients with Down's syndrome, a number of others (Mertz *et al.*, 1963; Kaufman and O'Brien, 1967; Bland *et al.*, 1968; Appleton, *et al.*, 1969) have confirmed this observation, as does our study. The mechanism of hyperuricaemia in Down's syndrome is not known. Pant *et al.* (1968) showed excessive uric acid excretion in 24-hour urine samples in Down's syndrome and suggested that overproduction of uric acid was the cause of the hyperuricaemia. However, their study was performed on an unrestricted diet. Appleton *et al.* (1969) showed high hypoxanthine and xanthine concentrations in plasma, and raised uric acid levels in sweat were reported by Danton and Nyhan (1966). They also assumed increased purine synthesis in Down's syndrome. In 1964, Hook and Engel pointed out the shorter life span and increased rate of turnover of leucocytes in Down's syndrome, and from leucokinetic studies with ^{32}P -labelled granulocytes Raab *et al.* (1966) found that the granulocyte half-life in patients with Down's syndrome was significantly reduced compared with that of normal adults. These findings suggest the possibility of increased purine metabolism in Down's syndrome.

Recently, the intracellular concentration of PRPP has been emphasized as an important factor determining the rate of *de novo* purine synthesis (Fox and Kelley, 1971). Becker *et al.* (1973) showed 2 brothers with clinical gout and with overproduction of uric acid, who had increased intracellular PRPP concentrations and high activity of the enzyme PRPP synthetase (EC 2.7.6.1). In patients with the

Table Plasma urate levels, erythrocyte glucose-6-phosphate dehydrogenase and erythrocyte PRPP levels, and PRPP synthesis from glucose in patients with Down's syndrome and controls (mean $\pm$ SD)

	Controls	Patients with Down's syndrome
No.	16	20
Age (years)	25.4 ± 5.9	23.1 ± 9.2
Plasma urate level (mg/100 ml)	4.15 ± 1.41	5.02 ± 1.43
Glucose-6-phosphate dehydrogenase (mU/ml packed red cells)	139.4 ± 42.0	174.4 ± 56.6
Synthesis of PRPP (nmol/ml packed red cells/h)	66.44 ± 21.48	71.00 ± 24.05
PRPP (nmol/ml packed red cell)	8.05 ± 3.02	6.23 ± 3.20

Conversion: Traditional units to SI—Urate: 1 mg/100 ml $\approx$ 0.0595 mmol/l.

Lesch-Nyhan syndrome high levels of PRPP have been recognized and raised PRPP is one of the factors in accelerated purine synthesis (Rosenbloom *et al.*, 1968). Increased production of PRPP also accounted for overproduction of uric acid in glycogen storage disease type I (Howell, 1965; Kelley *et al.*, 1968). PRPP is synthesized from ribose-5-phosphate via the pentose phosphate shunt in the erythrocytes. The enzyme G-6-PD which operates the pentose phosphate cycle from glucose is considered to be a key enzyme in PRPP synthesis (Johnson *et al.*, 1973). It was reported by Phillips *et al.* (1967) that G-6-PD activity was raised in erythrocytes in Down's syndrome. These findings suggest the possibility of increased PRPP synthesis from glucose in Down's syndrome. The present results for G-6-PD activity in erythrocytes of Down's syndrome did not show any significant increases. The synthesis of PRPP from glucose and the PRPP content of erythrocytes in Down's syndrome were the same as those of control subjects. These results are difficult to understand. Numerous enzymes require PRPP as a substrate. Possibly the utilization of PRPP is accelerated in Down's syndrome, the concentration of PRPP in erythrocytes is decreased and PRPP synthesis increased. This hypothesis was not proved in our study and further investigation will be necessary to clarify this proposed hypothesis of purine metabolism in Down's syndrome.

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