Pediat. Res. 8: 852–856 (1974)

Glutathione
neonate
pyroglutamic aciduria

Pyroglutamic Aciduria (5-Oxoprolinuria), an Inborn Error in Glutathione Metabolism

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Extract

Two sisters, one a neonate and the other 3 years of age, were found to suffer from pyroglutamic aciduria (5-oxoprolinuria). They had a chronic metabolic acidosis and required therapy with bicarbonate. Unlike their parents, both excreted large quantites of L-pyroglutamate in the urine.

No pyroglutamate could be demonstrated in a urine sample obtained from the pregnant mother at 30 weeks of gestation. Pyroglutamate was, however, detected in amniotic fluid and cord blood plasma as well as in urine from the neonate collected during the first few hours after birth. Metabolic acidosis developed during the 1st day of life and bicarbonate therapy was started. The patient also showed a moderate hyperbilirubinemia but no further complications were encountered in the neonatal period.

The 3-year-old girl had a daily excretion of pyroglutamate in urine corresponding to 195 mmol/1.73 m^2, i.e., identical with that found at 14 months of age. The excretion showed no correlation with feeding or physical activity. The concentrations of pyroglutamate in plasma and cerebrospinal fluid were 2.2 mM and 1.3 mM, respectively. She had no evidence of a disturbed renal transport of α-amino acids. Her psychologic and somatic development was normal, and she had no signs of neurologic damage.

Both patients presented evidence of increased hemolysis and their levels of glutathione in erythrocytes were markedly decreased, which indicated a defect in glutathione metabolism.

Speculation

In our patients with pyroglutamic aciduria, the levels of pyroglutamate-degrading enzyme (5-oxoprolinase) in leukocytes and cultured fibroblasts were found to be normal and turnover studies revealed a high capacity for the breakdown of pyroglutamate. These observations make a defect in pyroglutamate degradation somewhat unlikely. Instead, we believe that the patients have an increased production of pyroglutamate. The erythrocyte glutathione level was markedly reduced and it is suggested that pyroglutamate is formed in excessive amounts because of an enzymatic defect in the glutathione synthesis.

Pyroglutamic aciduria (5-oxoprolinuria) was originally described by Jellum *et al.* (7) in an adult male who suffered from metabolic acidosis and neurologic symptoms such as mental retardation, spastic tetraparesis, ataxia, and intentional tremor.

We have recently described a young girl who also had pyroglutamic aciduria and chronic metabolic acidosis but no detectable neurologic damage (6). This patient was studied at the age of 14 months, when her daily excretion of L-pyroglutamate in urine amounted to 50 mmol and her blood plasma concentration was 4.5 mM. Pyroglutamate turnover studies under steady state conditions showed that approximately 75% of the amount synthesized was metabolized by the patient. The level of 5-oxoprolinase in the patient's leukocytes was found to be normal.

Independent studies by Eldjarn and coworkers (2–5, 7, 16) on their adult patient have also failed to reveal the primary defect in pyroglutamic aciduria. The daily excretion of pyroglutamate in urine was approximately 270 mmol (4, 7) and was not affected significantly by variations in the dietary protein intake (2). After an intravenous dose of (^{14}C)pyroglutamate the patient expired considerably less $^{14}CO_2$ than a normal control subject, and this was taken as evidence of a block in the patient's capacity to metabolize pyroglutamate (2). However, the patient had a normal level of 5-oxoprolinase in cultured fibroblasts (16) and subsequent studies have revealed evidence of an increased production of pyroglutamate (3, 4).

The metabolic role of pyroglutamate has not been settled. Its involvement in the metabolism of glutathione has been proposed (14). A model for the transport of α-amino acids, referred to as the γ-glutamyl cycle, has been postulated (11, 14); it involves coupling of glutathione turnover to the translocation of α-amino acids across cell membranes, such as those of the renal tubular epithelium. However, neither patient with pyroglutamic aciduria had any signs of impaired reabsorption of α-amino acids in the kidney (6, 7). An attempt to demonstrate a correlation between pyroglutamate formation and renal tubular load of α-amino acids was unsuccessful in the infant with pyroglutamic aciduria (6). In the adult patient, on the other hand, an intravenous infusion of α-amino acids was followed by an increase in pyroglutamate excretion (2).

The present paper describes extended studies on the young girl with pyroglutamic aciduria and also on her newborn sister, who suffered apparently from the same metabolic error. In

view of the finding of decreased levels of glutathione in erythrocytes, we suggest that the primary defect in pyroglutamic aciduria is localized to the synthesis of glutathione which results in an overproduction of pyroglutamate.

CASE REPORTS

The parents of the two patients with pyroglutamic aciduria were healthy and unrelated. Neither parent showed detectable excretion of pyroglutamate in the urine.

Previous studies of the older patient (*JA*) at the age of 14 months have been published (6). She was now reinvestigated when 2 years 10 months old. The required daily dose of sodium bicarbonate was 25 mmole. Her weight and height were normal for age. Her self-adjusted diet had a high protein content, which was estimated to 5 g/kg body wt/24 hr. Otherwise her diet was normal. She had developed normally (DQ 100 according to the Bühler-Hetzer test compared with DQ 115 at 14 months of age). The patient showed no neurologic symptoms, her electroencephalogram and motor nerve conduction velocity were both normal.

From 1.5 years of age, the patient suffered from eczema localized to cheeks, neck, elbows, and hands. The intensity of the eczema varied but was considered to be mild. No specific allergen has been identified. *JA* also had signs of dental enamel hypoplasia but no active caries.

The second patient (*HA*) is the younger sister of *JA*. She was born after 39 weeks of uncomplicated pregnancy with a birth weight of 2,500 g. At 30 weeks of gestation a urine sample from the pregnant mother had been analyzed for the presence of pyroglutamate but none was detected, *i.e.*, less than 10 μM concentration was present. Immediately after a normal vaginal delivery, with Apgar scores of 10 points at 1 and 5 min, the patient was transferred to a neonatal ward for observation. Her acid-base balance was normal for 4 hr after birth, but she then developed metabolic acidosis (Fig. 1). At 20 hr of age the pH had decreased to 7.30, the standard bicarbonate concentration to 13 mM, the base excess to −10.5 mM, and the P_{CO_2} to 26 mm Hg. Oral administration of sodium bicarbonate was then started and in the course of the following days, the daily dose required to prevent remission of acidosis was found to be 25 mmol.

A urine sample obtained during the first hours of life, before feeding had started, was found to contain L-pyroglutamate at a concentration of 26 mM, which confirmed the diagnosis of pyroglutamic aciduria.

The baby developed jaundice with a maximal serum bilirubin level of 16 mg/100 ml at 50 hr; she was then treated with phototherapy, phenobarbital, and albumin. The reason for the hyperbilirubinemia was not apparent; sepsis, urinary tract infection, and blood group incompatibility were, however, ruled out. The jaundice disappeared in the course of the 1st week.

The baby was formula fed. After an initial fall to 2,310 g on the 2nd day, she gained weight steadily and was discharged from the hospital at 15 days of age with bicarbonate therapy for further controls at the out-patient department.

Hematologic data on the patients and their parents are given under *Results*.

METHODS

Routine analyses of blood and urine were performed by standard methods. L-Pyroglutamate was assayed as described previously (6); both gas-liquid chromatographic and enzymatic methods were used.

At the delivery of *HA*, samples were obtained from the amniotic fluid, cord blood plasma, and placental tissue, frozen immediately, and kept in the frozen state until analyzed.

The placental tissue was then thawed, minced, and homogenized in 3 volumes of water at 0–4°. The supernatant obtained after centrifugation at 30,000 × *g* for 30 min was deproteinized by centrifugation through a Dia-Flo membrane.

Oxidized and reduced glutathione was determined as described by Klotzsch (8).

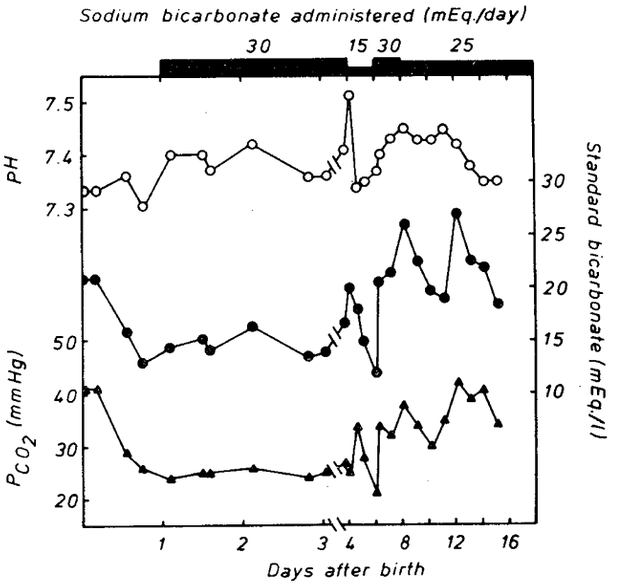

Fig. 1. Acid-base balance in the neonatal period of *patient HA*. Analyses were performed on capillary blood samples. o—o: pH; •—•: standard bicarbonate; ▲—▲: P_{CO_2}.

Table 1. *Concentrations of L-pyroglutamate in samples obtained at birth from two control subjects and patient HA*

Specimen	Concentration of L-pyroglutamate, mM	
	Control subjects	*Patient HA*
Amniotic fluid	<0.03	0.43
Cord blood plasma	<0.05	0.71
Placental tissue	<0.1[1]	5.2[1]
Urine (obtained during 1st day)	<0.03	26

[1] Millimoles per kilogram of wet weight tissue.

RESULTS

CONCENTRATIONS OF L-PYROGLUTAMATE

Markedly increased levels of pyroglutamate were detected in amniotic fluid, cord blood plasma, and placental tissue from *HA*, whereas specimens from control subjects contained insignificant concentrations (Table 1). Pyroglutamate was present in urine collected during the first hours after birth, before feeding had started. A 24-hr urine sample collected at 11 days of age contained 6.3 mmol pyroglutamate and at 3 months of age, 27 mmol (Table 2).

The elder sister, *JA*, had increased her excretion of pyroglutamate in urine from 51 mmol/24 hr at 14 months of age to 68 mmol at 2 years 10 months (Table 2). The excretion had, however, remained constant relative to her body surface. Urine samples collected fractionally during 24 hr showed some variation in the amount of pyroglutamate excreted per hour (average 2.85 mmol/hr, range 2.20–3.34) but there was no consistent pattern which correlated to the hour of day, intake of food, or physical activity. This was confirmed by analysis of

plasma levels; after fasting overnight, the concentration of pyroglutamate was found to be 2.24 mM, 2 hr after a meal while still in bed it was 2.29 mM, and subsequently after 2 hr of physical exercise (climbing a staircase), it was 2.02 mM. Compared with the plasma levels at 14 months of age (4.5 mM), the concentration had decreased. Based on an average plasma concentration of 2.15 mM, clearance was calculated to be 63 ml/min/1.73m^2. The corresponding clearance at the age of 14 months was 31 ml/min/1.73m^2.

In a sample of cerebrospinal fluid obtained after fasting overnight the concentration of pyroglutamate was 1.31 mM and in a sample of plasma obtained simultaneously it was 2.24 mM.

The concentrations of α-amino acids in plasma and cerebrospinal fluid were within normal limits, as were the quantities of α-amino acids excreted in the urine. Furthermore, there were no signs of any renal tubular defect; there was no proteinuria or glycosuria; normal urine osmolality was found in morning samples; acidification of the urine to pH 4.7 was observed repeatedly.

GLUTATHIONE LEVELS IN ERYTHROCYTES

During the neonatal period the elder sister (*JA*) developed anemia in addition to the metabolic acidosis. On the 3rd day of life her hemoglobin concentration was 15 g/100 ml and it decreased continuously to 7–8 g/100 ml at 3 weeks of age. At this time the reticulocyte counts were 8–9%. The anemia disappeared spontaneously after 2 months and the patient maintained hemoglobin concentrations of 11–13 g/100 ml. This was confirmed at 14 months of age; the reticulocyte counts were, however, increased (1.8–3.8%). Repeated hemoglobin determinations at the age of 2 years 10 months again showed a mild macrocytic anemia (hemoglobin 8.5–11.5 g/100 ml, erythrocytes 2.8–3.4·10^6/μl). No spherocytes were detected.

An analysis of glutathione in erythrocytes from *JA* indicated a drastically decreased level and a more thorough hematologic investigation was therefore done on all members of the family (Table 3). Both siblings presented evidence of enhanced hemolysis as shown by increased values for reticulocytes in combination with decreased concentrations of haptoglobin. The younger sister (*HA*) showed a certain degree of anemia, whereas *JA* appeared to compensate adequately for the accelerated breakdown of erythrocytes. Neither parent showed any evidence of increased hemolysis. All members of the family had normal serum bilirubin concentrations.

The levels of glutathione were markedly decreased in erythrocytes from both patients (Table 3). The blood samples had been deproteinized with perchloric acid immediately after withdrawal, transported in the frozen state, and analyzed 2 days later. In order to establish the effect of this procedure on erythrocyte glutathione, samples from five healthy control subjects were investigated. If the samples were analyzed immediately after withdrawal, the concentration of reduced glutathione was found to be 1.85 (SD 0.15) and oxidized glutathione 0.18 (SD 0.04) mmol/liter erythrocytes. These values are in good agreement with published data (12). After storage of the deproteinized samples at −20° for 2 days, the level of reduced glutathione was found to be 1.18 (SD 0.28) and oxidized glutathione 0.30 (SD 0.07) mmol/liter erythrocytes. Thus storage of the samples resulted in a 20% loss of glutathione residues in addition to a certain degree of oxidation of the reduced form. In spite of this, significantly decreased levels of glutathione were found in erythrocytes from both patients. Their father had an erythrocyte glutathione concentration of approximately 70% and their mother about 150% of the normal level.

Table 2. *Excretion of L-pyroglutamate in urine*

	Patient JA		*Patient HA*	
	At 14 mo	At 34 mo	At 11 days	At 3 mo
Daily excretion of L-pyroglutamate, mmol	51	68	6.3	27
Range	48–54	53–80		
mmol/1.73 m^2	210	195	58	165

DISCUSSION

The finding of pyroglutamic aciduria in two sisters strongly suggests that the defect is an inherited disease probably with autosomal recessive transmission. Apart from these two siblings, only one case has been described earlier (2–5, 7, 9, 16). A common feature seems to be a chronic metabolic acidosis and the presence of pyroglutamate in greatly increased concentrations in body fluids. The adult patient showed multiple symptoms of neurologic disturbance such as mental retardation, spastic tetraparesis, ataxia, and intentional tremor (7). The two affected children show no signs of neurologic damage so far. The adult patient was jaundiced and seriously ill in the neonatal period and from 1–2 years of age he showed neurologic symptoms and mental retardation (9).

Both affected children have shown a similar clinical course. The elder sister (*JA*) presented symptoms of metabolic acidosis at 3 days of age and has required substitution with bicarbonate ever since in a daily dose of 25 mmol. The younger sister (*HA*) was observed carefully for signs of acidosis in the neonatal period. She maintained a normal acid-base balance during the first hours of life but by the end of the 1st day she had developed a metabolic acidosis, which needed correction with bicarbonate (Fig. 1). The daily maintenance dose of bicarbonate was found to be 25 mmol; *i.e.*, the same as that of the older sister.

In *HA* the presence of pyroglutamate was demonstrated in amniotic fluid, placental tissue, and cord blood plasma as well as in urine obtained during the first hours of life, before feeding had started (Table 1). This was taken as evidence of

Table 3. *Representative peripheral blood values and erythrocyte glutathione content*

Subject	Age, yr	Hemoglobin, g/100 ml	Erythrocytes, ×10^6·μl	Hematocrit, %	Reticulocytes, %	Haptoglobin, mg/100 ml	Glutathione, mmol/liter erythrocytes	
							Reduced	Oxidized
HA	2/12	9.5	3.12	26.9	7.0	8.0	0.07	<0.10
JA	3-2/12	13.0	4.29	36.0	3.9	24	0.31	0.12
Mother	30	14.3	4.86	40.9	0.8	53	1.67	0.49
Father	30	16.0	5.15	44.9	1.5	52	0.96	0.11

increased concentrations of pyroglutamate already in the fetus. However, a urine sample obtained from the pregnant mother at 30 weeks of gestation failed to show excretion of pyroglutamate. This may well be explained by the great capacity for pyroglutamate degradation that has been demonstrated in healthy subjects (3, 4).

In the adult patient the daily excretion of pyroglutamate in urine amounted to approximately 35 g or 270 mmol/24 hr (4).

Both siblings excreted massive amounts of pyroglutamate in the urine, in the range of 60–200 mmol/1.73 m^2/24 hr (Table 2). In the younger sister (*HA*) there was a significant increase from 11 days to 3 months of age. If this reflects a postnatal maturation of the renal synthesis of pyroglutamate is open to speculation. In the elder sister (*JA*), the excretion has remained unchanged for 20 months in relation to her body surface, whereas the renal clearance of pyroglutamate has approximately doubled. Consequently, the plasma concentration of pyroglutamate has decreased from 4.5 mM to 2.2 mM.

Earlier studies of *patient JA*, have suggested that the accumulation of pyroglutamate is caused by an overproduction rather than a defect in its degradation (6). A turnover study under steady state conditions showed that the production amounted to approximately 200 mmol/24 hr, whereas the simultaneous excretion in urine was only 50 mmol/24 hr. Similar results from the adult patient have been published recently by Eldjarn *et al.* (3, 4), who also presented evidence that part of the pyroglutamate synthesis was localized to the kidneys. Furthermore, the activity of the pyroglutamate-degrading enzyme, 5-oxoprolinase, has been shown to be normal in leukocytes (6) and cultured fibroblasts (16, 18) from these patients. We have not been able to observe any relation between pyroglutamate excretion and food intake, protein loading, or physical activity, and it thus seems that the production of pyroglutamate is essentially a constant process in the metabolism. The concentration of pyroglutamate in spinal fluid was approximately 60% of that in plasma and the site of production of pyroglutamate remains obscure in our patient.

Kidney has been shown to have the highest specific activity of 5-oxoprolinase (17) and it has been speculated that pyroglutamic aciduria might be due to a decreased enzymatic activity in this tissue (2, 6). However, when (^{14}C)pyroglutamate was injected into patients who had had both kidneys removed (hemodialysis patients waiting for transplantation), they were able to convert the labeled precursor to CO_2 at the same rate as normal control subjects (3).

The metabolic role of pyroglutamate remains to be settled. Its involvement in the transport of α-amino acids across cell membranes has been suggested by Orlowski and Meister (14). In the proposed γ-glutamyl cycle the transport of α-amino acids across cell membranes is coupled to the turnover of glutathione, pyroglutamate being formed as an intermediate. However, the significance of the γ-glutamyl cycle in the transport of amino acids remains to be demonstrated. There are, however, no indications of defective transport of α-amino acids in the patients with pyroglutamic aciduria; there is no α-amino aciduria and normal concentrations of α-amino acids were found in plasma and cerebrospinal fluid. In addition, the growth and development of the 3-year-old patient have so far been normal. If the finding of decreased glutathione levels in erythrocytes is a generalized phenomenon, some other γ-glutamyl peptide must be able to substitute for glutathione as acceptor of the amino acids in the γ-glutamyl cycle, assuming that this mechanism is of fundamental importance.

Both siblings showed anemia in the neonatal period and subsequently had signs of increased hemolysis (Table 3). Their levels of glutathione in erythrocytes were drastically decreased. This finding is evidence in support of a defect in the synthesis of glutathione. The initial step in this pathway is catalyzed by γ-glutamylcysteine synthetase: L-glutamate + L-cysteine + ATP → L-γ-glutamyl-L-cysteine + ADP + P_i. It has been shown that this enzyme is also able to catalyze the synthesis of pyroglutamate in an incubation mixture which lacks cysteine (15). One possible explanation for the pathogenesis of pyroglutamic aciduria would be a genetic defect which results in a decreased affinity of the γ-glutamylcysteine synthetase for cysteine. Two adult siblings with γ-glutamylcysteine synthetase deficiency have been described recently (10). They showed hemolytic anemia, decreased levels of glutathione in erythrocytes, and markedly reduced activity of the γ-glutamylcysteine synthetase. They also had signs of spinocerebellar dysfunction. There were, however, no reports on metabolic acidosis or the presence of pyroglutamate in blood or urine. In conventional metabolic screening programs pyroglutamate might escape detection.

The second step in the synthesis of glutathione is catalyzed by glutathione synthetase: L-γ-glutamyl-L-cysteine + glycine + ATP → glutathione + ADP + P_i. A defect in this enzyme would lead to an accumulation of L-γ-glutamyl-L-cysteine. In the proposed γ-glutamyl cycle, this compound has to be protected from the action of the γ-glutamyl cyclotransferase, for which it is a good substrate, either by compartmentalization within the cell or by a close linkage between the two enzymes of glutathione synthesis (11). With an accumulation of this substance it is reasonable to expect it to be degraded by the cyclotransferase to pyroglutamate and L-cysteine. This would lead to a short circuit in the γ-glutamyl cycle, which could explain the increased production of pyroglutamate. Evidence in favor of this hypothesis has been obtained recently. Erythrocytes, placental tissue, and cultured fibroblasts from the two siblings with pyroglutamic aciduria were found to contain markedly decreased levels of glutathione synthetase (18).

SUMMARY

Two sisters, one neonatal and the other aged 3 years, both suffer from pyroglutamic aciduria (5-oxoprolinuria). They developed metabolic acidosis during the first days of life and have subsequently required substitution with bicarbonate. Their development has been normal to date and they have no signs of neurologic damage.

Both patients exhibited massive excretion of L-pyroglutamate in urine and increased levels of this metabolite in their body fluids. Increased levels of pyroglutamate were detected at birth in amniotic fluid, cord blood plasma, placental tissue, and urine. The excretion of pyroglutamate in urine showed no correlation to intake of food or physical activity. The patients had increased hemolysis and their erythrocyte levels of glutathione were less than 30% of normal. It is postulated that the primary defect in pyroglutamic aciduria is localized to a step in the synthesis of glutathione.

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19. The studies on human subjects were performed with their informed consent or, in the case of children, with the informed consent of their parents.
20. The authors wish to thank Mrs. Barbro Mattsson for her skillful technical assistance.
21. This work was supported by grants from the Swedish Medical Research Council (2583, 4272) and from the Karolinska Institutet.
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23. Accepted for publication June 28, 1974.

 Printed in U.S.A.