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Congenital Expression of Prolidase Defect in Prolidase Deficiency

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Summary

Newborn blood from three siblings with prolidase deficiency contained no detectable prolidase activity. Umbilical cord blood contained no prolidase activity in one sibling and only 6.8% of control activity in another sibling. In prolidase deficiency the enzyme defect is expressed at birth, well before the appearance of skin ulcers, and is demonstrable in filter paper specimens of blood obtained for routine screening.

Some genetic disorders are not clinically recognizable at birth or even during the first years of life. Unless a biochemical marker or other mutant gene product is recognized at or near birth, the congenital nature of the disorder is unproven. Examples of this include Huntington's Disease (2), Wilson's Disease (4), and Marfan's Syndrome (17). When a marker is identifiable, as in phenylketonuria (20), the disease is recognized as congenital.

Prolidase deficiency is a biochemical disorder that is inherited as an autosomal recessive trait. The disorder is characterized by severe skin ulcers that generally develop during childhood or adolescence. Reduced intelligence has also been observed in some patients. When brought to medical attention, affected individuals have iminodipeptiduria and a deficiency of prolidase activity (3, 6, 10, 15, 21). No patient has yet been studied at birth or shortly thereafter to determine whether the biochemical defect is indeed congenital.

We have examined prolidase activity in specimens of umbilical cord blood and newborn blood from three siblings with prolidase deficiency. Our results demonstrate that prolidase deficiency is biochemically expressed at birth; thus, prolidase deficiency can be detected by appropriate testing at birth and, most likely, could also be identified in the fetus through prenatal studies.

MATERIALS AND METHODS

Newborn blood specimens and patients. Filter paper specimens of dried blood that were used for routine newborn screening (13) and that were stored in envelops at room temperature were recovered. The samples included umbilical cord blood and newborn blood from two siblings with prolidase deficiency and newborn blood from a third sibling. Control umbilical cord and newborn blood specimens age-matched for each patient were also recovered from storage. The family came to attention when glycylprolinuria was detected by routine newborn urine screening in the youngest sibling (14). The two brothers, ages 3 and 4 yr, have mild developmental delay. The proband, a 19-mo-old girl, has mild hypotonia. The children are otherwise asymptomatic. All three have marked iminodipeptiduria and reduced erythrocyte prolidase activity (approximately 10% of normal).

Prolidase and prolinase assays. Prolidase (EC 3.4.3.7) is an iminodipeptidase which splits iminopeptides with C-terminal proline or hydroxyproline. It is most active with glycyl-L-proline, which is cleaved to glycine and L-proline (9). We measured prolidase activity by a modification of the method of Endo and Matsuda (8). Discs 3/16 inch in diameter were punched from each specimen and the blood was eluted at 4°C in a solution containing 0.250 ml of 0.1 M Tris-HCl buffer, pH 8.0, and 0.006 ml of 0.01 M $MnCl_2$. The duration of elution varied with the age of the specimen. The specimens stored for 9 mo were eluted for 24 h. With this elution time, however, enzyme activity was not detectable in eluates from control specimens stored for 3 and 4 yr, so elution with these and patient samples was continued for 1 wk. The eluates were preincubated at 37°C for 1 h after which 0.05 ml of 12 mM glycyl-L-proline (made up in 0.1 M Tris-HCl buffer, pH 8.0) was added. The volume of the final reaction mixture was 0.306 ml containing 0.196 mM $MnCl_2$ and 1.96

mM glycyl-L-proline. At zero time 0.02 ml of the reaction mixture was spotted on Whatman 3MM chromatography paper and 0.1 ml was deproteinized in 0.3 ml 3% sulfosalicylic acid. The remaining mixture was incubated at 37°C for 60 min at which time reaction mixture was again removed for spotting and deproteinization. Prolinase (EC 3.4.3.6), an analogous iminodipeptidase which cleaves *N*-terminal iminodipeptides, was measured by the same procedure except for the substitution of L-prolylglycine as substrate. Glycine was identified in the reaction mixture by unidimensional paper chromatography (7) and glycine and proline were measured by ion-exchange column chromatography (22).

RESULTS

Patient specimens were clearly distinguished from controls by paper chromatography of the reaction mixtures at 60 min incubation, based on the presence of glycine in controls and the absence of glycine in the patients. The prolidase activities of patients and controls are shown in Figure 1. No prolidase activity was detected in the newborn blood specimen from any of the patients. Prolidase activity in control newborn blood specimens was 463 ± 103 nmol glycine produced/disc/h in six specimens for 9 mo, 142 ± 52 nmol glycine produced/disc/h in 10 specimens stored for 3 yr, and 15 ± 12 nmol glycine produced/disc/h in 10 specimens stored for 4 yr. In cord blood, no prolidase activity was detectable in the specimen from the 3-yr-old sibling as compared with mean activity of 90 ± 32 nmol glycine produced/disc/h in 10 control specimens. Cord blood prolidase activity in the specimen from the youngest sibling was 33 nmol glycine produced/disc/h, which was only 6.8% of the matched control mean of 489 ± 126 nmol glycine produced/disc/h for six specimens.

Prolinase activity was increased in newborn blood from each of the siblings and in umbilical cord blood from the proband (Table 1). This increase was nearly 2-fold or greater as compared with matched control sepcimens.

We investigated possible reasons for the decrease in prolidase activity in filter paper specimens of blood as storage times increased (Fig. 1). Two possible explanations for this phenomenon are a reduced amount of eluted protein and loss of enzyme activity. To study the former possibility, the protein concentration was measured in eluates of filter paper newborn blood and cord blood specimens with storage times of 4 wk, 10 mo, 3 yr, and 4 yr. The specimens were eluted for 3 d and the protein concentration was determined by a modified Lowry method (18). These average concentrations were 6.3, 3.6, 2.5, and 0.76 mg/ml, respectively. When corrected for mean protein concentration in the eluate, the mean prolidase activities in filter paper newborn blood specimens stored for 10 mo, 3 yr, and 4 yr was 128.6, 56.8, and 19.7, respectively, expressed as μmol glycine produced/mg protein/h. The observed inverse relationship between prolidase activity and storage times of the specimens appear to result from both loss of enzyme activity and diminished protein elution.

Table 1. *Prolinase activity in filter paper specimens of dried umbilical cord blood and newborn blood from siblings with prolidase deficiency and controls. The control specimens were matched for age and length of storage with the corresponding specimen from the patient*

	Prolinase activity*	
Specimen	Newborn blood	Umbilical cord blood
Patient (10-mo-old)	428	348
Control	140	140
Control	240	172
Patient (3-yr-old)	172	
Control	64	
Control	28	
Patient (4-yr-old)	60	
Control	4	
Control	36	

* Nanomoles of glycine produced per disc per hour.

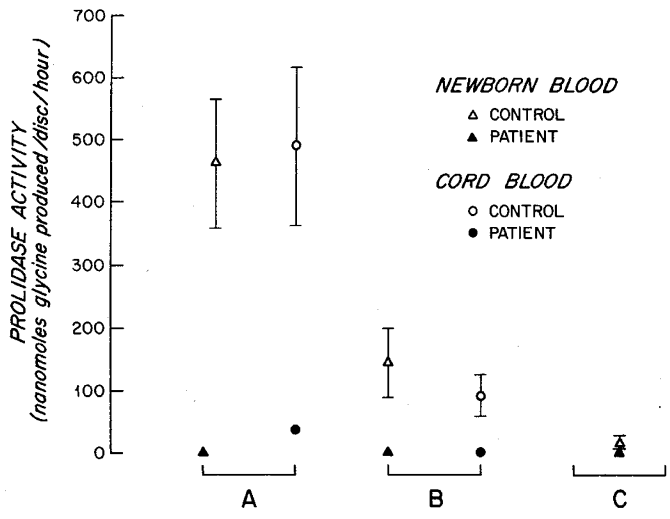

Fig. 1. The activities of prolidase in filter paper specimens of dried umbilical cord blood and newborn blood from siblings with prolidase deficiency and from age-matched control infants. Siblings A, B, and C and controls for each are represented within the respective columns. The prolidase activity in control specimens decreases substantially with storage (*A*, 9 mo; *B*, 3 yr; and *C*, 4 yr). There is a clear difference, however, between prolidase deficiency and matched controls.

DISCUSSION

Our results demonstrate the absence or marked reduction of prolidase activity in umbilical cord blood and newborn blood in prolidase deficiency. The enzyme abnormality in this disease is expressed at birth, and most probably, in the fetus, conforming to the model of an inborn error of metabolism. Furthermore, the proband in this family excreted glycylproline as early as 4 wk of age. This suggests that the complete biochemical phenotype in prolidase deficiency is present at birth or shortly therafter and at least several years before the clinical features appear.

Prolidase deficiency appears to be an inborn error of metabolism in the general category of disorders such as phenylketonuria, wherein the biochemical aberrations are present from birth and produce subclinical damage before clinical abnormalities are recognized (12). If proposed therapies for prolidase deficiency are to be optimally effective they probably will need to be initiated during early infancy (5, 9). Reduced or absent prolidase activity in blood can be used as a marker for the diagnosis of this disease at birth or in the neonatal period. Antenatal diagnosis may also be possible through the measurement of prolidase activity in cultured amniocytes obtained via amniocentesis.

The substantial increase of prolidase activity in blood from the patients with prolidase deficiency was unexpected. In other studies of patients, increased prolinase activities have been found in erythrocytes (11, 16), and cultured fibroblasts (1, 21) though this has not been a consistent finding (5, 21). If further investigations confirm an increase of prolinase activity in these individuals, one would question whether prolidase and prolinase are under a common genetic regulatory system with overproduction of one enzyme when production of the other is reduced (19). Altered levels of iminodipeptides could perhaps also affect prolinase production or activity. These levels have not yet been studied at birth or in the newborn period in patients with prolidase deficiency.

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Effect of Different Total Parenteral Nutrition Fuel Mixes on the Body Composition of Infant Miniature Pigs

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Summary

At 10 d of age miniature pigs were randomized to receive either of two total parenteral nutrition fuel mixes; oral feedings were discontinued. Both groups received 170 kcal·kg^{-1}·d^{-1} and 11 g·kg^{-1}·d^{-1} of synthetic amino acids. Nonprotein energy was supplied as glucose in group A, whereas in group B, it was divided equally between glucose and fat. Blood samples were drawn on the second and eighth postoperative days for hematologic, biochemical, and hormonal measurements. On the ninth postoperative day, total body water was determined and the animals were killed for carcass analysis. The animals tolerated the intravenous nutrition without ill effects as indicated by both clinical and biochemical parameters. Group A had significantly elevated levels of insulin and a higher insulin/glucagon ratio than group B. Cortisol levels did not differ significantly between groups. Total body fat, nitrogen, ash, K, Na, Cl, Ca, and P were similar between groups. TBW was significantly greater in group A compared with group B. Extracellular space calculated from body Cl and plasma Cl was similar between groups.

Abbreviations

CBC, complete blood count
TBW, total body water
TPN, total parenteral nutrition

TPN is a life saving means of nutritional support for patients who are unable to absorb adequate amounts of nutrients. The