Tohoku J. exp. Med., 1987, **151**, 465-475

Clinical, Biochemical and Enzymatic Studies in Type I Hyperprolinemia Associated with Chromosomal Abnormality

KAZUHIKO OYANAGI, AKIRA TSUCHIYAMA, YOSHIHIRO ITAKURA, YAEKO TAMURA, TOORU NAKAO, SHIGERU FUJITA* and HIROSHI SHIONO†

*Department of Pediatrics, Sapporo Medical College, Sapporo 060, *Department of Pediatrics, Kushiro Municipal Hospital, Kushiro 085 and ‡Department of Legal Medicine, Sapporo Medical College, Sapporo 060*

OYANAGI, K., TSUCHIYAMA, A., ITAKURA, Y., TAMURA, Y., NAKAO, T., FUJITA, S. and SHIONO, H. *Clinical, Biochemical and Enzymatic Studies in Type I Hyperprolinemia Associated with Chromosomal Abnormality.* Tohoku J. exp. Med., 1987, **151** (4), 465-475 —— A severe mentally retarded infant with type I hyperprolinemia associated with chromosomal abnormality is reported. The patient had a characteristic facial appearance of hyperprolinemia and suffered from convulsions after the age of 10 months. The child developed severe mental and motor retardation. The karyotype of the patient revealed partial duplication of the short arm in chromosome 10 using G banding techniques. The patient and her mother showed a fasting hyperprolinemia and an abnormal clearance curve after the proline load in the serum. The proline oxidase activities of the liver tissues obtained by biopsy in the patient was about 9% of those of controls. Kinetic studies and mixed experiments of the enzyme were with normal limits. Restriction of dietary proline at the age of 12 months revealed a prompt fall of the plasma levels of proline to the normal range, and a low proline diet was continued until the present time. During the period of dietary treatment, growth was satisfactory, but her mental development did not improve. From the developmental patterns of proline oxidase activities postnataly, we speculated that restriction of dietary proline intake should be relieved with age. ———— Hyperprolinemia; proline oxidase; chromosomal abnormality.

Several inborn errors of iminoacids metabolism have been reported in the past (Scriver et al. 1983). Hyperprolinemias can be divided into two types by locating the site of enzyme defect. Type I hyperprolinemia (McKusick 23 950) is caused by deficient proline oxidase activity, (EC number not assigned) and type II involves Δ^1-pyrroline-5-carboxylic acid dehydrogenase (EC 1.5.1.12). A type I hyperprolinemic patient was first described by Scriver et al. (1961), and about eleven families have since been reported (Scriver et al. 1983). The major clinical

Received May 9, 1986; accepted for publication March 9, 1987.

features associated with this disorder are hereditary nephropathy, mental retardation, photogenic epilepsy, and nerve deafness. The relationship between type I hyperprolinemia and the clinical manifestations remain unclear.

Recently, several families of hyperprolinemia type I and II without any clinical symptoms have been reported (Mollica et al. 1971; Pavone et al. 1975). Dietary treatment for hyperprolinemia was considered as an important factor for the improvement of the disorders.

We report upon an infant with mental retardation and chromosomal abnormality in whom very low levels of proline oxidase activity were found in the liver tissues obtained by biopsy.

The purpose of the present paper is to describe the clinical and biochemical findings of the patient with proline oxidase deficiency.

Case Report

The female infant in this case was born after the previous five pregnancies had ended in spontaneous miscarriage. She was the product of a normal pregnancy and delivery, and weighed 2,816 g at birth. At the age of five months, she was unable to smile and to support her neck. She developed convulsions frequently at the age of ten months. The girl was admitted to our hospital at the age of eleven months because of mental and motor retardation as well as elevated proline levels.

The patient was well nourished and had a normal body weight, but exhibited a short status (-3.5 S.D.). She had a high and full forehead, wide and prominent nasal foot, hyperterolism, low-set ears and a small jaw (Fig 1). The cardiovascular and respiratory systems were normal. The liver and spleen were not palpable. Her eyes showed normal movement. She could not support her head and her extremities were hypotonic. The

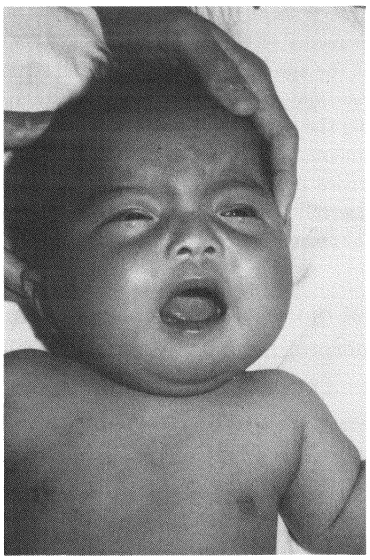

Fig. 1. Front view of the patient. Note a high and full forehead, wide and prominent nasal foot, hyperterolism, low-set ears and small jaw.

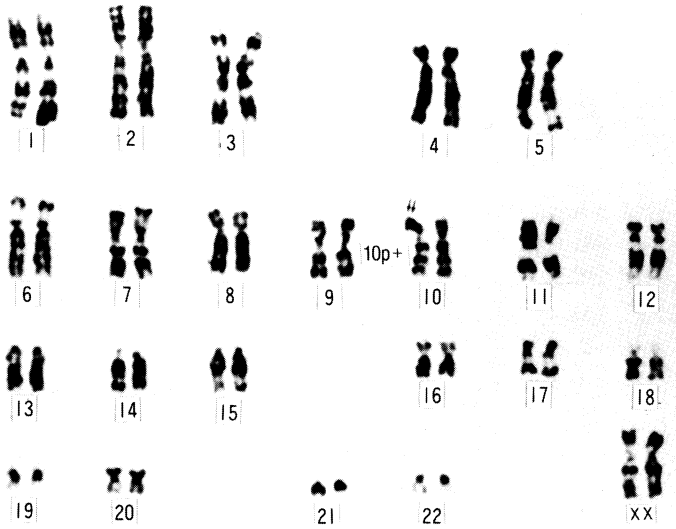

Fig. 2. G-banded karyotype of patient. Arrows show that the distal portion of the short arm in chromosome 10 is duplicated.

reflexes were normal and her pathological reflexes were absent.

Routine peripheral blood analysis and urinalysis were all within normal limits. Liver function tests were normal. Blood glucose, lactate, ammonia, alkaline phosphate, copper, BUN, creatinine, electrolytes, total protein, and protein elctrophoresis were all normal. The cerebrospinal fluid was clear and showed no abnormality in either cell counts or protein contents. Fundoscopic examination revealed no abnormality. Renal function tests were also normal. Intravenous urography and renal scintigram showed normal pictures. Auditory brain stem responce (ABR) and otopharyngeal examinations of the patient were normal. Electroencephalographic findings showed sporadic spikes and high voltage slow waves. Computed tomography of the brain showed diffuse symmetrical dilatation of the ventricles and slightly cortical atrophy. Developmental quotient of the patient was markedly decreased to 36.

Chromosome preparations were obtained from peripheral blood lymphocytes, and revealed 46, xx, +10 p (Fig. 2). The origin of the additional region of the short arm of chromosome 10 was not obscure. Both parental karyotypes were normal.

Methods and Results

Quantitative analysis of amino acids in serum, urine and cerebrospinal fluid were performed by means of an automatic amino acid analyzer (JCL-6AH type, Joel Co., Tokyo). Serum concentrations of proline in the patient after fasting were in the range of 472–829 μ mole/liter (Table 1). Urinary amino acids analysis in the patient was within normal limits and did not show any iminoglycinuria (Table 2). Proline in the cerebrospinal fluid of the patient was not detected.

Oral loading tests of L-proline were carried out in the patient and her parents. After overnight fastings, 100 mg of L-proline per kilogram body weight was given orally and then serum and urine amino acids were measured, respectively. The results obtained are shown in Fig. 3 and Table 3. The serum concentrations of proline of the patient and her mother before the load were apparently higher than those of the controls and showed an abnormal clearance curve as well. Serum proline levels of her father showed a similar clearance curve

TABLE 1. *Seurm amino acid level (μmole/liter)*

Amino acids	Patient	Controls ($n=28$)
His	86.9	73.1± 14.5
Orn	67.8	85.7± 33.3
Lys	141.3	170.2± 54.7
Arg	84.3	88.2± 31.6
Cys	4.2	10.7± 7.7
Met	17.6	31.3± 19.7
Ileu	51.2	73.7± 24.2
Leu	150.5	128.1± 40.5
Tyr	56.1	73.0± 32.0
Phe	47.2	89.7± 39.2
Pro	830.8	185.8± 77.6
Gly	455.1	310.3±156.5
Val	403.2	231.5± 66.8
Tau	228.9	105.3± 41.3
Asp	22.7	15.3± 8.2
Thr	134.1	125.0± 56.3
Aspn	76.7	69.8± 57.8
Ser	191.3	161.2± 43.2
Glu	150.0	100.7± 47.6
Gln	531.9	555.2±237.6

as those of the controls. Proline, hydroxyproline and glycine excretions in urine specimens of the patient and her father were the same as those of the controls before and after the load. However, prominent proline excretions in the urine of her mother were observed after load.

Examination of urine for Δ^1-pyrroline-5-carboxylic acid (5 PC) was carried out by the method of Efron (1965), and 5PC was not detected in urine while the patient and her parents were receiving a normal diet nor during the proline load test.

Liver tissues from the patient for enzyme assay were obtained by an open biopsy at twelve months of age. The mitochondrial fractions from the liver tissues were prepared by the method of Hogeboom (1955).

Proline oxidase activities in the liver tissues were measured by method of Herzfeld et al. (1977). Reaction mixtures contained a 53 mM potassium phosphate buffer (pH 8.0), 158 mM-L-proline (Wako Chemical Co., Osaka), and 1.6 μM cytochrome C (Sigma Chemical Co., St. Louis, MO, USA) in a final volume of 1.9 ml. The reaction was incubated at 37°C for 30 min with shaking in air and stopped with 1.0 ml of 10% (w/v) trichloroacetic acid. The 5 PC formed was determined by the addition of 0.1 ml of 0.2 M *o*-aminobenzaldehyde (Sigma Chemical Co.), centrifugation and reading the A_{440} by the spectrophotometer (Hitachi, Model 200-20, Tokyo) in the supernatant fraction after 30 min. The enzyme activities were calculated as nanomoles of 5 PC formed per min per mg of protein.

Lactate dehydrogenase (LDH) activities, one of the marker enzymes of cytosol fraction and glutamate dehydrogenase (GDH) activities as a mitochondrial marker enzyme were assayed in the liver tissues, respectively (Kornberg 1955; Strecker 1955).

The protein content of the liver tissues was estimated by the method of Lowry et al. (1951).

TABLE 2. *Urinary amino acid analysis (μmole/g creatinine)*

Amino acids	Patient	Controls ($n=8$)
His	2,851	2,036-3,294
Orn	32	76- 191
Lys	355	318- 696
Arg	239	166- 203
Cys	93	107- 147
Met	95	56- 77
Ileu	125	177- 212
Leu	58	121- 131
Tyr	294	466- 637
Phe	88	163- 188
Pro	94	Not detect
Hypro	Not detect	Not detect
Gly	1,912	1,504-3,447
Ala	806	700-1,007
Val	101	108- 146
Tau	435	607-2,300
Asp	188	379- 607
Thr	626	330- 533
Aspn	654	68- 284
Ser	1,239	1,045-1,253
Glu	105	50- 137
Gln	2,089	1,041-1,526

TABLE 3. *Urinary iminoglycine excretion before and after proline loading (μmole/g creatinine)*

	Proline		Hydroxyproline		Glycine	
	Before	After	Before	After	Before	After
Patient	51	191	0	0	2,607	4,001
Mother	69	3,765	0	0	1,194	1,977
Father	0	74	0	0	1,051	1,977

proline 100 mg/kg weight.

Enzymatic analysis of the liver tissues in the patient and age-matched controls are shown in Table 4. The ratio of mitochondrial fractionation of the liver tissues in the patient and controls was above 90%. Proline oxidase activity of the mitochondrial fraction in the patient's biopsied liver sample was decreased to about 9% of the age-matched controls, whereas the enzyme activities of other fractions were not detected. The Km for proline of the patient exhibited a slight elevation compared with that of the controls (Fig. 4).

The presence of an activator of proline oxidase was ruled out by the mixing experiment.

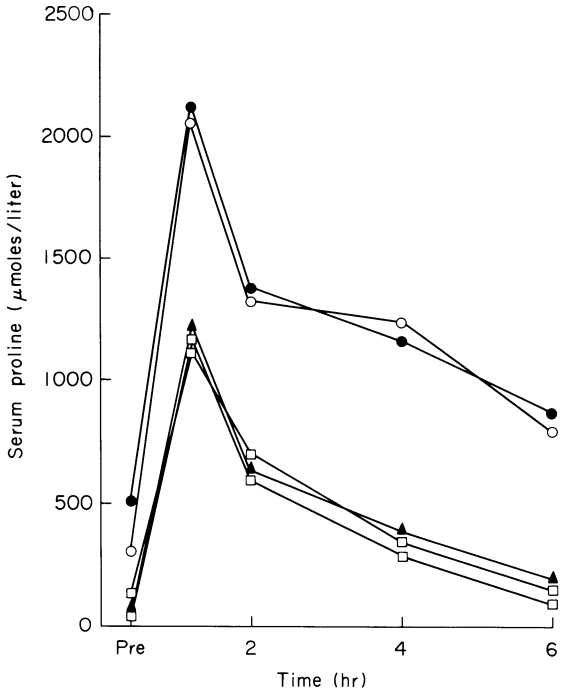

Fig. 3. Oral loading tests of L-proline (100 mg/kg body weight).
●——●, patient; ○——○, mother; ▲——▲, father; □——□, controls.

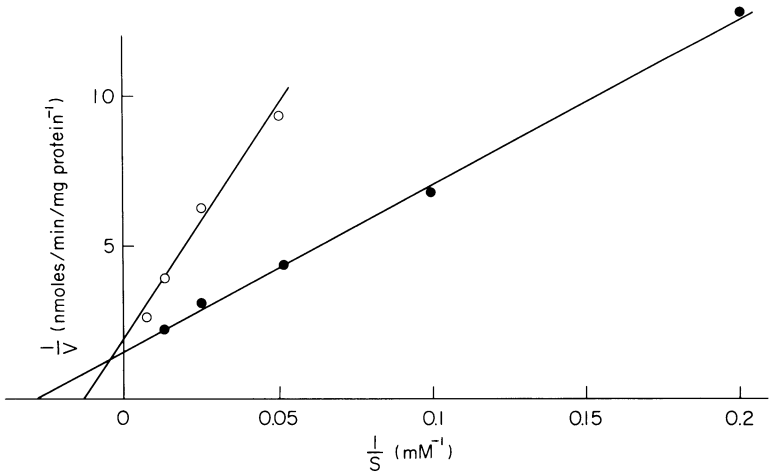

Fig. 4. Lineweaver-Burk plot of proline oxidase in human liver. ○, type 1 hyperprolinemia (Km=77.9 mM); ●, control (Km=38.5 mM).

Similarly, L-valine and L-leucine were not effective as inhibitors of measured proline oxidase activity in the liver tissues of the patient.

Proline oxidase activity were measured in fetal and postnatal liver tissues. Fetal liver tissues were obtained from legal abortions. The specific activity of proline oxidase increased 5 to 10 fold in the liver tissues form fetal to postnatal life (Fig. 5). The enzyme activities of the liver tissues in infants and children were higher than those of neonates.

TABLE 4. *Hepatic proline oxidase activity in type I hyperprolinemia*

	Proline oxidase activity (n moles/min/mg protein)	
	Pellet	Supernatant
Patient	0.36 (7% of control)	0
Controls ($n=5$)	5.56±2.46	0

Enzymes	Patient				Controls ($n=3$)
	Mitochondria	Cytosol	Microsome	Nucleus	Mitochondria
Proline oxidase (nmoles/min/mg protein)	0.97 (9% of control)	0	0	0	10.56-9.83
Lactate dehydrogenase (μmoles/min/mg protein)	0.040 (6.9%)	0.575 (91.7%)	0.002 (1.4%)	0	0.031-0.045
Glutamate dehydrogenase (μmoles/min/mg protein)	45.7 (94.1%)	2.7 (5.9%)	0	0	53.4-39.8

Mixed experiment	Proline oxidase activity (nmole/min/mg protein)	Protein incubated (mg)
Patient	0.97	2.47
Control	2.60	5.09
Patient+control		
Predicted	3.57	
Actual	3.36	3.78

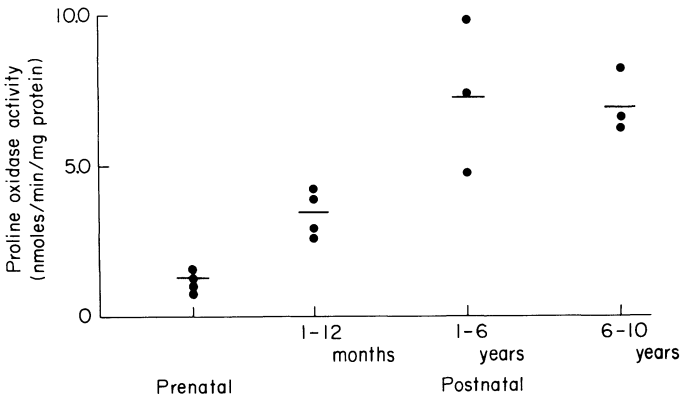

Fig. 5. Specific activities of proline oxidase in human liver of different ages.

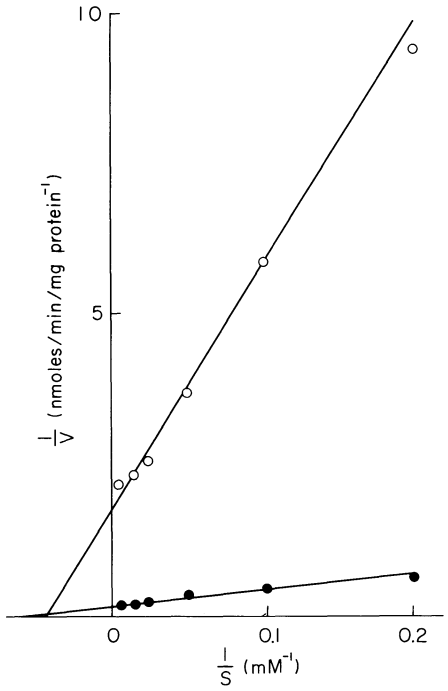

Fig. 6. Lineweaver-Burk plot of proline oxidase activity in fetal and postnatal liver. ○, fetal (Km=17.9 mM); ●, postnatal (Km=22.7 mM).

The kinetic data demonstrated the same Km for fetal and postnatal proline oxidase activities in mitochondria (Fig. 6).

The daily intake of proline was restricted to about 50 mg per kg body weight and was started at the age of 12 months. Serum proline levels of the patient decreased and were maintained in the range of 174-348 μmole/liter.

Discussion

Proline oxidase, a mitochondria bound enzyme, oxidizes L-proline to Δ^1-pyrroline-5-carboxylate (5PC) which can be sequentially dehydrogenated to glutamine and α-ketoglutarate to serve as a course of energy for cells. The human metabolic disorder of type I hyperprolinemia is characterized by a very low rate of proline oxidation due to a deficiency of mitochondrial proline oxidase (Efron 1965).

In most cultured cells, proline oxidase is undetectable (Dowing et al. 1976), and so the definite diagnosis of type I hyperprolinemia was due to the measurement of enzyme activity in liver tissues. A single case on the assay of proline oxidation in a type I hyperprolinemia was reported by Efron (1965). The autopsied liver tissue of the reported patient indicated a reduced proline oxidation compared with the control human liver samples. Conversion of proline to

glutamate was estimated to be not more than 10% in liver of patient. Our case was the second report whose proline oxidase in liver tissue clearly decreased to about 9% compared with age-matched controls. There was considable variation in the proline oxidase activity of the controls in Efron's study (1965). Since proline oxidase activity in human liver tissue is variable by ages (Fig. 5), control data of the enzyme activity should be prepared as an age-matched one.

The specific activities of proline oxidase increased 5 to 10 fold in human liver from fetal to postnatal (Fig. 5), and the developmental patterns of this enzyme in human liver was comparable to those of rat liver (Kowaloff et al. 1976). However, Km values for proline in a human liver were 8 to 16 folds higher than those of rat liver enzyme. The proline oxidase activity is variable by ages. It increases with age in the human liver. This difference may relate to the varied rate of utilization of proline for protein synthesis. In rapidly proliferating young tissue, it is speculated that the synthesis of collagen and other proteins is derived chiefly from proline utilization (Kowaloff et al. 1976).

The major clinical manifestation in a type I hyperprolinemic patient reported here, is the impairment of the central nervous system. The relationship between the biochemical defect and neurological abnormalities is not yet clear. Recently, several hyperprolinemic patients without any clinical manifestations have been reported (Mollica et al. 1971 ; Pavone et al. 1975). The PRO/Re mice in which hepatic proline oxidase deficiency developed normally without dietary treatment showed no abnormalities of their ranal morphology (Kanwar et al. 1975). Whelan and Conners (1980) described two healthy children of untreated maternal type I hyperprolinemia. Mollica and Pavone (1976) reported that the hyperprolinemias could be considered a benign condition that did not warrant any treatment. Whether mental retardation of our type I hyperprolinemia is due to hyperprolinemia or chromosomal abnormality is uncertain. However, low proline diets have been used in a patient with the type I defect and an improvement of the growth and normal state were reported during the period of diet treatment (Harries et al. 1971). Low proline dietary therapy of our patient was started from twelve months of age and serum proline concentrations of the patient were decreased and maintained to within normal levels. During the period of dietary treatment, growth of the patient was satisfactory, but mental development did not improve. From the developmental patterns of proline oxidase activities, we speculated that restriction of dietary proline intake will be relieved with age.

Genetic fashion of the hyperprolinemias is proposed an autosomal recessive trait in which some heterozygotes exhibit hyperprolinemia (Scriver et al. 1983). Our patient's mother also showed of fasting hyperprolinemia and an abnormal clearance curve after the proline load in the serum. The mother was suspected as a heterozygote. Recently, Valle et al. (1976) clarified the significantly decreased levels of 5PC dehydrogenase activity in the leukocytes extracts and the cultured skin fibroblasts from obligate heterozygotes for type II hyperprolinemia and that

this disease is inherited in an autosomal recessive fashion. However, it is impossible to determine the heterozygote of type I defect from the enzyme analysis, since proline oxidase enzyme is not present in blood leukocytes or skin fibroblasts (Dowing et al. 1976).

A type I hyperprolinemic patient associated with chromosomal abnormality has not been reported until now. Metabolic diseases have been mapped to specific autosomes. Glutaminate-γ-semi-aldehyde (GS) which is one of the intermediates in the proline synthesis and the degradation and GS synthetase exists on chromosome 10 from the human gene map (McKusick 1978). Our type I hyperprolinemic patient is associated with chromosome 10 abnormality and it is difficult to presume that proline oxidase enzyme is linked to chromosome 10 or not.

References

1) Dowing, S.J., Phang, J.M., Kowaloff, E.M., Valle, D. & Smith, R.J. (1976) Proline oxidase in cultured mammalian cells. *J. cell. Physiol.*, **91**, 369-376.
2) Efron, M.L. (1965) Familial hyperprolinemia. Report of a second case, associated with congenital renal malformations, hereditary hematuria and mild mental retardation, with demonstration of an enzyme defect. *New Engl. J. Med.*, **272**, 1243-1254.
3) Harries, J.T., Piesowicz, A.T., Seakings, J.W.T., Francis, D.E.M. & Wolff, O.W. (1971) Low protein diet in type I hyperprolinemia. *Arch. Dis. Childh.*, **46**, 72-81.
4) Herzfeld, A., Melz, V.A. & Knox, W.E. (1977) Enzymes metabolizing Δ^1-pyrroline-5-carboxylate in rat tissues. *Biochem. J.*, **166**, 95-103.
5) Hogeboom, G.H. (1955) Fractionation of cell component of animal tissues. *Meth. Enzymol.*, **1**, 16-19.
6) Kanwar, K.S., Krakower, C.A., Manaligod, J.R., Justice, P. & Wong, P.W. (1975) Biochemical, morphological and hybrid studies in hyperprolinemic mice. *Biomedicine*, **22**, 209-216.
7) Kornberg, A. (1955) Lactate dehydrogenase of muscle. *Meth. Enzymol.*, **1**, 441-443.
8) Kowaloff, E.M., Granger, A.S. & Phang, J.M. (1976) Alterations in proline metabolic enzymes with mammalian development. *Metabolism*, **25**, 1087-1094.
9) Lowry, O.H., Rosebrough, N.J., Farr, A.L. & Randall, R.J. (1951) Protein measurements with the Folin phenol reagnet. *J. biol. Chem.*, **193**, 265-275.
10) McKusick, V.A. (1978) The human gene map. In: *Mendelian Inheritance in Man*, edited by V.A. McKusick, The Johns Hopkins University Press, Baltimore-London, pp. 66-77.
11) Mollica, F. & Pavone, L. (1976) Hyperprolinemia: A disease which does not need treatment? *Acta paediat. scand.*, **65**, 206-208.
12) Mollica, F., Pavone, L. & Antener, I. (1971) Pure familial hyperprolinemia: Isolated inborn error of amino acid metabolism without other anomalies in a sicillian family. *Pediatrics*, **48**, 225-231.
13) Pavone, L., Mollica, F. & Levy, H.L. (1975) A symptomatic type II hyperprolinemia associated hyperglycinemia in three sibs. *Arch. Dis. Childh.*, **50**, 637-641.
14) Scriver, C.R., Schafer, I.A. & Efron, M.L. (1961) New renal tubular amino-acid transport system and a new hereditary disorder of amino-acid metabolism. *Nature (Lond.)*, **192**, 672-673.
15) Scriver, C.R., Smith, R.J. & Phang, J.M. (1983) Disorders of proline and hydroxyproline metabolism. In: *The Metabolic Basis of Inherited Disease*, edited by J.B. Stanbury, J.B. Wyngaarden, D.S. Fredrickson, J.L. Gldstein & M.S. Brown, McGrow-Hill, New York, pp. 360-381.

16) Strecker, H.J. (1955) L-glutamic dehydrogenase from liver. *Meth. Enzymol.*, **2**, 220-225.
17) Valle, D., Goodman, S.I., Applegrath, D.A., Shih, V.E. & Phang, J.M. (1976) Type II hyperprolinemia Δ^1-pyrroline-5-carboxylic acid dehydrogenase deficiency in cultured skin fibroblasts and circulating lymphocytes. *J. clin. Invest.*, **58**, 598-603.
18) Whelan, D.T. & Conners, N.C. (1980) Matarnal hyperprolinemia. *Lancet*, **1**, 981.