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Persistent Tyrosinemia Associated with Low Activity of Tyrosine Aminotransferase

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Summary

A son of related Turkish parents had grossly elevated serum tyrosine concentration and excreted tyrosine and *p*-hydroxyphenolic acids into the urine, whereas neither succinylacetone nor succinylacetoacetate could be demonstrated. The tyrosine concentration was normalized by a proper diet. This was not followed strictly at home. During the first 2 years of life, the patient had severe undulating nystagmus that disappeared later. No skin lesions were present and there was only slight corneal clouding of the eyes. At the age of 5, the patient had attained the maturity of a 4-year-old, showing a balanced profile.

Specific tyrosine aminotransferase (EC 2.6.1.5) was present in the liver; the K_m value for tyrosine was normal. However, the total activity was less than 10% of normal, a situation similar to that observed in fetal human liver. A younger sister of the patient also has tyrosinemia and low hepatic tyrosine aminotransferase activity.

Abbreviation

pHPP, *p*-hydroxyphenylpyruvate

Tyrosine, the precursor amino acid of thyroid hormones, catecholamines, melanin, and tyramine, is catabolized in the liver via a pathway beginning with transamination in the cytosol by L-tyrosine:2-oxoglutarate aminotransferase (EC 2.6.1.5). This reaction yields pHPP which is then sequentially oxidized by pHPP dioxygenase (EC 1.13.11.27) and homogentisate 1,2-dioxygenase (EC 1.13.11.5) to yield homogentisate and maleylacetoacetate, respectively. The latter compound is sequentially attacked by maleylacetoacetate isomerase (EC 5.2.1.2) and fumarylacetoacetase (EC 3.7.1.2) with final cleavage to fumarate and acetoacetate. Tyrosine aminotransferase is the rate-limiting step of this sequence (11, 13, 30), and its activity is controlled in a complicated fashion. It is induced by glucocorticoids, glucagon, catecholamines, and tyrosine in rat liver (2, 5, 19, 25, 27, 29) and in fetal human liver in organ culture (3, 6, 13).

The metabolism of tyrosine is impaired in liver cirrhosis (7, 26) and in 30% of premature newborns (9, 17). The significance of these types of tyrosinemia has been under dispute (17, 24). Two types of hereditary tyrosinemia have been described in the literature. The more common type, tyrosinemia I, manifests itself initially by failure to thrive, jaundice, hepatosplenomegaly, hematuria, and edema. If the patient lives, severe nodular liver cirrhosis, Fanconi syndrome, and hypoglycemia occur; hepatomas are common (17, 36). Tyrosine, pHPP, *p*-hydroxyphenyllactate, succinylacetoacetate, and succinylacetone are excreted into the urine (10, 17). This disease is inherited in an autosomal recessive fashion and is due to deficiency of fumarylacetoacetase (8, 22).

Tyrosinemia II (Richner-Hanhart syndrome, tyrosinosis of Oregon type) is rare; only a few cases have been described in the literature (14, 16, 21, 33). This syndrome is characterized by mental retardation, nystagmus, and palmar and plantar hyperkeratosis and erosions; clouding, erosions, and dendritic ulcerations of the cornea have been described. Tyrosine and excessive amounts of pHPP are excreted into the urine (14). This disease has been reported to be caused by an absolute deficiency of hepatic tyrosine aminotransferase (14). The excretion of pHPP, the product of the missing enzyme, has been explained by transamination by the mitochondrial isoenzyme of aspartate aminotransferase (EC 2.6.1.1) in extrarenal and extrahepatic tissues that do not have pHPP dioxygenase activity (14, 20). This mitochondrial isoenzyme of aspartate aminotransferase that can transaminate tyrosine only at unphysiologically high concentration (25, 28) can be separated from tyrosine aminotransferase by virtue of its high pI (31). In three cases, tyrosine aminotransferase activities have been measured in liver biopsy samples and have been reported to be totally absent in the cytosol (14, 18, 21).

In the present investigation, we describe two siblings with persistent tyrosinemia and partial deficiency of cytosolic tyrosine aminotransferase.

CASE REPORTS

The first patient was the son of healthy Turkish parents. The father and the maternal grandmother were first cousins. The paternal grandmother (not related to the mother) had a suspected metabolic disease. The patient had two younger sisters, one of which will be described below. The other sister was apparently healthy.

The boy was born at term in normal delivery after an uncomplicated pregnancy and was appropriate for gestational age (3340 g, 52 cm). A moderate unconjugated jaundice resolved spontaneously at the age of 1 month. Routine neonatal screening revealed elevated serum tyrosine levels while the concentrations of other amino acids were normal. The child was hospitalized at 1 month of age. A mild hepatosplenomegaly was found without any further clinical symptoms. Administration of vitamin C did not influence serum tyrosine levels. A diet free of tyrosine and low in phenylalanine was introduced at the age of 1 month. However, it was not followed strictly at home (Fig. 1). The levels of serum tyrosine in relation to diet are indicated in the figure. Introduction of this diet always resulted in a normalization of serum tyrosine levels in 3–4 days.

An intensive undulating nystagmus of sudden onset arose at the age of 5 months after 3 months of normal diet and high serum tyrosine levels. The nystagmus lasted for 1½ years and faded away slowly. From the second year on, the boy adopted a strange, torticollis-like posture when concentrating on near objects. During his toddler years, the boy had frequent febrile

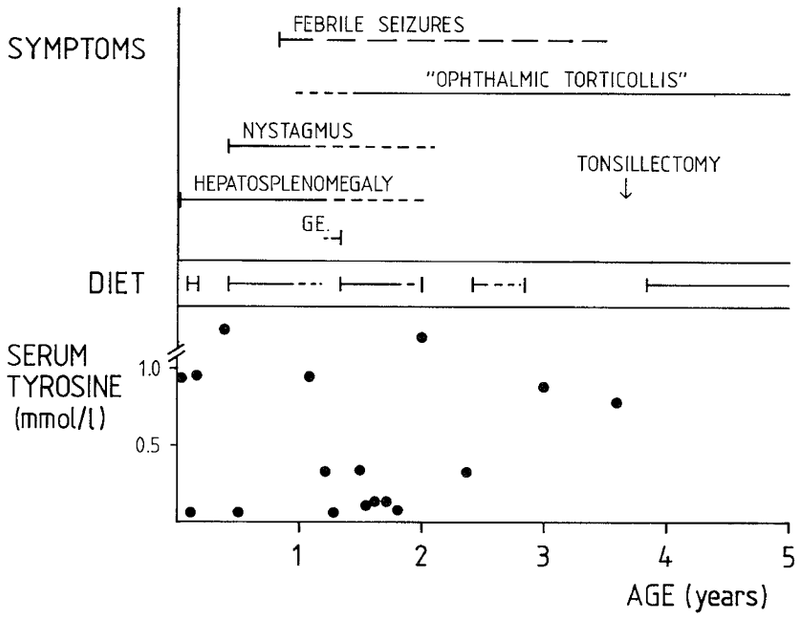

Fig. 1. Summary of clinical findings of the first patient in relation to diet and plasma tyrosine concentrations. *GE*, gastroenteritis.

seizures but the electroencephalography was normal on repeated occasions. A moderate psychomotor retardation observed during the first year of life was improved during the next few years. At the age of 5, the boy has reached the maturity of a 4-year-old, showing a balanced profile.

During the first months of life, the boy had slightly elevated levels of serum transaminases. Liver biopsy according to Menghini was performed on four occasions: at the ages of 1 and 4 months, 1½ years, and 5 years. No histological changes could be revealed by light microscopic examination. The patient has not had any signs of hyperkeratosis or any other skin lesions.

The second patient, the younger sister of the first patient, was born without complications with a birth weight of 4200 g. Neonatally, her plasma amino acid concentrations were normal and no organic acids were found in her urine. She was fed on normal formula and no check-ups were performed. At the age of 6 months, she was hospitalized because of infectious diarrhea. Three weeks after her recovery, she was found to be generally retarded in her psychomotor development. The liver was palpated 3–4 cm below the costal margin. She had elevated serum transaminases. Her plasma tyrosine concentration was slightly above normal level. No organic acids were found in the urine.

The actual investigation was performed at the age of 13 months. In her clinical status, a hepatomegaly was noticed and her psychomotoric development was generally somewhat retarded. Biochemically, she had normal serum transaminase levels and a markedly elevated serum tyrosine concentration. Needle biopsy of the liver showed no pathological changes under the light microscope. The electroencephalography was normal.

BIOCHEMICAL STUDIES

The boy had a plasma tyrosine concentration of 794 μmol/liter (normal range, 39–77 μmol/liter) at the age of 3⁷/₁₂ years when he was not on the tyrosine-free diet. The plasma concentrations of the other amino acids were normal. The urinary excretion of tyrosine was 155 μmol/24 h (normal < 132 μmol/24 h) and that of *p*-hydroxyphenylphenolic acids was 1.9 mmol/24 h (normal < 1.0 mmol/24 h). Succinylacetoacetate and succinylacetone could not be demonstrated in the urine. Erythrocyte porphobilinogen synthetase and urinary porphobilinogen excretion were normal. These determinations were done as described in (22).

A liver biopsy by the method of Menghini was performed on the boy at the age of 5 years with the informed consent of the parents. The sample (21.5 mg) was homogenized in 20 volumes of 320 mM glycerol, 5 mM potassium phosphate, pH 7.6, using a Potter-Elvehjem glass-Teflon homogenizer. The homogenate was centrifuged at 20,000 × *g* for 10 min and the supernatant fraction was kept frozen until analyzed. Tyrosine aminotransferase activity was assayed by a modification (31) of the method of Diamondstone (12) and aspartate aminotransferase activity and protein as described previously (23, 34). Total "tyrosine aminotransferase activity" of the supernatant fraction was 1.65 nmol of product/mg of protein/min. For comparison, in a previous study from our laboratory, the corresponding activities in human fetuses of 12–22 weeks of gestational age averaged 0.53 and those for adults 19.8 nmol of product/mg of protein/min (6). The activity is age-dependent in infancy but is on an adult level at the age of 5 (32). The electrofocusing pattern (31) of tyrosine aminotransferase activities from normal liver and from our patient are shown in Figures 2 and 3. In both cases, a peak of aspartate aminotransferase activity is seen at pH 9. These peaks also had low "tyrosine aminotransferase" activity as can be expected in the light of the broad substrate spectrum of this enzyme (31, 35). The enzyme is of no significance in normal metabolism of tyrosine because of its low affinity towards this nonphysiological substrate (28, 31, 35). As shown in Figure 2, specific cytosolic tyrosine aminotransferase activity was divided into subforms at pH 5 as expected (4, 31). A comparatively low peak (fractions 4–7) corresponding to cytosolic tyrosine aminotransferase is evident in Figure 3. The K_m of these combined fractions towards tyrosine was 1.1 mM (Fig. 4), which is close to the value obtained with purified enzyme (4). The corresponding determinations for fractions 5–9 (tyrosine aminotransferase) and 24–26 (mitochondrial aspartate aminotransferase) from the experiment shown in Figure 2 are displayed for comparison in Figure 4.

A liver biopsy was also obtained from the patient's younger sister at the age of 1 year. Due to the small size of the sample, the presence of specific tyrosine aminotransferase could not be demonstrated. Total tyrosine aminotransferase activity was clearly below that of the brother but the exact level could not be accurately determined.

DISCUSSION

The boy excreted neither succinylacetone nor succinylacetoacetate and his erythrocyte porphobilinogen synthetase activity was normal in clear distinction from tyrosinemia I. The clinical

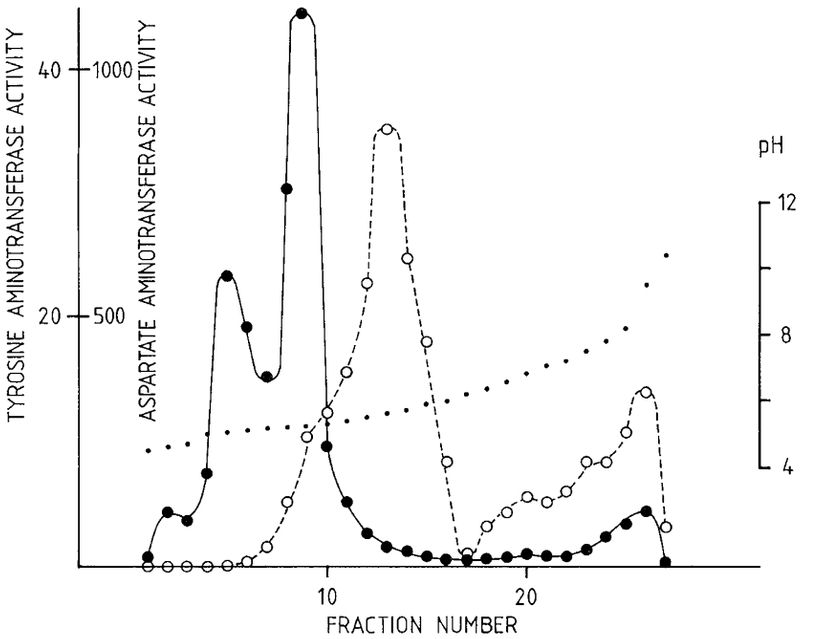

Fig. 2. Isoelectric focusing of normal human liver tyrosine aminotransferase activity. A sample of the liver of a healthy 32-year-old man (accidental death) was homogenized in 2 volumes of 320 mM glycerol, and 5 ml of the 100,000 × *g* supernatant fraction was focused in a glycerol density gradient (total volume, 100 ml) in the pH range 4–6 (1% Ampholine) for 40 h at 400 V. Fractions were collected and analyzed for tyrosine aminotransferase and aspartate aminotransferase activities as described in the text. Enzyme activities are expressed as nanomoles of product/ml/min. ●——●, tyrosine aminotransferase; ○– –○, aspartate aminotransferase; · · · · ·, pH.

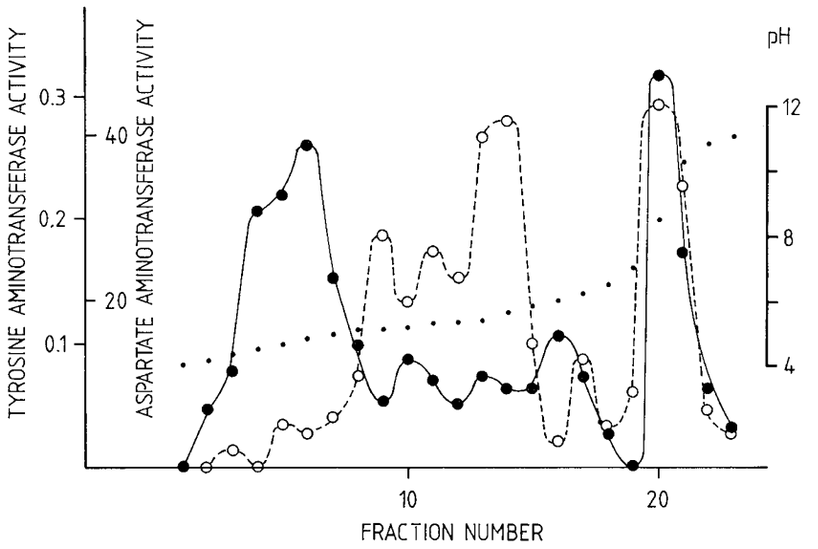

Fig. 3. Isoelectric focusing of tyrosine aminotransferase activity from the liver of the first patient. Two hundred twenty-five μl of the supernatant fraction described in the text were electrofocused in the pH range 4–6 (1% Ampholine) in a total volume of 15 ml for 15 h at 400 V. The *symbols* and *units* are as in Figure 2.

picture is different from tyrosinemia I as well as from Richner-Hanhart syndrome in which palmar and plantar hyperkeratosis as well as corneal ulcerations and mental retardation are present. A case resembling those of our patients' has been described by Goldsmith *et al.* (16) who found lowered activity of tyrosine aminotransferase in the liver of an adult patient with tyrosinemia. Also, while this manuscript was in preparation, Giardini *et al.* (15) described a patient with tyrosinemia. Specific tyrosine aminotransferase was reported to be present in the patient's liver but the activity was low compared to normal (V_{max} 0.37 *versus* 0.88 μmol/mg of protein/h). *p*-Hydroxyphenylpyruvate dioxygenase activity was reported to be absent. Unfortunately, our samples were too small to allow determinations of this enzyme activity in our patients.

We have shown previously (6) that the specific enzyme tyrosine aminotransferase is present in fetal human liver at the end of the first trimester. However, the activity is low in fetal and premature neonatal human liver (6, 30). Although the enzyme is inducible by, *e.g.*, glucocorticoids in organ culture of fetal liver, it cannot be induced by intraperitoneal injections of these hormones *in utero* in the rat. Therefore, it has been suggested that there is a factor in fetal liver that suppresses the induction by glucocorti-

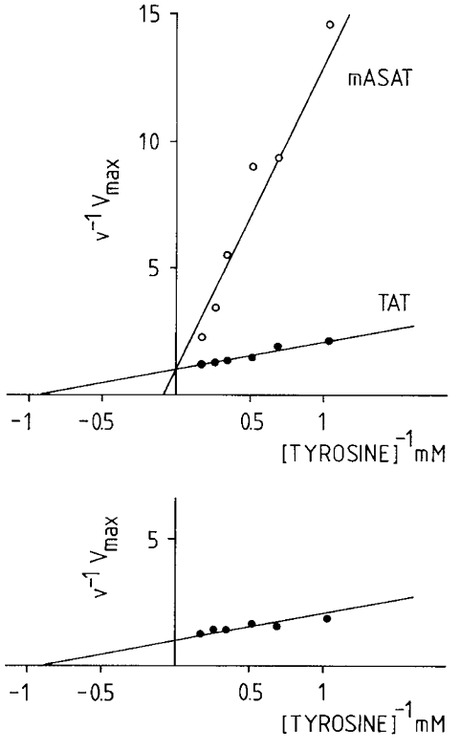

Fig. 4. Determination of K_m values for tyrosine of specific tyrosine aminotransferase (*TAT*) from normal human liver (above) and from the liver of the first patient (below). See text for explanations. *mASAT*, mitochondrial aspartate aminotransferase.

coids (37). In the case of our patients, the situation is similar to that in fetal and premature neonatal liver. They both have low liver tyrosine aminotransferase activities. It was shown, however, that the boy has a specific tyrosine aminotransferase that does not accept aspartate or oxaloacetate as substrate and has the same isoelectric point and K_m value for tyrosine as the adult enzyme (4). Therefore, it is possible that the defect is not in the coding for tyrosine aminotransferase itself but in the regulatory mechanism of this enzyme.

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