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Immunochemical Study of Ornithine Transcarbamylase Deficiency

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Deficiency of ornithine transcarbamylase (OTC, EC 2.1.3.3) (McKusick 31125), a mitochondrial enzyme of the urea cycle, appears to be one of the most frequent causes of inherited ammonia intoxication, and evidence indicates that the enzyme is X-linked. Recently, different kinds of mutation have been reported (Cathelineau *et al.*, 1972; Briand *et al.*, 1982). We carried out detailed studies on the molecular properties of the enzyme from two heterozygous females, using an antibody to bovine OTC which cross-reacted with the human enzyme.

MATERIALS AND METHODS

Liver samples from two female patients who suffered from hyperammonaemia due to an OTC deficiency have been studied. The diagnoses of OTC deficiency were performed before death. Patient 1, whose detailed clinical course was previously reported (Kodama *et al.*, 1983), died at the age of 10 years. Patient 2, who was from a different family from patient 1, died at 5 years of age. The liver samples were obtained at autopsy within a few hours and afterwards were kept at −80 °C until use. Control liver samples were obtained from infants who died from congenital heart disease.

The liver was homogenized with 4 volumes of 1% Triton X-100 containing 1 mmol l^{-1} dithiothreitol and centrifuged at 105 000 *g* for 60 min at 4 °C to remove insoluble materials. OTC activity was measured according to the method of Nuzum and Snodgrass (1976).

Bovine liver OTC was purified to homogeneity by the method of Marshall and Cohen (1972). Antibovine OTC was raised in rabbits by injecting 6 mg of the purified enzyme mixed with Freund's complete adjuvant subcutaneously twice every 2 weeks.

Aliquots (15 µl) of bovine, human control, and patients' liver extracts were analysed by Ouchterlony double immunodiffusion against antibovine OTC rabbit serum. The amounts of cross-reactive materials against the antiserum were measured by Mancini radial immunodiffusion.

For immunotitration, varying amounts of extracts from the livers of the patients and controls were added to constant amounts of antibovine OTC rabbit serum in 20 mmol l^{-1} potassium phosphate buffer (pH 7.4) containing 5 mmol l^{-1} ornithine and 1 mmol l^{-1} dithiothreitol. Following a preliminary incubation for 15 min at 37 °C, the mixtures were incubated overnight at 4 °C. After removal of the immunoprecipitates by centrifugation, the OTC activity in each of the supernatants was measured.

RESULTS AND DISCUSSION

The OTC activity of both patients 1 and 2 was found to be very low (180 and 150 µmol h^{-1} (g wet weight)$^{-1}$ at pH 8.0, respectively) when compared with normal control values (4200 ± 900 µmol h^{-1} (g wet weight)$^{-1}$ at pH 8.0). The OTC activity of the patients varied with pH (6.6–10.0), but they were always about 5% of those of the normal control.

Antibovine OTC rabbit serum reacted with human OTC and gave a cross-reaction between bovine and human OTC. However, the same amounts of liver extracts from both patients did not react with the antiserum. The contents of OTC proteins in the patients' livers calculated from radial immunodiffusion were 0.1 and 0.12 µg mg wet weight^{-1} of liver as bovine OTC, respectively, whereas that of the control liver was 1.8 + 0.6 (mean ± SD). These results showed that the amounts of cross-reactive material from both patients were about 5% of those from control livers (Figure 1). As shown in Figure 2, the OTC activity of the patients could be immunotitrated by antibovine OTC rabbit serum, suggesting that the cross-reactive materials in the patients' livers had OTC activity. Thus we concluded that low activity of OTC in the present patients results from the deficiency of OTC protein.

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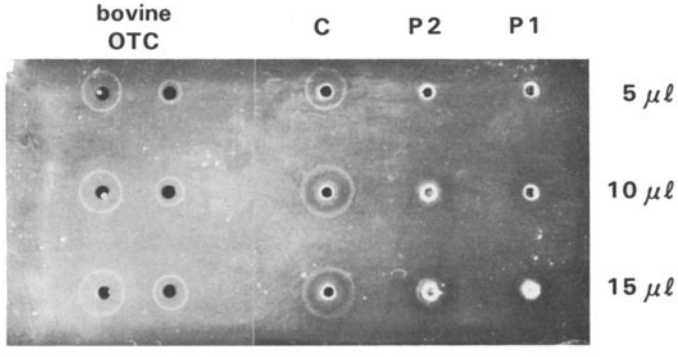

Figure 1 Radial immunodiffusion of bovine OTC and liver extracts from the patients and control. 5, 10 and 15 µl of each extract and various amounts of bovine OTC were placed

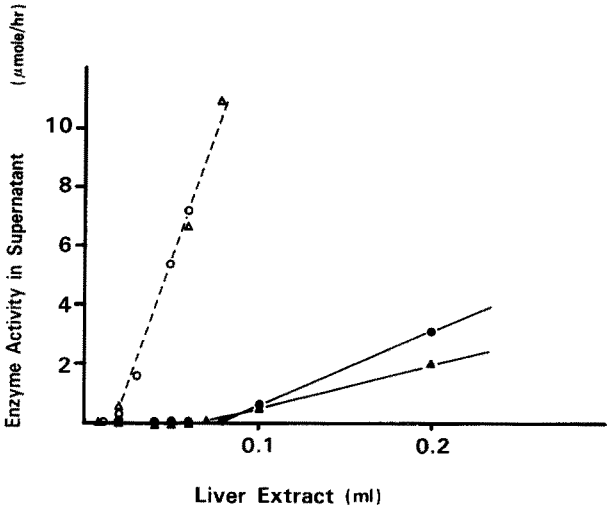

Figure 2 Immunotitration of OTC in liver extract (×5) of patients 1(●–●), 2(▲–▲) and control (– – –): A mixture (1 ml) contains varying amounts of extract, antibovine OTC, 20 mmol l^{-1} potassium phosphate pH 7.4, 5 mmol l^{-1} ornithine and 1 mmol l^{-1} dithiothreitol

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