ORNITHINE TRANSCARBAMYLASE, AN ISOELECTRIC POINT (pI) ISOZYME IN HUMAN LIVER AND ITS DEFICIENCY.

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Summary

The crude extract of human liver ornithine transcarbamylase, obtained from a patient with hyperammonemia due to enzyme deficiency, was studied by the isoelectric focusing method. The activity of ornithine transcarbamylase in the patient at pH 8.0 was only slightly reduced.

After electrolysis, two main peaks which were isoelectric at pH 3.2 and pH 4.4 were observed in the control, while only one peak at pH 2.8 was found in the patient.

Nine cases of hyperammonemia with ornithine transcarbamylase deficiency have been reported. Of these cases 3 were considered to be due to variant or mutant enzymes (1,2). However, biochemical studies were not conclusive in these cases.

In our previous report (3), we described an 8 1/2 month female infant with hyperammonemia and ornithine transcarbamylase deficiency in whome several enzymatic studies were performed.

Ornithine transcarbamylase activity in the patient at pH 8.0 was only slightly reduced compared with that in normal control. The Michaelis constant (Km) of the enzyme for carbamyl phosphate in the patient liver showed approximately 4 fold values of the control, while the Km value for ornithine was similar in the patient and in the control.

It was suspected from these observations that ornithine transcarbamylase of the patient liver was a mutant enzyme.

This paper deals with a further investigation on ornithine transcarbamylase of the patient liver which was carried out by the isoelectric focusing method.

Material and Method

Post mortem samples of the liver were obtained from the patient with hyperammonemia and also from control infants without liver disease within a few hours after their death. All materials used in this study were stored for more than 6 months at -20°C. The liver tissue was homogenated with 0.1 % (w/v) cetyltrimethylammonium bromide solution and centrifuged. The supernatant was divided into two parts. In one part of this solution ornithine transcarbamylase activity was estimated by the method of Brown and Cohen (4), and the other part was used for isoelectric fractionation (5). Two ml of the supernatant was applied in a LKB 8101 Ampholine Column of 110 ml capacity containing LKB 8151 Carrier Ampholine which was selected to give a pH gradient between pH 3 and pH 10. Focusing and separation of the enzyme was carried out at 0°C. Stabilization against convection was achieved by using a density gradient prepared from one dense and one less dense solution. After focusing for 40 hours, beginning at 800 V to a final potential of about 600 V, the contents of the column were cut into one ml fractions. Immediately after fractionation, the pH of each fraction was measured at 0°C with a radio pH meter Hitachi-Horiba F5 with a relative accuracy of ± 0.02 pH units.

The fractions were then analyzed for ornithine transcarbamylase activity by the method of Brown and Cohen (4), in which, however, 0.6 ml of the fraction instead of 0.2 ml in the original method was used in order to obtain a desirable optical density for estimation of citrullin at 490 mu.

Table

Liver enzyme

	Patient		Control		
Carbamyl phosphate synthetase	123		100		
Ornithine transcarbamylase					
pH 8.3	4760	* 4800	6850	6080	
pH 7.0	1130		4500		
Arginin synthetase system	84		84	138	
Argininosuccinate cleavage enzyme	386		386		
Arginase	6910		4000	7550	8000

units : μmoles / gm. wet weight / hr

* The value estimated in the present study

Results and Discussion

The ornithine transcarbamylase activity of the patient liver in the present study was found to be the same as previously reported (Table). This observation might suggest that 6 months storage or over at -20°C does not affect the enzyme activity.

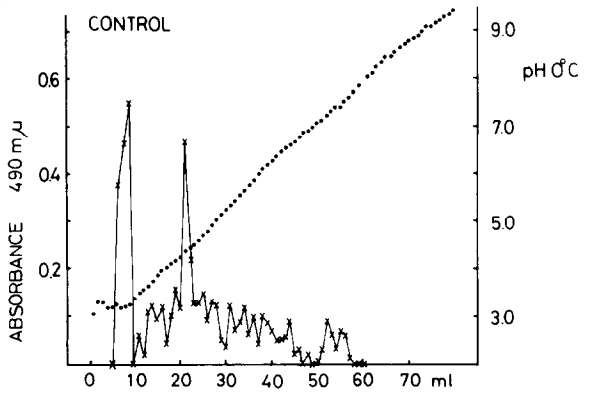

Figure 1 Isoelectric fractionation of ornithine transcarbamylase in human liver. ✕——✕ control human liver, ••• pH at 0°C.

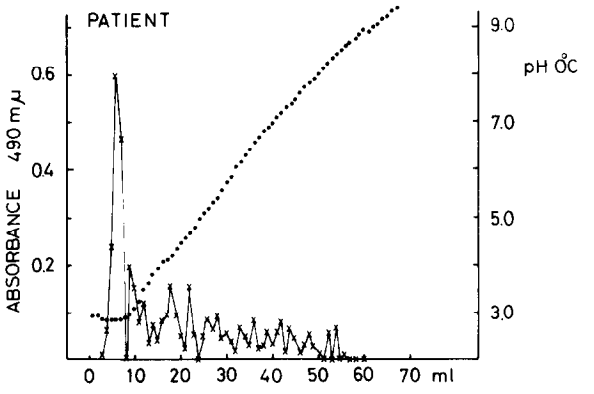

Figure 2 Isoelectric fractionation of ornithine transcarbamylase in patient liver. ✕——✕ patient liver, ••• pH at o°C.

Approximately 70 - 80 per cent of the total ornithine transcarbamylase activity before separation remained after electrolysis. Therefore, no great loss of activity occurred

during electrolysis. After electrolysis, two main peaks which were isoelectric at pH 3.2 and at pH 4.4 were observed in the control liver enzyme. (Figure 1) On the other hand one main peak at pH 2.8 was found in the patient liver enzyme. (Figure 2) Further investigation on the Km value estimation in each peak fraction was impossible, because the patient liver material was exhausted in the previous and the present studies. Therefore it would be very difficult to conclude whether a reduction of affinity of the enzyme for carbamyl phosphate, as was observed previously, is directly connected with a defect of the peak at pH 4.4 in the isoelectric fractionation. (Figure 3) The present observations might support our prior consideration that the liver ornithine transcarbamylase in the patient is a mutant enzyme.

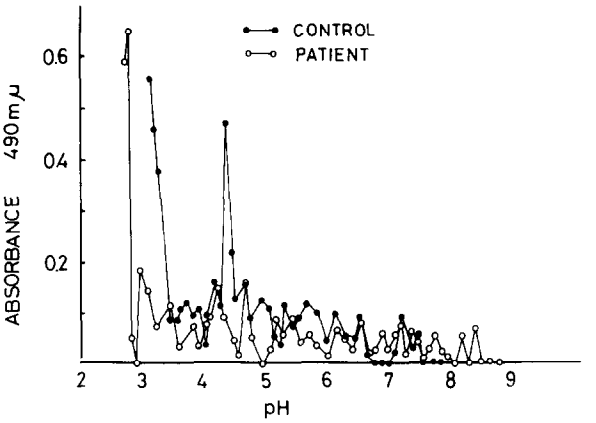

Figure 3 Comparison of pI - isozyme pattern of ornithine transcarbamylase.

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