ELECTROPHORETIC ABNORMALITY OF GALACTOSE-1-PHOSPHATE URIDYL TRANSFERASE IN GALACTOSEMIA.

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SUMMARY

In two siblings with clinical galactosemia and incomplete deficiency of galactose-1-phosphate uridyl transferase in the red cells, an abnormally slow moving enzyme was detected upon electrophoresis. This indicates that the transferase molecule is modified in its structure, a heretofore undescribed finding in galactosemia. The name of "Rennes" is tentatively given to this type.

Galactose-1-phosphate uridyl transferase (transferase) is present in red blood cells. This allows ready detection of galactosemia, a recessive inherited condition due to a deficiency of transferase. Recently several types of mutants have been described (1)(2)(3). One of them, the Duarte variant, which does not affect the clinical status, was identified not only by its abnormal red cell enzyme activity, but also by an abnormally fast electrophoretic mobility (4). However in galactosemia no electrophoretic detection of the enzyme has been reported thus far.

In this paper we present an electrophoretic study of red cell transferase from two siblings with clinical galactosemia. In both cases the enzyme displayed an abnormally slow mobility,

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a result which indicates that the deficiency may be, at least in these subjects, due to a structural abnormality of the enzyme.

MATERIAL AND METHOD

The patients studied were two siblings : a 16 months old boy and his sister, 2 months old. Both exhibited clinical and biological symptoms of galactosemia(x).

The transferase activity was estimated by the uridine-diphospho-glucose (UDPG) consumption method, adapted from Beutler (6). Electrophoresis was carried out using technique of Mathai and Beutler (4) in which slight modifications were introduced : horizontal instead of vertical gel and incorporation of 0.01 M β-mercaptoethanol in the gel. Hemolysates, prepared by freezing and thawing washed red cells, were applied to Whatman paper strips. The blotted strips were inserted into the gel. Several dilutions of hemolysates were also further concentrated by pervaporation through dialysis tubing against dry Sephadex G 25. After electrophoresis, the transferase activity was localized on the sliced gel at 37°C by the use of the reagent mixture devised by Mathai and Beutler (4), in which cysteine was replaced by 0.1 M dithioerythritol. After a variable period of time, a fluorescent band corresponding to the enzyme activity was seen upon examination of the gel under long-wave ultra-violet light. When the substrate (galactose-1-phosphate) was omitted in the mixture no band at all was apparent.

RESULTS

The transferase activity of the two patients with galactosemia was not completely abolished. The amount of UDPG consumed

(x) We wish to thank Pr. Coutel and Dr. Jezequel for their clinical study of these patients. Their results will be fully published elsewhere.

per hour and per g of hemoglobin was 1.0 micromole, the normal activity in our laboratory being 14.05 (2 σ = 4.82 micromoles).

Upon electrophoresis of deficient hemolysates and specific revelation for transferase activity, a very slight fluorescent band was detectable with a slower mobility than normal enzyme. (Figure 1)

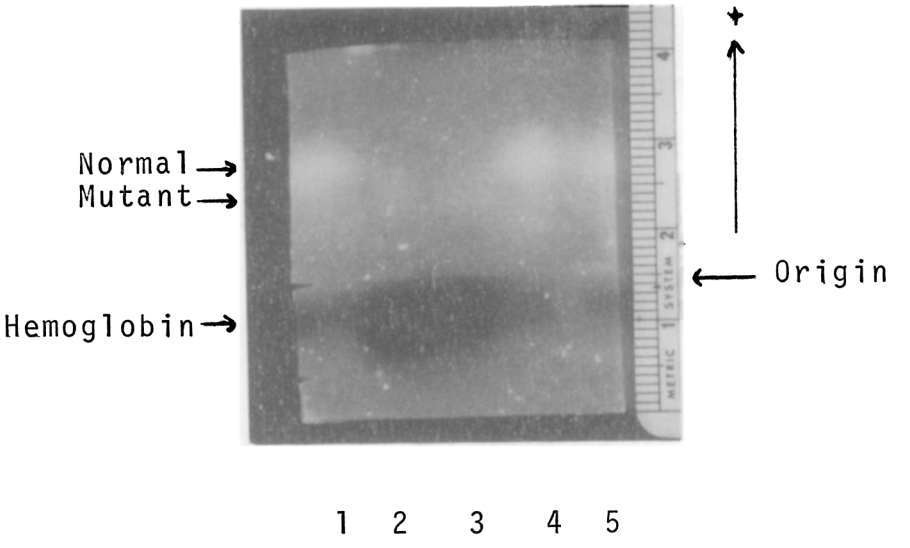

Fig. 1 Starch gel electrophoresis of transferase:1, 4, 5 : normal hemolysates ; 2, 3 : hemolysates from galactosemic siblings. Channel 2 and 3 are overloaded with hemolysate in order to make visible the weak transferase activity.

This weak band was more visible in deficient hemolysates concentrated against dry Sephadex, as shown in Figure 2.

In diluted, undiluted and concentrated normal hemolysates no such band was detected, the normal band retaining its regular mobility. Therefore, increasing the concentration of hemolysate does not modify the transferase mobility. Experiments carried out with concentrated hemolysates, from normal subjects, with and without substrates, rule out the possible presence of a weak slower minor band in normal red cells as well as that of an artefact.

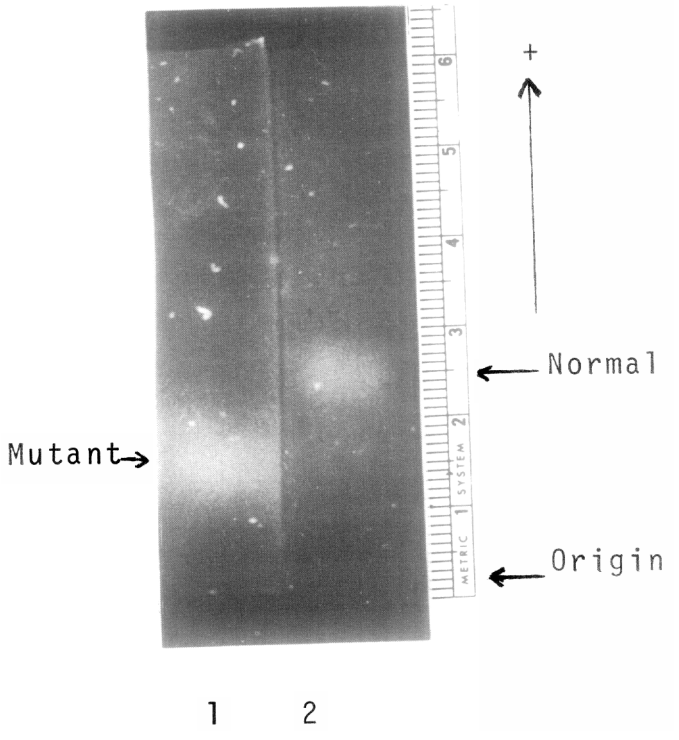

Fig. 2 Starch gel electrophoresis of highly concentrated hemolysates.
Channel 1 ; galactosemic ; channel 2 : normal (same intensity of bands has been obtained by shortening incubation time of channel 2).

DISCUSSION

Our results show in two galactosemic patients with incomplete loss of transferase activity, an electrophoretic abnormality of the enzyme, with less anodic mobility than normal. This indicates that the molecule is structurally abnormal. It is not possible to decide at present whether this abnormality is responsible for the loss activity through a modification of the active site or through an in vivo decrease of stability of the enzyme (7). The presence of an immunologically detectable protein, almost completely deprived of enzymatic activity as found for instance in fructose intolerance (8) and with pseudo-cholinesterase (9) is to be sought. This problem is now under current investigation.

For it was not possible to study the parents, no conclusion can be drawn at present regarding the genetic significance of our findings. On the other hand, more cases should be examined in order to determine whether the electrophoretic abnormality of transferase that we found is common to all cases of galactosemia or represents only a rare variant. For the time being we propose to name this type "Rennes" which is the birthplace of our patients.

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