References and Notes

1. M. Sundaralingam, *Biopolymers* **7**, 821 (1969).
2. J. Karle and H. Hauptman, *Acta Crystallogr.* **9**, 635 (1956).
3. D. C. Rohrer and M. Sundaralingam, *ibid.* **B26**, 546 (1970).
4. M. Sundaralingam, *J. Amer. Chem. Soc.*, in press.
5. ——— and J. Abola, *Acta Crystallogr.*, in press; M. Stroud, *ibid.*, in press, and unpublished result.
6. U. Thewalt, C. E. Bugg, R. E. Marsh, *ibid.* **B26**, 1089 (1970).
7. In a similar fashion, the 2′-deoxyribosides, 5′-deoxycytidine monophosphate monohydrate (M. A. Viswamitra, B. Swaminatha Reddy, G. H. Y. Lin, M. Sundaralingam, *J. Amer. Chem. Soc.*, in press), deoxyadenosine monohydrate [D. G. Watson, D. J. Sutor, P. Tollin, *Acta Cryst.* **19**, 111 (1965)], and thymidine [D. W. Young, P. Tollin, H. R. Wilson, *ibid.* **B25**, 1423 (1969)] exhibit the conformations ${}_3T_2$, ${}_3T^2$, and ${}_3T^2$, respectively. The primary pucker is C(3′)-*exo* in these compounds, and this conformation appears to be unique to the deoxyribosides and relevant to the structure of DNA. Thus, for the first time, evidence for the occurrence of characteristic conformations for the ribosides on one hand and the deoxyribosides on the other has been found.
8. In dihydrothymidine [J. Konnert, I. L. Karle, J. Karle, *Acta Crystallogr.* **B26**, 770 (1970)], which is not a tRNA component, a similar disorder of O(5′) was found, but it occurred between the conformations *gt* and *gg* in the ratio 0.66 : 0.34. Thus, even here the predominant conformer was *gt*, supporting the observations in dihydrouridine. However the 2′-deoxyribose in dihydrothymidine exhibits a novel conformation, ${}^{O}E$ [O(1′)-*endo*] in terms of the best four-atom plane C(1′)–C(2′)–C(3′)–C(4′), and ${}^{O}T_4$ [O(1′)-*endo*–C(4′)-*exo*] in terms of the three-atom plane C(1′)-C(2′)-C(3′).
9. We thank Dr. Douglas Rohrer for growing the crystals. Supported by NIH grant GM-17378.

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Galactosemia: Evidence for a Structural Gene Mutation

Abstract. *Identical immunoprecipitin reactions appeared in double immunodiffusion between rabbit antibodies to human galactose-1-phosphate uridyl transferase and red cell preparations from both normal and galactosemic individuals. The galactosemic erythrocyte preparations quantitatively absorbed the antibody that immunoprecipitates enzymatically active galactose-1-phosphate uridyl transferase.*

Galactosemia is a human autosomal recessive disorder due to a functional deficiency of galactose-1-phosphate uridyl transferase (transferase) (*1*). Every tissue of affected individuals that has been studied is devoid of, or markedly deficient in, transferase activity (*2*). The absence of transferase activity in cells of galactosemic individuals poses the question of whether the genetic defect is an alteration or deletion of the locus that codes for the structural protein, or is an abnormality of the regulatory factors that control the rate of synthesis or degradation of the transferase protein. This report presents evidence that the defect in galactosemia is a "point" mutation in the structural gene which has resulted in the synthesis of a catalytically inactive transferase protein.

Human transferase was purified 1000-fold (specific activity = 1 unit per milligram of protein) from liver by a procedure that includes homogenization, precipitation with protamine sulfate, fractionation with ammonium sulfate, gel filtration, and column electrophoresis in Sephadex G-200 (*3*). Antibody to this purified enzyme was produced in a male New Zealand albino rabbit by four biweekly intramuscular injections of 1 mg of enzyme protein dissolved in 0.5 ml of 50 m*M* glycylglycine, *p*H 8.5, mixed with 0.5 ml of Freund's complete adjuvant. One week after the fourth injection an immunoprecipitin to transferase protein was detected by double diffusion in agar, and 50 ml of blood was collected by ear vein puncture. The serum was collected and fractionated on Sephadex G-200 with 50 m*M* glycylglycine, *p*H 8.5, as the eluting buffer. The fractions containing antibody as determined by double immunodiffusion and precipitation of transferase activity were pooled, lyophilized to dryness and redissolved in water at one-half their concentration in whole serum.

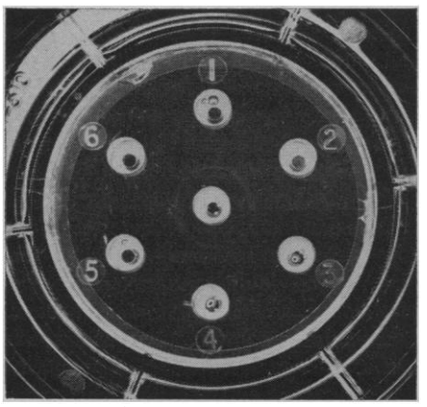

Fig. 1. Double-immunodiffusion cell photographed 24 hours after loading: 25 μl antitransferase after G-200 gel filtration was placed in the center well. Outer wells contained purified human liver transferase preparations (well 1), and the following erythrocyte preparations; 3-month-old male Caucasian galactosemic (well 2), normal (well 3), 3-year-old male Caucasian galactosemic (well 4), normal (well 5), and a 3-year-old female Negro galactosemic (well 6).

Hemoglobin-free red cell preparations were obtained as follows. Red cells from heparinized blood, washed twice with 0.85 percent sodium chloride, were lysed in an equal volume of water and then frozen and thawed. The lysate was diluted with an equal volume of 10 m*M* phosphate buffer, *p*H 7.0, which was then mixed with two volumes of a 50 percent suspension of diethylaminoethyl (DEAE)-cellulose (Whatman DE 52) equilibrated in the same phosphate buffer. The mixture was stirred for 1 hour and poured into a chromatographic column. The hemoglobin fraction was eluted with the same buffer until there was no detectable adsorbance at 280 nm. The fraction containing transferase activity was then eluted with phosphate buffer containing 50 m*M* ammonium sulfate. The first protein peak detected after the addition of buffered ammonium sulfate contains the transferase enzyme and consistently yields 70 to 100 percent recovery of enzyme activity. This fraction was lyophilized to dryness and redissolved in water just before use.

Double immunodiffusion was performed in high-resolution agarose cells (Cordis Laboratories). The wells with the red cell preparations contained 150 μg of protein and the well with the purified transferase enzyme contained 20 μg of protein (Fig. 1). All red cell preparations were assayed for transferase activity by the uridine diphosphoglucose–consumption assay (*4*) before and after fractionation on DEAE-cellulose. The preparations from galactosemic individuals had no detectable transferase activity, and galactokinase activity before fractionation was normal in all preparations regardless of transferase genotype. A single band of identity is present between all red cell preparations which is continuous with that of the purified liver transferase. After several days a second precipitin band appears between the purified liver preparation and the antibody. No additional precipitins appear between red cell preparations and the antibody.

To demonstrate specificity of the antibody for transferase protein, a preparation from a normal individual with a constant amount of normal (Gt^+/Gt^+) transferase activity was mixed with increasing amounts of antibody, incubated at 37°C for 1 hour and then at 4°C for 2 days. After centrifugation, the supernatants were assayed for

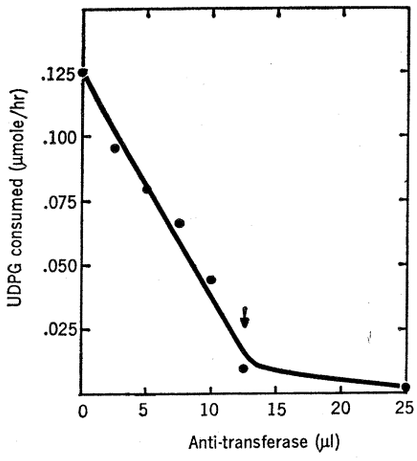

Fig. 2. Normal transferase activity remaining in the supernatant after precipitation of the enzyme with increasing amounts of antitransferase. Replicate portions of 300 μg of normal red cell protein were mixed with increasing amounts of antitransferase in a final volume of 150 μl. The tubes were incubated for 1 hour at 37°C and 2 days at 4°C, centrifuged, and 50 μl of supernatant assayed for transferase activity by uridine diphosphoglucose (*UDPG*)–consumption assay. The arrow indicates the amount of antitransferase used for the data in Fig. 3.

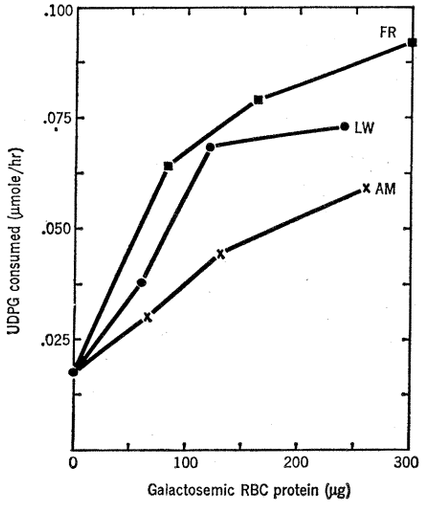

Fig. 3. Normal transferase activity remaining in the supernatant after precipitation with a constant amount of antitransferase that was first treated with increasing amounts of galactosemic red cell protein from three different galactosemics. Portions of galactosemic red cell protein as indicated were mixed with a constant amount (12.5 μl) of antitransferase in a volume of 100 μl. The tubes were incubated at 37°C for 1 hour and 4°C for 3 days, centrifuged, and 300 μg of normal red cell protein in 50 μl was added to each tube. After incubation as before, the tubes were centrifuged and 50 μl of supernatant was assayed for transferase activity by uridine diphosphoglucose (*UDPG*)–consumption assay. *AM*, 3-month-old male Caucasian galactosemic; *FR*, 3-year-old male Caucasian galactosemic; *LW*, 3-year-old female Negro galactosemic; *RBC*, red blood cell.

transferase activity. The removal of transferase activity from the supernatants is proportional to antibody concentration (Fig. 2).

To confirm the presence of a cross-reacting material (CRM) to transferase enzyme protein in the galactosemic preparations, increasing amounts of galactosemic red cell protein were mixed with preparations having a constant amount of the antibody (12.5 μl) determined by the precipitation data in Fig. 2. After 1 hour at 37°C and 3 days at 4°C the tubes were centrifuged and a constant amount of normal (Gt^+/Gt^+) transferase activity identical with that used for the data of Fig. 2 was added to each tube. The tubes were incubated as before and the transferase activity remaining in the supernatant was then determined. The CRM transferase protein in the galactosemic erythrocyte preparations formed a precipitin with the same antibody that complexes with the normal, active enzyme (Fig. 3).

Whole immune serum before fractionation on Sephadex G-200 as well as serum obtained from the same rabbit before immunization did not precipitate transferase activity. However, the whole immune serum, before fractionation, does form precipitin bands in double immunodiffusion gels against transferase protein whereas the control serum does not.

The identification of a CRM transferase protein in galactosemic cells indicates that the nature of the defect in this enzyme-deficiency disease is a "point" mutation which renders the enzyme protein catalytically inactive but has little or no effect on the antigenic properties of the molecule. The possibility was previously suggested by studies with antitransferase prepared with calf liver enzyme (*5*), and is further supported by the report on interallelic complementation at the transferase locus in which cultured fibroblasts from certain patients with galactosemia produced active transferase after hybridization (*6*).

It is reasonable to approach the therapy of inborn errors of metabolism in which the involved enzyme protein is present, albeit malfunctioning, by attempts at activation or stabilization of the affected molecule. It is therefore important to identify such diseases where possible; such an identification has now been made of galactosemia.

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References and Notes

1. H. M. Kalckar, E. P. Anderson, K. J. Isselbacher, *Biochim. Biophys. Acta* **20**, 262 (1956).
2. D. Y. Y. Hsia, Ed., *Galactosemia* (Thomas, Springfield, Ill., 1969); T. A. Tedesco and W. J. Mellman, *J. Clin. Invest.* **48**, 2390 (1969).
3. T. A. Tedesco, in preparation.
4. ——— and W. J. Mellman, in *Galactosemia*, D. Y. Y. Hsia, Ed. (Thomas, Springfield, Ill., 1969), p. 66.
5. R. G. Hansen, *ibid.*, p. 55.
6. H. L. Nadler, C. M. Chacko, M. Rachmeler, *Proc. Nat. Acad. Sci. U.S.* **67**, 976 (1970).
7. We thank K. Miller for technical assistance and Drs. G. Morrow III and L. Baker for providing galactosemic specimens. Supported by NIH grants HD 00588 and RR 240.

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Does DDT Inhibit Carbonic Anhydrase?

Abstract. *At a concentration of 50 to 100 micrograms per milliliter,* p,p'-*DDT (and* p,p'-*DDE) did not inhibit the rate of hydration or dehydration of carbon dioxide by carbonic anhydrase. At concentrations greater than 500 micrograms per milliliter, partial inhibition of the rate of dehydration of carbonic acid was observed, but this involved precipitation of drug in the reaction vessel. This degree of inhibition suggests that DDT may not inhibit carbonic anhydrase effectively at the usual concentrations found in tissue after exposure of organisms to DDT in the environment.*

Birds of prey have been found to lay thin-shelled eggs, a phenomenon which has been ascribed by Peakall (*1*) and by Bitman *et al.* (*2*) to inhibition of carbonic anhydrase in the shell gland by DDT (*3*). No direct studies of inhibition were done; the shell gland or oviduct of treated birds had about 60 percent (*1*) or 18 percent (*2*) reduction in enzyme activity when taken for in vitro analysis. This is not usually enough reduction for physiological in-