GALACTOSEMIA, A CONGENITAL DEFECT IN A NUCLEOTIDE TRANSFERASE

by

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Galactosemia is a hereditary disease of childhood which manifests itself biochemically as a disorder in the metabolism of galactose[1,2,3,4]. It was recently found[5] that α-galactose-1-phosphate (Gal-1-P)[6,7] accumulates in the erythrocytes of galactosemic children if they receive galactose or milk. Galactokinase must therefore be present in the galactosemic organism.

According to LELOIR and his group[8] the conversion of Gal-1-P to Cori ester, α-glucose-1-phosphate (G-1-P), involves the following steps in which a specific nucleotide, uridinediphospho-glucose (UDPG or in equations abbreviated URPPG) is required:

$$\text{Gal-1-P} + \text{URPPG} \rightleftharpoons \text{G-1-P} + \text{URPPGal} \quad (1)$$

$$\text{URPPGal} \rightleftharpoons \text{URPPG} \quad (2)$$

Step No. 2 is catalyzed by an enzyme described by LELOIR in 1951[9], called galacto-waldenase. Step No. 1 was found to be catalyzed by a highly specific transferase. It is not identical with the transferase which brings about cellular synthesis of UDPG. The latter catalyzes the following reaction in which UTP (URPPP) and inorganic pyrophosphate (PP) are involved[10,11]:

$$\text{G-1-P} + \text{URPPP} \rightleftharpoons \text{PP} + \text{URPPG} \quad (3)$$

This transferase has been named by us PP-uridyl transferase or simply PP transferase. The enzyme catalyzing step No. 1 we term PGal-uridyl transferase or simply PGal transferase. It has been found in galactose-adapted yeast[11,12] and in the mammalian liver[13]. Assays have recently been developed for a study of galactose metabolism in cell lysates or tissue suspensions[14]. By means of these assays the activity of the enzymes catalyzing reactions No. 1 and 2 has been determined in human hemolysates. Since galacto-waldenase has been found to be absent, or at least not manifest within the conditions under which the assay is performed[14], we are not able to make any statements concerning the role of this enzyme in galactosemia. The lack of galacto-waldenase has on the other hand, greatly facilitated measurements of the two uridyl transferases, especially those of the PGal transferase, and added to the accuracy of the assay especially with respect to specificity and reproducibility**.

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** See Addendum, p. 268.

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METHODS

Intact red blood cells were incubated with free galactose for 3 hours at 37° C to get a measure of Gal-1-P accumulation. For the estimation of the activity of the PGal transferase, hemolysates of red blood cells or frozen and thawed whole blood were incubated for 30 minutes at 37° C with the various substrates. One sample was incubated with Gal-1-P and the second with UDPG alone. The missing component was added after deproteinization. The third sample was incubated with both substrates. When PP transferase was measured, the procedure was the same but PP was substituted for Gal-1-P. After denaturing and precipitating the hemoglobin and other proteins by addition of an equal volume of 90% ethanol containing 10% chloroform[15] the respective substrates omitted during the incubation were added to the various controls. The coagulum was reextracted with 30% ethanol (0.5 volume) and the combined filtrates spun clear and extracted with peroxide-free ether, which removes ethanol and chloroform; the residue was then evaporated to a proper volume.

The enzymic determination of Gal-1-P in the filtrates after incubation of red blood cells with free galactose was performed according to the following principle. When Gal-1-P in the presence of fractionated liver PGal transferase is incubated with UDPG, and phosphoglucomutase, 6-phosphoglucose dehydrogenase, and triphosphopyridine nucleotide (TPN) are added as an indicator system for G-1-P (or G-6-P), each mole of Gal-1-P reacting with UDPG brings about a liberation of one mole G-1-P from the latter[11]. The determination is somewhat complicated by a high blank of G-6-P which is usually present in blood filtrates. For the enzymic spectrophotometric determination of the UDPG formed or utilized during the incubations, aliquots of the concentrated filtrates were added to buffer pH 8.5, containing cysteine and an indicator system consisting of diphosphopyridine nucleotide (DPN) and UDPG dehydrogenase[16]; for the additional determination of UDPGal in most of the assays, purified galacto-waldenase[13,17] was subsequently added as the final component. By this method the two transferases were each assayed in both directions. Although the estimation of G-1-P could also have been used as an assay method, too low values are often encountered by this method since G-1-P is rapidly metabolized in hemolysates, regardless of the presence of uridine nucleotides. For the same reason both transferases are preferably assayed in the direction of UDPG disappearance having G-1-P as a reaction product rather than as a reactant. Even the estimation of UDPG disappearance may tend to underestimate the rate of activity of the transferases because often more than 80% of the UDPG is utilized. In order to insure that UDPG formation did not originate through galacto-waldenase, which could not be detected but might be activated in the complete incubates, UDPG labeled with ^{14}C in the glucose[18] was used and the distribution of radioactivity in the nucleotide and non-nucleotide fraction was determined. The separation between the nucleotide and the non-nucleotide fraction was obtained by means of norite as described previously[11,19,20]. The specific radioactivity of the nucleotide fraction was obtained by determining UDPG in this fraction by enzymic spectrophotometry as described. In some cases ^{32}P was introduced into the G-1-P and its uptake into the nucleotide fraction as a result of the action of PGal transferase was measured.

In the great majority of the cases the first and the second sample contained, within the limits of error, the same amount of UDPG in the filtrates. The same applies to the distribution of counts. In a few cases there was a 20% disappearance of UDPG in the second sample. However, no UDPGal formation could be detected. The second sample was usually taken as the reference and the difference values ($\pm \Delta$) were based on comparison between the second and the third sample.

MATERIALS

α-Galactose-1-phosphate was prepared according to COLOWICK's method[7]. The preparation used in the present study was synthesized by Dr. E. MAXWELL from crystalline 1-bromotetraacetyl galactose kindly provided by Dr. HEWITT G. FLETCHER, Jr. α-Glucose-1-phosphate and uridine diphosphoglucose (UDPG) were commercial preparations from Sigma Chemical Company. UDPGalactose was made according to MAXWELL, KALCKAR AND BURTON[13]. UDPG with ^{14}C in the glucose was prepared enzymically[18]. G-1-^{32}P was prepared from glycogen and ^{32}P phosphate with muscle phosphorylase by Dr. R. W. WHEAT. UDPG dehydrogenase was purified from liver[21,22]. Galacto-waldenase was also prepared from calf's liver by Dr. E. MAXWELL by a method of purification to be published shortly[17]. Glucose-6-phosphate dehydrogenase was prepared by Dr. E. MAXWELL and crystalline phosphoglucomutase was generously supplied by Dr. V. A. NAJJAR.

RESULTS

Table I confirms and extends the observations by SCHWARTZ *et al.* It can be seen that blood cells from galactosemic children accumulate Gal-1-P when incubated with

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galactose *in vitro*. The same experiment performed with normal blood showed no such accumulation.

TABLE I

Micromoles Gal-1-P accumulation per ml red blood cells per h, 37°

T. R., 58 yrs., male, normal	< 0.005
V. McC., 5 months, female, milk allergy	< 0.005
Family T.:	
Father, 35 yrs., normal	< 0.005
Mother, 30 yrs., normal	< 0.005
Son, 6 yrs., galactosemic	0.108
Family O'D.:	
Father, 33 yrs., normal	< 0.005
Mother, 28 yrs., normal	< 0.005
Daughter, 6 yrs., normal	< 0.005
Son, 4 yrs., galactosemic	0.091
Daughther, 2 yrs., 6 months, galactosemic	0.119
Son, 3 months, galactosemic	0.116

The accumulation could be due to: (1) an increase in galactokinase activity as compared with that of the enzymes catalyzing the subsequent steps; (2) a block in the PGal uridyl transferase; (3) a block in the galacto-waldenase. The latter would also give an accumulation of UDPGal. However, since the content of UDPG in blood cells is very small[14], the main accumulation would in this case also be found in the Gal-1-P fraction. Since we have not found galacto-waldenase even in hemolysates of normal erythrocytes, we are not able in the present article to elaborate on the question of what role this enzyme plays in the disease (see, however, Addendum).

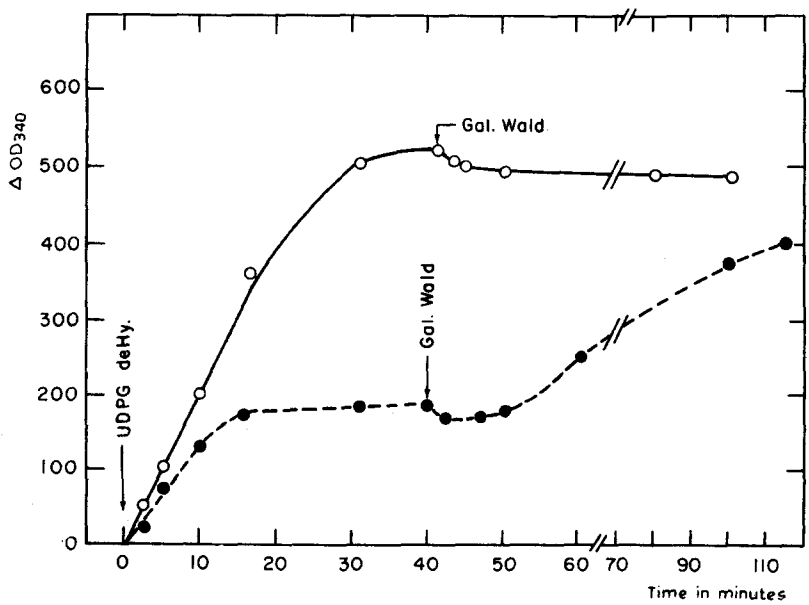

Fig. 1. Normal mother, 30 yrs., of galactosemic boy (family T.), enzyme analyses of filtrates. O—— O Hemolysate incubated with UDPG, ●——● Hemolysate incubated with UDPG + Gal-1-P.

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We have focused our main attention on the occurrence in hemolysates of the two uridyl transferases, PP transferase and PGal transferase. An investigation of the activity of the two enzymes in erythrocytes with special emphasis on PGal transferase was undertaken. Fig. 1 gives an illustration of the procedure involved in analyzing a hemolysate for PGal transferase. The graph of one of the controls is given; the other control incubated with Gal-1-P alone and having UDPG added after deproteinization is not illustrated since it was identical within 10% to that represented on the graph. The complete sample showed a utilization of UDPG of 60 to 65%.

Table II summarizes the balances of determinations of PGal transferase. It can be seen that the balance based on radioactivity in the nucleotide fraction agrees well with that obtained from enzymic spectrophotometry. This excludes any UDPG formation brought about by galacto-waldenase.

In Table III the formation of ^{32}P-labelled nucleotide from G-1-$\overset{*}{P}$ and UDPGal also points to a UDPG formation on the basis of PGal transferase. The dilution of radioactivity here stems presumably from G-1-P formation from blood glucose.

TABLE II

BALANCE OF INCUBATION CORRESPONDING TO EQUATION:

$$\text{Gal-1-P} + \text{URP}\overset{*}{\text{P}}\text{G} \rightleftharpoons \overset{*}{\text{G}}\text{-1-P} + \text{URPPGal}$$

URP$\overset{*}{\text{P}}$G contained $122 \cdot 10^3$ counts per μM. Rates expressed in μM per h (37°) and ml red blood cells

	Normal (Family T., Mother)	*Galactosemic (Family T., Son)*
Δ URPPG consumed (μM)	0.57	$<$ 0.02
Δ URPPGal formed (μM)	0.50	— 0.02
Δ ^{14}C-URP$\overset{*}{\text{P}}$G disappearance (μM)	0.50	$<$ 0.005

TABLE III

BALANCE OF INCUBATION CORRESPONDING TO EQUATION:

$$\overset{*}{\text{G}}\text{-1-P} + \text{URPPGal} \rightleftharpoons \text{Gal-1-P} + \text{URP}\overset{*}{\text{P}}\text{G}$$

Balance is made with reference to sample which was incubated with G-1-$\overset{*}{P}$ alone and URPPGal added afterwards. Control had URPPGal incubating with hemolysate and G-1-$\overset{*}{P}$ added afterwards. Complete sample was incubated with both components. Rates expressed in μM per h (37°) and ml red blood cells.

		Normal (H.M.K.)	*Galactosemic (Family O'D., Son)*
URPPG formed (in μM)	Control	+ 0.04	0.02
	Complete	+ 1.30	0.02
^{32}P-nucleotide formed (in μM UDPG)	Control	— 0.08	0.005
	Complete	+ 0.40	0.005

The activities in hemolysates from persons of different age, sex and on different diets (special reference to galactose-free diets) and afflicted with diseases different from galactosemia are given in Table IV. It can be seen that the PGal transferase is invariably present in blood cells, even in cases in which children have been on a galactose-free diet on account of disturbances different from galactosemia such as

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milk allergy. The occurrence of PGal transferase in blood is therefore not dependent on the administration of galactose. Neither does its presence seem to vary with age or sex.

The two uridyl transferases were also determined in blood from galactosemic patients and from their healthy relatives. As Table V shows, PGal transferase is absent from the blood of galactosemic persons, whereas the PP transferase is present in abundant amounts. In one case of galactosemia (Family T., son, 6 yr.) the test was repeated two months later and it was again found that PGal transferase was absent. In two cases of galactosemia the incubation time was extended from 30 to 60 minutes. Yet, even in these incubates, no PGal transferase could be detected.

TABLE IV

ACTIVITY OF PGAL AND PP TRANSFERASES IN RED BLOOD CELL HEMOLYSATES

Rates expressed as uridyl transfer, micromoles per ml red blood cells per h, 37°.

		PGal transferase	*PP transferase*
J. W.,	6 yrs., male, normal	0.60	1.25
J. R.,	7 months, male, normal	0.45*	1.20
A. F.,	6 yrs., male, normal	0.87	0.82
T. F.,	8 yrs., male, normal	0.87	
K. F.,	7 months, female, normal	1.00	
B. M.,	4 yrs., female, normal	> 0.75	
B. M.,	3 yrs., male, normal	0.87	
H. K.,	47 yrs., male, normal	1.30	
V. McC.,	5 months, female, milk allergy	0.60*	
M. B.,	7 months, male, milk allergy	0.75*	
C. M.,	13 months, male, milk allergy	1.00	1.48
N. J.,	6 yrs., male, nephritis	0.90	
M. L.,	24 yrs., female, chorion epithelioma metastatic (+ lactation)	0.92	

* Reaction run in the reverse direction, *i.e.*, G-1-P + URPPGal ⇌ Gal-1-P + URPPG.

TABLE V

ACTIVITY OF PGAL AND PP TRANSFERASES IN HEMOLYSATES OF RED BLOOD CELLS FROM NORMAL AND GALACTOSEMIC SUBJECTS

Rates expressed as uridyl transfer, micromoles per ml red blood cells per h, 37°.

	PGal transferase	*PP transferase*
Family O'D.:		
Father, 33 yrs., normal	1.12	1.25
Mother, 28 yrs., normal	0.73	
Daughter, 6 yrs., normal	0.67	1.25
Son, 4 yrs., galactosemic	< 0.02	2.50
Daughter, 2 yrs., 6 months, galactosemic	< 0.02	2.40
Son, 3 months, galactosemic	< 0.02	1.75
Grandfather, 66 yrs., milk "intolerance"	0.75	1.50
Family T.:		
Father, 35 yrs., normal	0.63	
Mother, 30 yrs., normal	0.57	1.40
Son, 6 yrs., galactosemic	< 0.02	1.25
Family W.:		
Son, 5 yrs., normal	0.80	1.16
Daughter, 2 yrs., galactosemic	< 0.02	1.25
T. B., 24 yrs., male, galactosemic	< 0.02	1.75
R. N., 2 yrs., male, galactosemic	< 0.02	1.60
R. T., 3 yrs., male, galactosemic	< 0.02	2.20

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The healthy relatives of the patients show a normal picture, *i.e.* both transferases are present just as in the control group. With the assay described it has not been possible so far to detect any "trait" (partial block) for the disease. However, as discussed above, there is reason to believe that the values for the activites of the two transferases represent average rather than maximum rates. The initial rate was measured in 3 cases (2 normal and 1 milk allergy) and it was found that the rate is linear for 10 minutes, and usually 2 to 4 times higher than the average values based on 30 minute incubates. The kinetic aspect of this reaction is under further study. The data do not, therefore, exclude the possibility that lower values for the PGal transferase might be found in one of the parents of a child afflicted with galactosemia. This aspect of the problem is under investigation.

DISCUSSION

The following points seem particularly pertinent to the present problem. Is it certain that the lack of PGal transferase in the erythrocytes of the galactosemic subjects reflects the same condition in the liver? This is a question which cannot definitely be answered at present. However, the fact that in galactosemia the galactose tolerance is extremely low and that galactose-1-phosphate accumulates in the erythrocytes supports such a view. Nevertheless, it would be desirable to investigate biopsy specimens of livers from normal and galactosemic subjects in order to settle this question more definitely and also prove that the disease does not result from any other metabolic defects such as a block of galacto-waldenase. An interference with the latter would, however, make it difficult to understand why galactosemic subjects develop apparently normally on a galactose-free diet. (See Addendum.)

The genetics of the disease has been studied by KOMROWER and his co-workers[4]. It is interesting that in practically all the families with one or more galactosemic children, at least one of the parents showed a low galactose tolerance test. It would probably be most advisable to follow possible "traits" by means of this test, whereas the afflicted persons may rather be screened by the test described here since a loading of these subjects with galactose is in general not without hazard. Whether the method could embrace a study on "traits" as well is, as discussed above, a feature which deserves further investigation. We have, so far, not been able to find any indications that blood from galactosemic subjects contained inhibitors (such as anti-enzymes) against the PGal transferase of normal blood. Neither is there at the present time basis for the assumption that the disease should be a result of a block in the synthesis of a cofactor of the PGal transferase. Normal blood heated to 70° C for 1 to 2 minutes is unable to activate galactosemic blood with respect to PGal transferase. Most likely, therefore, the biosynthesis of a catalytically active PGal transferase protein is blocked in galactosemia.

ACKNOWLEDGEMENTS

Our thanks are due to Drs. C. F. CORI, G. T. CORI, V. O'DONNELL, J. KETY, H. H. MASON, R. HARRIS AND A. HARTMAN for their kind help in obtaining the galactosemic cases, and to Miss BODIL WAAGE-JENSEN who rendered valuable assistance as a trainee under the Scandinavian-American Foundation through a grant-in-aid generously made available to one of us (H.M.K.) by the Eli Lilly Laboratories.

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SUMMARY

Galactosemia seems to furnish an example of a congenital human metabolic disease in which a specific enzyme is missing. The enzyme which catalyzes the exchange of α-galactose-1-phosphate with uridinediphospho-glucose, forming α-glucose-1-phosphate and uridinediphospho-galactose is absent in blood from galactosemic subjects. It is known that this enzymic exchange is an important reaction by which administered galactose is used in general carbohydrate metabolism. Several of the metabolic manifestations of the disease might readily be explained on the basis of this enzymatic defect.

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Addendum (March 2, 1956):
Recently Dr. E. MAXWELL (*cf. J. Am. Chem. Soc.* (1956), in press) has found that liver galacto-waldenase requires catalytic amounts of DPN. We have subsequently found (KURT J. ISSELBACHER, ELIZABETH P. ANDERSON, HERMAN M. KALCKAR AND K. KURAHASHI: unpubl. work) that galacto-waldenase activity can be detected in hemolysates if 0.5–1 μM DPN is added to 5 ml hemolysate. With this technique it has been possible to demonstrate galacto-waldenase in hemolysates from normal and from galactosemic subjects.

Received December 23rd, 1955