GLUCURONYL TRANSFERASE AND GLYCOGEN DEFICIENCY IN LIVER OF GUNN RATS

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It has been demonstrated that the enzymatic defect in Gunn's strain of jaundiced rats is a deficiency of glucuronyl transferase (1). This enzyme transfers the glucuronide moiety from uridine diphosphate glucuronic acid (UDPGA) to bilirubin, and thus facilitates the excretion of the latter. Other workers (2, 3), have found that glucuronyl transferase activity is greatest in the microsomal fraction of the liver. Attempts to purify and characterize this enzyme, however, have been unsuccessful. We have found that glucuronyl transferase activity is extremely variable in microsomes obtained by differential centrifugation, but that a much more constant activity is obtained when microsomes are precipitated together with other proteins by ammonium sulfate fractionation.

In recent studies on uridine nucleotides, Leloir, (4) has demonstrated that the enzyme which utilizes uridine diphosphate glucose (UDPG) as the glucose donor for the synthesis of glycogen is itself attached to particulate glycogen. We have now found that glucuronyl transferase is similarly bound to glycogen. Since particulate glycogen is in part centrifuged down together with the microsomes, this results in an erroneous impression of a microsome localized enzyme.

In order to locate the enzyme more precisely, Leloir's fractionation technique was utilized. Rabbit liver homogenates were fractionated by centrifugation into particulate glycogen, microsomes, mitochondria, and three supernatant fractions. The particulate glycogen was obtained as a precipitate at 23,000 x g for 15 minutes. The transferase activity was measured using phenolphthalein as the glucuronic acid acceptor. The assay medium contained 0.20 μmoles of UDPGA, 0.15 μmoles of phenolphthalein, 50 μmoles of $MgCl_2$, and 50 μmoles of tris (hydroxymethyl aminomethane) buffer, pH 8.0, and 0.2 ml. of the liver fraction to be assayed. Protein determinations were done using the biuret reaction (5); glycogen was determined with van Wagtendonk's technique (6); and nucleic acids by the method of Littlefield (7). The results of the analysis are in Table 1.

TABLE 1

Localization of Transferase Activity

Fraction	Phenolphthalein Conjugated	Protein	Glycogen	Specific Activity	Activity Glycogen
	$\frac{\mu\text{moles}}{0.2\text{ ml}}$	$\frac{\text{mg}}{1.0\text{ ml}}$	$\frac{\text{mg}}{1.0\text{ ml}}$	$\frac{\mu\text{moles}}{\text{mg protein}}$	$\frac{\mu\text{moles}}{\text{mg glyco}}$
700 x g x 10min. supernatant solution	0.044	53.6	10.2	0.0041	0.0215
5000 x g x 10min. supernatant solution	0.059	46.5	10.5	0.0063	0.0281
Mitochondria	0.009	19.9	5.1	0.0022	0.0088
23000 x g x 15min. supernatant solution	0.032	46.0	5.6	0.0035	0.0286
Microsomes	0.085	54.1	15.0	0.0076	0.0283
Particulate Glycogen	0.027	3.5	8.7	0.0386	0.0155

The highest specific activity was found in the particulate glycogen fraction. Glucuronyl transferase activity was quite high in the microsomal fraction, a finding consistent with the high glycogen content here. The enzyme activity in the microsomes and in the three supernatant fractions appears roughly proportional to the concentration of glycogen in each of them.

TABLE 2

Glycogen Content and Transferase Activity

Specimen	Glycogen	Protein	Phenolphthalein Conjugated	Specific Activity
	mg / 100 mg liver	mg / 0.1 ml	µmoles / 0.1 ml	µmoles / mg protein
Control 1	2.190	6.7	0.048	0.0072
Control 2*	3.405	6.6	0.039	0.0059
Control 3	5.450	6.1	0.043	0.0072
Control 4	4.451	5.2	0.024	0.0046
Control 5	5.060		0.049	
Heterozygous Gunn rat 1	6.516	5.7	0.013	0.0023
Heterozygous Gunn rat 2	4.903	5.5	0.021	0.0039
Homozygous Gunn rat 1	0.280	5.5	0.000	0.0000
Homozygous Gunn rat 2	0.076	4.6	0.003	0.0006
Homozygous Gunn rat 3*	0.340	5.4	0.000	0.0000
Homozygous Gunn rat 4**	1.074	5.7	0.003	0.0006

*Specimen stored at -15° for 6 months.
**Although this rat was homozygous, it was an older animal, and at necropsy it did not show the jaundiced tissues typical of homozygous rats.

Since it was now evident that glucuronyl transferase activity was concentrated in the glycogen fraction, it was of interest to measure the glycogen content of Gunn rat livers. Liver glycogen in normal controls, heterozygous Gunn rats, and homozygous Gunn rats (all fed ad libitum on the same diet) was measured and found to greatly decreased in homozygous rats as compared to heterozygous rats and controls. (Table 2.)

Rather than assume that Gunn rats manifested two separate and distinct metabolic defects, i. e. a deficiency of glucuronyl transferase and an inability to manufacture liver glycogen, an attempt was made to relate these to a single enzymatic defect earlier in their common metabolic pathway.

Uridine diphosphate glucosepyrophosphorylase (UDPGpyrophosphorylase) was assayed according to standard techniques (8). Phosphoglucomutase (PGM) activity was determined by measuring the reduction of TPN^+ at 340 millimicrons in the presence of glucose 1-phosphate and glucose 6-phosphate dehydrogenase. UDPGpyrophosphorylase activity was moderately reduced in homozygous Gunn rats. The reduction however, does not seem to be sufficient to account for the quantitative decrease in liver glycogen. PGM activity was within normal limits.

Further steps in the metabolic pathways are being investigated.

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