of ghost plasma suspensions plus the inability of the latter to utilize glucose affords a convenient system in which to measure extra-glycolytic activities remaining in the post-hemolytic cell.

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Galacto-waldenase and the enzymic incorporation of galactose 1-phosphate in mammalian tissues

It has been found previously[1,2] that extracts of galactose-adapted *Saccharomyces fragilis* catalyze the incorporation of gal-1-P*, ** into uridylic-bound galactose according to the following equation:

$$\text{gal-1-P} + \text{UDPG} \rightleftharpoons \text{UDPgal} + \text{G-1-P} \qquad (1)$$

In the conversion of gal-1-P to G-1-P, UDPG acts catalytically since it can be regenerated by LELOIR's galacto-waldenase[4]:

$$\text{UDPgal} \rightleftharpoons \text{UDPG} \qquad (2)$$

Reaction (1) is a non-pyrophosphorolytic type of uridyl transfer catalyzed by an enzyme which we call GP uridyl transferase in contrast to PP uridyl transferase[3] which catalyzes the reversible pyrophosphorolysis of UDPG:

$$\text{UDPG} + \text{PP} \rightleftharpoons \text{UTP} + \text{G-1-P} \qquad (3)$$

We have now demonstrated the presence of large amounts of both GP uridyl transferase and galacto-waldenase in rat and calf liver. Moreover, since the transferase is able to resist heating to 50° centigrade at pH 5.9, it can be separated from the heat labile galacto-waldenase. The separation of the two enzymes makes it possible to prepare UDPgal of sufficient purity for use in a simple spectrophotometric assay for galacto-waldenase. UDPgal was formed from UDPG in the following system: liver GP uridyl transferase (10 mg protein), gal-1-P 28 μmoles, UDPG 10 μmoles, TPN 20 μmoles, glucose 1,6-diphosphate 0.01 μmoles, cysteine 300 μmoles, $MgCl_2$ 500 μmoles, phosphoglucomutase and glucose-6-phosphate dehydrogenase in 20 ml of 0.1 *M* tris buffer pH 8. G-1-P formed in this system (Equation 1) was removed by the successive action of phosphoglucomutase and glucose-6-phosphate dehydrogenase. The overall course of the reaction was followed by measuring TPN reduced. Four μmoles of UDPgal containing 8% impurity of UDPG was isolated from the reaction mixture by adsorption and elution from Darco[5] followed by paper chromatography in neutral ethanol ammonium acetate solvent[6]. UDPgal was assayed by conversion to UDPG which is able to reduce DPN in the presence of UDPG dehydrogenase[7] (see Fig. 1).

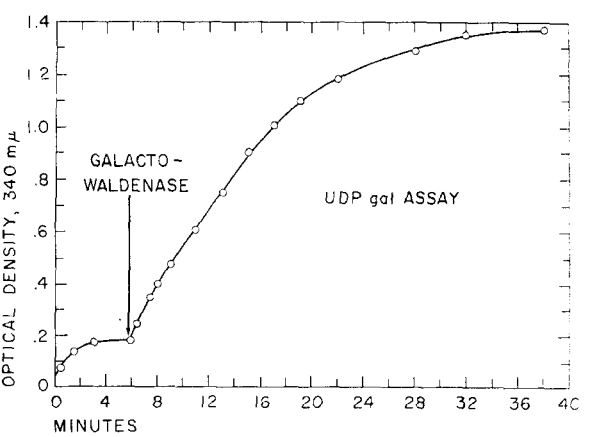

Fig. 1. Assay mixture contained: UDPgal, DPN 0.5 μmoles, and cysteine 5 μmoles in 0.5 ml 0.1 *M* glycine buffer at pH 8.6. UDPG dehydrogenase added at 0 time. Galacto-waldenase added at indicated time.

The rate of conversion of UDPgal to UDPG by galacto-waldenase can be measured by DPN reduction in the presence of an excess of UDPG dehydrogenase. Using this assay, galacto-waldenase has been partially purified from a water extract of calf liver acetone powder by repeated ammonium sulfate fractionation at different hydrogen ion concentrations.

Galacto-waldenase has been demonstrated also in other mammalian tissues. Mammary glands from lactating rats contained about twice as much enzyme as the corresponding amount of protein from mammary glands of non-lactating rats (see Table I). Interestingly enough the same preparations were unable to incorporate gal-1-P into uridyl nucleotide according to reaction (1), although they contained PP uridyl transferase and were able to form UDPG and UDPgal from G-1-P and UTP. It is known that G-1-P is used in lactose synthesis[8]. Similar results were encountered in experiments using brain extracts from two weeks old rats, *i.e.*, active galacto-waldenase and active PP uridyl transferase were present but no reaction occurred between galactose-1-phosphate and UDPG. The livers of these animals contained, in addition to galacto-waldenase and PP uridyl transferase, the enzyme GP uridyl transferase which catalyzes the reaction between gal-1-P and UDPG.

TABLE I

GALACTO-WALDENASE IN MAMMARY GLAND

Assay mixture contained: Crude protein fractions from mammary glands 0.4 mg protein, UDPgal 0.04 μmoles, DPN 0.5 μmoles, cysteine 5 μmoles, and UDPG dehydrogenase in 0.5 ml 0.1 *M* glycine buffer pH 8.6.

Time (minutes)	*UDPG formed (μmoles)*	
	*non-lactating**	*lactating***
10	0.006	0.013
20	0.012	0.023
30	0.016	0.030

* Values obtained with pooled mammary glands from 6 rats.

** Average of values obtained with mammary glands from 3 rats tested separately and agreeing within 10%.

Galacto-waldenase has also been found in *H. influenza* B and *E. coli*, when these organisms were grown on glycogen or glucose as the sole carbohydrate sources. It is known that *E. coli* contains galacto lipids[7].

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** The following abbreviations are used: gal-1-P = α-D-galactose-1-phosphate, G-1-P = α-D-glucose-1-phosphate, UDPgal = uridine diphospho galactose, UDPG = uridine diphospho glucose, TPN = triphosphopyridine nucleotide, DPN = diphosphopyridine nucleotide, UTP = uridine triphosphate, PP = inorganic pyrophosphate.