CHAPTER SIX

GENERAL DISCUSSION
GENERAL DISCUSSION:

The existence of the two forms of UDPglucose-4-epimerase in goat liver might be a general feature of mammalian livers since we have noted that beef liver epimerase can also be separated in two similar fractions both by treatment with calcium phosphate gel and by eluting on a DEAE-cellulose column. The relative proportions of the two forms in goat and in beef liver are also similar. There are however certain minor differences between the two sources. Thus, whereas the major form of the goat liver enzyme was inhibited by Gal-6-P, the major form of the beef liver remained unaffected under identical conditions. (Fig. 3.3). The physiological significance, if any, of these two forms of the enzyme is not clear at the moment. We attempted to carry out experiments with developing and experimental galactosemic rats to investigate into any possible effect of these conditions on the relative proportions of these two forms. However, our initial attempts to obtain these two forms in reasonably pure conditions and without any significant loss, has not been very successful. Cuatrecasas and Segal (94) had shown that the kinetic properties of galactokinase changed significantly in rats during the first 30 days after birth. From these data, these authors tentatively concluded that a new
form of the enzyme might be developing with age. They, however, failed to physically separate these forms.

Our work and work by other groups of workers (113, 124) have shown that the activity of epimerase from different sources is significantly modulated by a large number of closely related metabolites which include NADH, UMP, G-6-P, G-1-P, Gal-1-P and UDPGal itself. Inhibition by NADH is observed only when the required NAD is supplied from the medium and can not therefore be of metabolic significance in case of the yeast enzyme. Relative ratio of NAD to NADH may however be of crucial importance in mammalian cells (199). Strong and competitive inhibition by UMP is a common feature of all the epimerases including for both the forms from the liver (Table 3.2). Inhibition by substrate was previously reported for the yeast enzyme. Similar inhibition by UDPGal was observed only in case of the major form from the liver (Fig. 3.5).

Activation of epimerase from any source by sugar phosphates was not reported before. Metabolic significance of these interactions is not clear at the moment. But a closer study of these interactions has revealed the possible existence of an effector site for the yeast enzyme. Enzymes which catalyze freely reversible reactions are not generally known to have a separate effector site. This is consistent with the general experience that
regulatory enzymes usually catalyze practically irreversible reactions. Establishment of equilibrium in an *in vivo* situation will, however, depend on the relative concentrations of the substrate and the product. The relative concentrations of UDPG and UDPGal have not been determined for *S. fragilis*. Obviously, it is important that the relative concentrations of these nucleotide sugars and the relevent sugar phosphates be accurately determined under various physiological conditions of growth for this organism.

The reconstituted yeast enzyme shows some very interesting properties. Unlike in case of the *E. coli* enzyme (115), the reconstituted yeast enzyme failed to recover the full activity of the native enzyme and completely lost the native fluorescence (120). We have also shown that unlike the native enzyme it gives a sigmoidal saturation kinetics (Fig. 4.9) which changes over to normal Michaelis kinetics in presence of G-6-P. Obviously, the reconstituted enzyme assumes a conformation that is distinctly different from that of the native enzyme, even though the molecular weight of the two forms are the same. It has not yet been clearly established whether NAD is bound to the reconstituted enzyme or is supplied from the medium for the catalysis. Further work on the reactivation process and the reconstituted enzyme may considerably enhance our understanding of the epimerase as a protein.
The presence of the new enzyme Gal-6-phosphate dehydrogenase in relatively high amounts in mammalian liver opens up the possibility that this enzyme along with other undiscovered enzymes might be responsible for an alternate route of galactose catabolism. According to Beutler and his group (32), 6-phosphogalactonic acid, the oxidized product of Gal-6-P in presence of hexose-6-phosphate dehydrogenase, is a dead-end product of metabolism. Our preliminary characterization of the product has clearly eliminated 6-phosphogalactonic acid as the product of this reaction (Fig. 5.2). The product is most likely a keto-aldose, phosphorylated at the C6 position. Several transformations of such an intermediate leading to known intermediates of metabolism can be visualized but in absence of any experimental data they will be highly speculative in nature. Assuming that formation of Gal-6-P from Gal-1-P via phosphoglucomutase is metabolically an insignificant process (164), the possibility of the existence of new kinase or a mutase leading to the formation of Gal-6-P can not be ruled out. Using the highly purified galactose-6-phosphate dehydrogenase in a coupled assay system we are at present looking for the existence of such an enzyme.