SUMMARY AND CONCLUSION

The occurrence of fatty acid binding proteins, required for intracellular lipid transport and metabolism, is well established. These proteins have been shown to overcome the detrimental effects exerted by long chain fatty acids and their acyl-CoA derivatives on many enzymes. It is also well known that glucose-6-phosphate dehydrogenase, which is the key enzyme of HMP shunt pathway, furnishes NADPH for lipid biosynthesis and is inhibited by long chain fatty acids and their acyl-CoA esters. This enzyme plays an important role in developing tissues. However, studies on FABPs during human embryogenesis are rather limited, and their role in regulating G6PD activity in developing human placenta and fetal liver have not yet been fully understood.

The present investigation has been undertaken to isolate, purify and characterize FABP from developing human placenta and fetal liver and to investigate the role of FABP in regulating G6PD activity in these two tissues.

Findings of the study are summarized below :

1] FABP is present in human placenta and fetal liver cytosols. Three fractions of FABP, viz. DE-I, DE-II and DE-III, can be detected in each of these two tissues by DEAE-cellulose chromatography. DE-I is weakly adsorbed on the ion-exchanger and eluted with the eluting buffer, i.e. 0.01 M Tris-HCl, pH 8.5. DE-II and DE--III are eluted with a linear gradient of NaCl up to 0.3 M in 0.01 M Tris-HCl, pH 8.5.

2] FABPs are present in human placenta and fetal liver from the early periods of gestation, i.e. 5-10 weeks onwards. In placenta, concentrations of DE-I, DE-II and DE-III increase steadily up to 30th week. After that, the rate of increase in DE-III contents remains unaltered even at term but those of DE-I and DE-II decline. Concentrations of all the three fractions of FABP in liver increase gradually till term. Such an increase in FABP contents with gestation may be due to the increasing demand of fatty acid transport

and lipid synthesis during development. In placenta, the rate of increase in FABP contents declines near term, which probably reflects the ageing of the tissue.

3] Mobilities of DE-I, DE-II and DE-III are found to be different in PAGE. This observation suggests that there exist charge differences between these proteins.

4] It is found from the molecular weight determination of DE-I, DE-II and DE-III by gel filtration and SDS-PAGE that all these proteins are single chain polypeptides with a molecular weight 14,200 Dalton. Adult human liver FABP also has the same molecular weight.

5] TLC analysis of endogenous lipids reveals that fatty acids are the only detectable lipid classes in DE-II and DE-III. All bound fatty acids are extracted by organic solvents and can not be detected in the acid hydrolysate of the proteins suggesting non-covalent binding between fatty acids and FABPs. DE-I has been shown to bind almost no lipid.

6] GLC analysis of individual fatty acids indicates that binding characteristics of DE-I, DE-II and DE-III are totally different in both the tissues. DE-I contains no endogenous fatty acid, DE-II binds many long chain fatty acids, e.g. palmitic acid, stearic acid, oleic acid and linolenic acid, and DE-III is mainly an arachidonic acid carrier. Purification of DE-II and DE-III from human placenta and fetal liver is noteworthy because these two carry mainly long chain unsaturated fatty acids, which are the precursors of many biological modulators. Thus, it is essential for the fetus to import these fatty acids through the placenta. Though DE-I is almost lipid free, it has been grouped with DE-II and DE-III, since according to Takahashi *et al*. (270) these three forms are at least partially interconvertible.

7] Triton X-100 and ethylene glycol increase the binding of 12-(9-anthroyl)stearic acid by DE-II and DE-III in human placenta and fetal liver. EDTA does not have any effect. On the other hand, glycine decreases the binding ability of FABP.

8] FABP from human placenta and fetal liver have been found to be heat stable when heated at 80^0C for 10 minutes. Amount of bound 12-(9-anthroyl)stearic acid remains unaltered before and after the heat treatment.

9] Both in human placenta and fetal liver FABPs exist in three forms differing in isoelectric points and bound ligands. Isoelectric points of DE-I, DE-II and DE-III are 7.9, 6.8 and 5.4, respectively.

10] Ouchterlony double immunodiffusion studies reveal that three forms of FABPs are immunochemically identical and no change occurs in the antigenic site of DE-II during intrauterine development. Moreover, human placental and fetal liver FABPs are immunochemically identical. These findings are in agreement with the facts that placenta and fetal liver are complementary to each other and they together form the feto-placental unit.

11] G6PD has been found to be present in human placenta and fetal liver throughout the gestation. Its activity is high during early period of life, i.e. 5-10 weeks in both the tissues. These results are in conformity with the fact that in growing and developing tissues, activity of HMP shunt pathway is high to provide more ribose and erythrose for nucleic acid synthesis and more NADPH for reductive biosynthesis of lipids, amino acids, etc.

After 10th week, the activity of G6PD decreases in both the tissues, though another increase in G6PD activity is observed with a peak at 25-30 weeks of gestation. Activation of G6PD at the beginning of the third trimester may be another instance where reactivation of an enzyme occurs according to the tissue

requirement. During the later part of the third trimester, G6PD activity declines in both the tissues. These results are supported by the observations of others that around birth, cell differentiation slows down, and hence requirement of nucleic acid synthesis also decreases. On the other hand, more glucose is oxidized through the glycolytic pathway rather than through the HMP shunt pathway as gestation proceeds.

12] Concentration-dependent inhibition of G6PD by PAL-CoA, as well as by oleic acid, has been observed. PAL-CoA is more potent inhibitor of G6PD than oleic acid. Such inhibition of the enzyme is related to its subunit structure, which responds to these inhibitors by undergoing changes from tetrameric form to dimeric ones.

NADP, but not G6P, has been found to protect G6PD from PAL--CoA inhibition.

13] DE-II and DE-III fractions of FABP can reverse the inhibition by displacing PAL-CoA or oleic acid from the enzyme, thereby regenerating the dehydrogenase tetramer. Thus, inhibition by PAL-CoA or oleic acid is reversible in nature and FABP can reverse the inhibition by removing the inhibitor rather than affecting the enzyme directly.

14] DE-II and DE-III both have almost similar effects in reversing PAL-CoA inhibition of G6PD in human placenta and fetal liver. But in case of oleic acid inhibition, DE-II has been found to be more effective than DE-III in removing the inhibitor. These findings indicate that affinities of DE-II and DE-III for palmitic acid are almost similar, but in case of oleic acid the affinity of DE-II is higher than that of DE-III.

15] In absence of any exogenous PAL-CoA or oleic acid, G6PD is activated by DE-II and DE-III indicating the binding of endogenous inhibitors by these proteins. These results may explain why these inhibitory effects of long chain fatty acids and fatty acyl-CoA

thioesters are not seen in intact cells. These modulatory effects of FABPs on G6PD activity can be observed throughout the gestation. Such regulation of G6PD by FABP is important, because this enzyme is one of the main sources of NADPH for lipid biosynthesis during embryogenesis.

Hence, in the light of the experimental observations on FABP and its role in regulating G6PD in human placenta and fetal liver, the following conclusions may be made :

FABP exists in both fetal liver and placental tissues in three immunologically identical forms, which have the same molecular weight but differ in isoelectric points and binding affinities.

Since DE-II and DE-III bind mainly long chain unsaturated fatty acids, they may be indispensible in transporting these essential nutrients during human embryogenesis.

Immunological studies as well as other physicochemical properties indicate that human placental and fetal liver FABPs may be identical and they may have similar types of physiological functions.

By protecting G6PD from the inhibition by long chain fatty acids and fatty acyl-CoA thioesters, FABP not only regulates the lipid metabolism but also indirectly protects the unborn child because G6PD deficiency affects reproductive processes, causes favism, acute hemolytic anemia etc. in new-borns and increases prenatal and neonatal mortality.