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Developmental changes of the glycolytic enzymes in the human fetal heart

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Abstract. The ontogeny of hexokinase, phosphofructokinase, phosphoglucoisornerase, aldolase, pyruvate kinase and lactate dehydrogenase activities which are associated with glycolysis, an important energy yielding process, has been studied in human fetal heart for periods ranging from 13 weeks to above 33 weeks of gestation. Hexokinase, phosphoglucoisomerase and pyruvate kinase activities show similar developmental profiles exhibiting maximum activity at 25-28 weeks ofgestation. Phosphofructokinase activity, on the other hand, shows a minimum at this period and exhibits a peak value at early stages (13-16 weeks of gestation). Though considerable activity for aldolase is observed at an early period, it declines thereafter, but again increases in the later period. The probable role and correlations of these glycolytic enzymes with energy demand and general functional development in human fetal heart in ontogeny are evaluated.

Keywords. Glycolysis; hexokinase; phosphoglucoisomerase; phosphofructokinase; aldolase; pyruvate kinase; lactate dehydrogenase; human fetal heart; development.

Introduction

In the past few years, a great deal of interest has been centred upon carbohydrate metabolism, particularly on the glycolytic pathway during development in various species. Similar to other organs, the relative importance of the glycolytic pathway, in comparison with other pathways, has been demonstrated in normal heart slices (Charles and Sidney, 1956). Like adult brain, adult cardiac muscle also shows a great dependence on this pathway for deriving energy (Beatty *et al.,* 1972). Hahn and Skala (1971) and Cox and Gunberg (1972) have stressed the importance of this glycolytic pathway in early life of rat fetal heart also. Similarly, Wittels and Bressler (1965) have demonstrated that energy required for contraction of bovine fetal heart is mainly supplied by carbohydrate metabolism.

Despite all the impressive evidence regarding the existence and function of the glycolytic pathway in developing mammalian heart, little attention has been paid to a study on human fetal heart. It has been pointed out that in adult human heart, the glycolytic pathway is active (Jolley *et al.,* 1958). Therefore, the present study was

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Abbreviations used: NAD, Nicotinamide adenosine dinucleotide; ATP, adenosine triphosphate.

undertaken to assess the developmental pattern of some key enzymes involved in the glycolytic pathway in human fetal heart. Such a study may enable a correlation of biochemical parameters to the structural and functional ontogeny of human fetal heart and may also provide a gauge for the developmental process and the modifications in the enzymes of the glycolytic pathway. Six important glycolytic enzymes namely, hexokinase, phosphoglucoisomerase, phosphofructokinase, aldolase, pyruvate kinase and lactate dehydrogenase have been selected for this study and their activities have been followed in the isolated human fetal heart during different gestational period.

Materials and methods

The human fetuses of different gestational ages were obtained as therapeutic abortions from different MTP (Medical termination of pregnancy) clinics and hospitals in and around Calcutta. Fetuses above 20 weeks were collected as stillborn. Beginning from 13 weeks, the groups were divided at 3 week intervals up to 32 weeks and one group included a period of 33 weeks and above (also see Das *et al.,* 1979). Six samples were experimented in each group. Immediately after the heart tissue was removed it was left in the deep freeze (–20°C) for 7–8 h. The tissue was then homogenised with deionized water (10%, w/v) and after centrifugation at 1,000 *g* for 15 min at 0–5°C the supernatant fraction was used as the source of various enzyme activities studied.

Hexokinase was determined by the coupled enzyme assay method of Joshi and Jagannathan (1966) with glucose and adenosine triphosphate (ATP) as substrates and glucose-6-phosphate dehydrogenase as the second enzyme. Phosphoglucoisomerase was assayed by the method of Roe *et al.* (1949) and pyruvate kinase was assayed by the method of Weber *et al.* (1965). Phosphofructokinase was determined according to the coupled enzyme assay method of Ling *et al.* (1966) with fructose-o-phosphate and ATP as substrates and aldolase as the second enzyme.

Aldolase was determined by the colorimetric method of Beck (1955) and lactate dehydrogenase was assayed by the method of Neilands (1955) with lactate as substrate and nicotinamide adenosine (NAD) as the coenzyme. The data in the figure 1 have been expressed in international units (milliunits/mg protein). Protein content of the samples was determined by the method of Lowry *et al.* (1951).

Results

The developmental patterns of hexokinase, phosphoglucoisomerase and pyruvate kinase activities in human fetal heart are illustrated in figure 1A. It is apparent from this figure that both hexokinase and phosphoglucoisomerase exhibit a similar time sequence during development. Both the enzymes have a high level of activity at early stages of development (*i.e.* 13–16 weeks of gestation) and drop sharply during 17–24 weeks of gestation. The maximum activity for both the enzymes is observed at 25–28 weeks of gestation, after which the activities fall to lower levels and at 33 weeks and above it represents about 38 % of the maximum activity in both cases. The activity-pattern of pyruvate kinase is almost similar to that of hexokinase and phos-

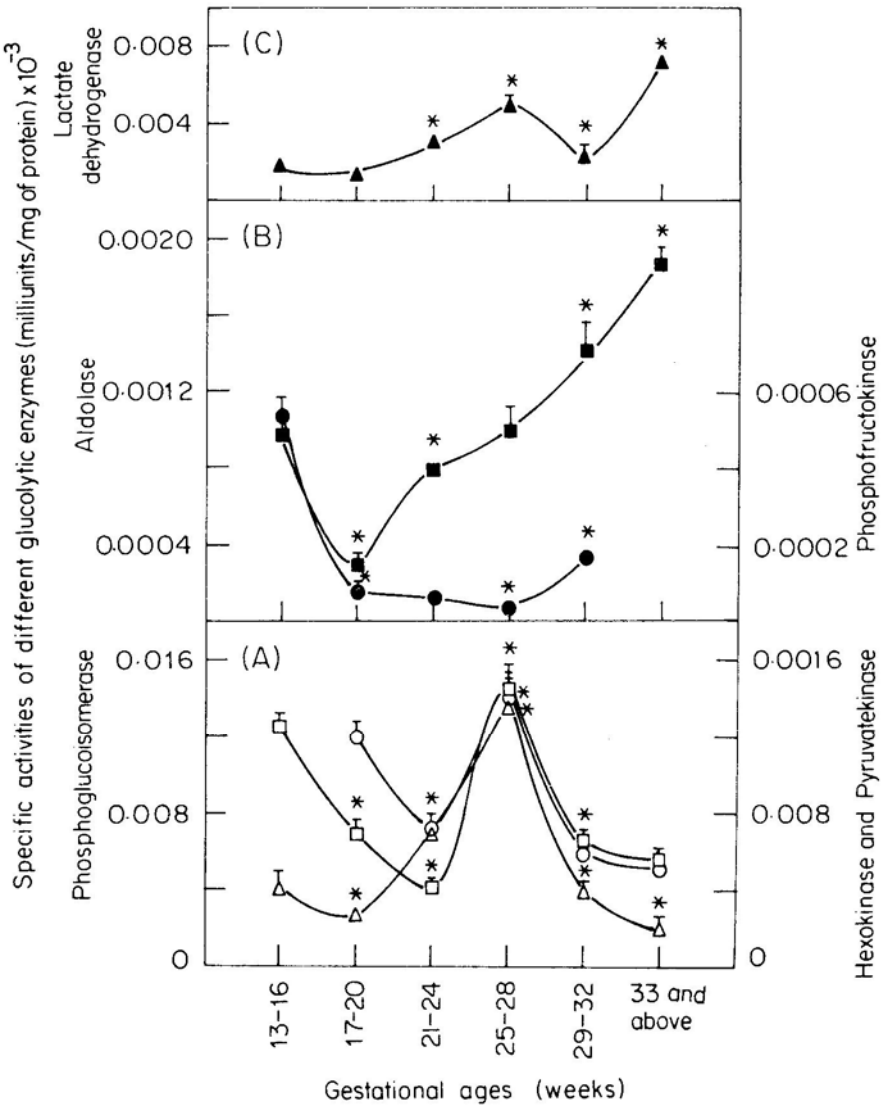

Figure 1. A. Developmental changes of hexokinase (O), pyruvate kinase (Δ) and phos-Phoglucoisomerasein (□) human fetal heart. **B.** Developmental changes of aldolase (■) and Phosphofructokinase (●) in human fetal heart. C. Developmental changes of lactate dehydrogenase (▲) in human fetal heart.

Values represent mean and vertical bars represent S.D. (n = 6 in each group). * P < 0·001 (highly significant).

phoglucoisomerase, but in contrast to these enzymes, the rise in activity of pyruvate kinase begins earlier (after 20 weeks of gestation).

Figure 1B reveals the developmental profile of phosphofructokinase and aldolase which are quite distinct from the pattern observed in figure 1A. The phos-

phofructokinase activity is found to be maximum even during 13–16 weeks of gestation and thereafter declines sharply at 17–20 weeks and remains at this low level till 25–28 weeks. After this stage, it shows a tendency to increase. The developmental changes in aldolase activity follow a pattern similar to phosphofructokinase up to 17–20 weeks showing a minimum at this stage, but its pattern abruptly changes thereafter showing a sharp increase even at gestation periods above 33 weeks.

Figure 1 C shows the developmental time course of lactate dehydrogenase activity in human fetal heart. Like pyruvate kinase, lactate dehydrogenase starts with a low activity and after 17–20 weeks of gestation rises markedly up to 25–28 weeks of gestation. It drops to a low level at 29–32 weeks after which it shows a second increase till 33 weeks and above.

Discussion

The results presented herein provide a clear picture regarding the developmental profile of enzymes, associated with glycolysis in human fetal heart. Except for aldolase and phosphofructokinase, the other enzymes studied show a peak at 25–28 weeks of gestation. It has been reported in pig heart that both the metabolites glucose-6-phosphate and fructose-6-phosphate inhibit phosphofructokinase activity (Werner *et al.,* 1983). The maximum activity of hexokinase and phosphoglucoisomerase at 25–28 weeks of gestation indicating a greater production of glucose-6-phosphate and fructose-6-phosphate at this stage and the minimum activity of phosphofructokinase at 25–28 weeks of gestation are in good agreement with the above evidence. Besides, it has been well established that the inhibition of phosphofructokinase leads to the accumulation of glucose-6-phosphate which in turn inhibits the activity of hexokinase (Weil-Malherbe and Bone, 1951). The inverse relationship between hexokinase activity and phosphofructokinase activity lends support to the findings cited above.

Neely and Morgan (1974) showed that a decrease in fructose-6-phosphate results in an increase in fructose- 1,6-diphosphate associated with faster glycolytic flow. The developmental behaviour of aldolase follows more or less a similar pattern with phosphofructokinase (figure 1B) except for a sudden increase after 17–20 weeks of gestation, the peak being observed at later stages of gestation in fetal heart.

Besides the divergent behavioural profile of lactate dehydrogenase at different stages of gestation may be explained from the turnover characteristics of this enzyme and its multiple form in fetal tissue.

In conclusion it can be said that all the enzymes studied here except phosphofructokinase show very high activities at 25–28 weeks of gestation which may correspond to the appearance of a rapid movement of human fetus and high fetal heart rate during this period indicating a rapid development of human fetal heart (Sorokin *et al.,* 1982).

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