STUDY OF ACID AND ALKALINE PHOSPHATASES IN HUMAN FOETAL ORGANS DURING DEVELOPMENT

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SUMMARY

In embryo sugar phosphates are metabolized by the enzyme phosphatase in variable pH from the very onset of gestation. In the present study this enzyme assay in different organs like brain, liver, heart etc. of the foetus of different period of gestation upto 24 weeks were studied. The specific activity of the enzyme in both acidic and alkaline media was separately noted and the increment of the activity was observed throughout the period of gestation with advancement of time.

Introduction

Break down of Phosphorylated metabolites like sugar, phosphates etc. is being catalyzed by the enzyme(s) phosphatase(s). These dephosphorylated products and inorganic phosphates are utilized for fulfilment of the requirement in several biochemical events. Since the enzyme(s) is operative under the influence of variable pH as the intracellular environment required for some specific reactions — an attempt has been made to look for the activity of the enzyme(s) both in acidic and alkaline pH from different human foetal organs during development — the prenatal stage which is characterized by its high demand for these products.

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Material and Methods

Human foetal samples of different gestation (determined from last menstrual period and physical examination) were obtained from mothers undergoing 'hysterotomy and ligation at Sadar Hospital, Suri and the samples above 20 weeks gestation were obtained from premature labour.

Foetal organs were dissected aseptically and stored at 0°C within one hour of operation and these were kept frozen until further investigation.

β — glycerophosphate (disodium salt), bovine serum albumin, Tris (hydroxymethyl) aminomethane (Tris), were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

2 — mercaptoethanol was purchased from BDH, England. Ammonium molybdate was purchased from J.T. Baker Chemical Company, Phillipsburg, N.J., U.S.A.

All other Chemicals used were the best analytical grade available in the market.

Preparation of Enzymes

All steps were carried out at 0°C to 4°C. Human foetal tissues were minced separately and suspended in 2 volume of chilled 0.154 M KCL having 0.2 mM mercaptothanol. Homogenization was carried out in a Remi Type M 153 homogenizer for 2 to 3 minutes.

The crude homogenate was centrifuged at 10,000 xg for 30 minutes in a Janetzki K24 refrigerated centrifuge. The pellet was discarded and the supernatant fraction filtered through a pack of glass-wool to remove lipid particles.

The filtrate was dialyzed for 3 to 4 hours against 500 volume of the same buffer used for homogenization.

After completion of dialysis, the fraction which was recovered from the dialysis bag was used as the enzyme source.

Enzyme assay

The colorimetric assay procedure was designed according to the following procedure. The quantity of inorganic phosphate released by action of acid/alkaline phosphatase from the phosphorylated substrate was measured.

The incubation mixture contained in a total volume of 1.0 ml with the following components. 50 mM Tris-acetate buffer, pH 8.0 (for alkaline phosphatase assay) or 50 mM Sodium — acetate buffer, pH 6.0 (for acid phosphatase assay), 2 mM β — glycer-

TABLE I

Pattern of Acid Phosphatase Activity in Different Organs During Development of Human Fetus

Gestation period (weeks)	Range of body weight (gms.)	No. of Samples Examined	Corrected specific activity for acid phosphatase in human fetal organs (micromole) Pi/h/mg protein		
			Brain	Liver	Heart
4- 8	1-10	4	0.0	0.18 ± 0.03	0.0
8-12	10-15	5	0.002 ± 0.001	0.24 ± 0.01	0.0
12-16	15-165	3	0.03 ± 0.014	0.47 ± 0.1	0.15 ± 0.01
16-20	165-400	2	0.08 ± 0.03	0.40 ± 0.18	0.04 ± 0.02
20-24	400-500	3	0.1 ± 0.05	0.45 ± 0.08	0.03 ± 0.01

TABLE I (*Contd.*)

Gestation period (weeks)	Corrected specific activity for acid phosphatase in human fetal organs (micromole) Pi/h/mg protein				
	Lungs	Kidney	Stomach	Spleen	Placenta
4-8	0.01 ± 0.005	0.07 ± 0.02	0.0	0.0	0.58 ± 0.3(?)
8-12	0.02 ± 0.01	0.16 ± 0.07	0.04 ± 0.001	0.12 ± 0.05	0.14 ± 0.06
12-16	0.04 ± 0.03	0.25 ± 0.08	0.08 ± 0.01	0.1 ± 0.04	0.36 ± 0.17
16-20	0.03 ± 0.02	0.28 ± 0.1	0.14 ± 0.018	0.08 ± 0.03	0.52 ± 0.12
20-24	0.03 ± 0.01	0.3 ± 0.2	0.19 ± 0.02	0.08 ± 0.02	0.55 ± 0.15

TABLE II

Pattern of Alkaline Phosphatase Activity in Different Organs During Development of Human Fetus

Gestation period (weeks)	Range of body weight (gms)	No. of samples examined	Corrected spcific activity of alkaline phosphatase in human fetal organs (micromole Pi/h/mg Protein)		
			Brain	Liver	Heart
4-8	1-10	5	0.0	0.16 ± 0.04	0.04 ± 0.01
8-12	10-15	5	0.005 ± 0.001	0.29 ± 0.05	0.08 ± 0.03
12-16	15-165	5	0.042 ± 0.015	0.58 ± 0.11	0.07 ± 0.02
16-20	165-400	5	0.062 ± 0.021	1.2 ± 0.23	0.08 ± 0.01
20-24	400-550	5	0.069 ± 0.019	1.15 ± 0.11	0.08 ± 0.02

TABLT II (*Contd.*)

Gestation period (weeks)	Corrected specific activity of alkaline phosphatase in human fetal organs (micromole Pi/h/mg Protein)				
	Lungs	Kidney	Stomach	Spleen	Placenta
4-8	0.0	0.09 ± 0.02	0.0	0.0	0.14 ± 0.02
8-12	0.03 ± 0.02	0.15 ± 0.06	0.0	0.0	0.11 ± 0.06
12-16	0.06 ± 0.019	0.20 ± 0.03	0.03 ± 0.01	0.26 ± 0.04	0.9 ± 0.03
16-20	0.07 ± 0.04	0.21 ± 0.02	0.08 ± 0.01	0.30 ± 0.05	1.1 ± 0.08
20-24	0.06 ± 0.03	0.24 ± 0.01	0.09 ± 0.02	0.26 ± 0.036	1.0 ± 0.2

osphosphate and an appropriate protein aliquot (100—200μg). The reaction was initiated by addition of substrate immediately after the enzyme with proper mixing. Duplicate tubes were run along with two appropriate blanks (without enzyme) and two zero minute controls in which 0.25 ml. of 20% Chilled TCA (Trichloroacetic acid) was added.

The enzyme incubation was carried out for one hour at 38°C.

After one hour, the reaction was terminated according to the procedure applied for zero-minute control. The denatured proteins were precipitated out by low speed centrifugation and the resultant supernatant was again incubated for one hour at 38°C in presence of a reagent (6N H_2SO_4 : 10% ascorbic acid : 2.5% ammonium molybdate : H_20 = 1 : 1 : 1 : 2) for estimation of inorganic phosphate according to the method of Chen *et. al.* Enzyme dependent release of inorganic phosphate was measured by substracting the zero minute value from the experimental set.

Protein was estimated by the method of Lowry *et. al.* using BSA as standard.

Specific activity of acid/alkaline phosphates is defined as μ mole inorganic phosphate released per hour per milligram protein.

Results and discussion

The activity of acid and alkaline phosphatases varies considerably in different human foetal organs during development with respect to gestation period. A comparative account of this change focussed an idea towards understanding the rate of synthesis and/or activation of these enzyme poteins related to phosphate metabolism in developing foetal organs. Barring few

exceptions, these enzymes were found to be operative in each and every organ throughout the intrauterine life and there was a tendency of increment of specific activity at the two pH values from the very begining of development onwards (Table 1 and Table 2).

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