LACTATE DEHYDROGENASE ISOENZYME PATTETNS IN HUMAN FOETAL TISSUES DURING DEVELOPMENT

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Summary : The changes in lactate dehydrogenase isoenzyme fractions in various tissues from human foetuses were studied at different stages of development. The isoenzyme pattern changes with the gestation period. The adult pattern is attained at birth in heart, liver. muscle and kidney. Brain shows predominance of cathodic fractions even at birth, but becomes aerobic after 6-12 months. LDH_1/LDH_5 ratio shows a similar trend. It appears that the adult pattern of LDH isoenzymes consists of predominantly either the faster (aerobic tissues) or slower (anaerobic tissue moving isoenzymes resulting from a gradual shift during foetal and neonatal life.

Key words : lactate dehydrogenase, isoenzymes, brain, muscle, kidney, human foetuses, heart, liver, ontogeny

INTRODUCTION

During the growth and development of an organism several alterations in metabolic pathways occur. These changes probably facilitate generation of energy stores for the development of the particular organ. Among these, changes in enzymes and isoenzymes are important which eventually determine the function of that particular organ in fully developed organism. These changes have been seen both in intrauterine and neonatal stages. Generally, the oxygen consumption in foetal or immature brain is considerably lesser than in adult brain tissue. Respiration is also lower in human foetal brain tissue than in the adult. A sharp increase in the rate of oxygen consumption occurs during development coincident with critical phase of differentiation. New born rats, cats, rabbits, dogs and guinea pigs have been shown to have greater tolerance to exposure to an atmosphere of nitrogen (1). The rate of glycolysis, however, decreases during the postnatal

period and is lower in adult than in young animals. These developmental changes in respiration and glycolysis are paralleled by changes in the oxidative enzymes in developing brain.

Interest in isoenzyme studies in tumor biochemistry increased when it was found that tumor pattern resembled that of foetal pattern. Since predominance of anaerobic glycolysis has been reported in tumors, the study of lactate dehydrogenase (LDH) isoenzymes (IE) in tumors has received attention of many workers. In our earlier studies we have noted increased anaerobic glycolysis in brain tumors similar to foetal tissues (2). Most of the metabolic studies so far conducted were on animal tissues and data on human material is meagre. For a meaningful extrapolation to human system and clinical practice, it is very essential to have similar data on human tissues. In order to study alterations of LDHIE in human tissues during ontogeny the LDHIE patterns in various organs of human foetuses were studied at different stages of gestation.

MATERIAL AND METHODS

The aborted human foetuses from 16 to 40 weeks of gestation were collected from Vanivilas Hospital, Bangalore within one hour of medical termination of pregnancy and kept in ice. The period of gestation was determined by (a) Period of amnenorrhoea for the mother (b) Clinical assessment of the period of gestation (c) The crown heel and crown rump length of the foetus (3) and (d) Gross developmental features of brain (4). Any specimen with gross features of birth anoxia or any other developmental anomalies were excluded from the study. The brain samples were collected from the parasagittal area of the frontal cortex and the skeletal muscle from the middle one third of the gestrocnemius muscles of the foetuses. Samples were also obtained from liver, kindney and heart from relatively constant area. Similarly, specimens were obtained, within 6-8 hrs postmortem from 5, 13, 30, 50 and 55 years old individuals of both sexes. The matured tissue of these specimens is denoted as adult tissue in this paper. They died of acute spinal cord injury or a sudden death due to nonneurological disorder

The tissues collected were immediately washed with cold buffered saline (pH 7.4) to remove the adhering blood and kept at $-$ 20°C till analysis. The total LDH was estimated immediately while the isoenzyme separation was done within a week.

A 5% homogenate of the tissue in water was made. The homogenate was centrifuged at low speed and the supernatant was used for the assay of both total LDH and isoenzyme separation. Total LDH was assayed by the method of Wroblowsky and La Due (5). The absorbance change at 340 *nm* was measured by using Stasar III System-4-

Spectrophotometer with programmable printer. With timed absorbance mode the change in absorbance was measured over a period of time and the △ absorbance per minute was used in the calculations. The values were calculated as units per litre of the 5% homogenate for all the tissues.

LDH iscenzymes were separated by Cellulose acetate electrophoresis in tris barbital buffer (pH 7.4) by using Beckman Microzone system. The isoenzymes were visualized by the TNBT and PMS reaction of Rcsalki (6). After develcpment, the percentage of each fraction was calculated from the densitometric recordings.

RESULTS

In our earlier study we found no significant change in isoenzyme fractions when tissues were stored for 1-2 weeks in saline at either 0°C or −20°C. The presence of saline did not contribute to the dissociation of the isoenzyme fractions (unpublished data). Only LDH_1 and LDH_5 levels are depicted in the tables. There is no appreciable change in the intermediate fractions.

The LDH isoenzyme pattern of foetal heart and brain tissues is given in Table I.

TABLE I: LDH isoenzyme distribution in foetal heart and brain median values.

LDH isoenzyme	*Heart*								*Brain*							
distribution	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Total LDH U/L	225	180	175	200	160	130	140	185	200	260	240	260	280	320	280	240
LDH_1 %	17	16	15	14	15	17	27	32	18	13	13	15	16	15	15	23
LDH_5 %	12	12	11	10	10	10	6	5	8	6	6	12	13	15	16	6
1/5	1.4	1.3	1.4	1.4	1.5	1.7	4.5	6.5	2.3	2.2	2.2	1.3	1.2	1.0	0.9	4.0

Nos. 1 to 8 under each tissue represent the stages of the foetal tissues in weeks and adult in years and the figures in parenthesis the number of samples as : 1.16 weeks (2), 2.20 weeks (2), 3.24 weeks (2), 4.28 weeks (2) 5.32 weeks (1), 6.36 weeks (1), 7.40 weeks (1) and 8. Adult 5 years to 55 years (5).

Heart : It is seen from the table that in the early stages, LDH_1 and LDH_5 fractions almost remain unchanged upto 30 weeks. Then at the age of 36 weeks to 40 weeks the LDH_1 fraction increases and LDH_5 decreases. The ratio of LDH_1/LDH_5 is found to be almost constant upto 32 weeks and then it is elevated nearer to as that of adult heart pattern.

Brain : It is observed from the table that the LDH pattern varies with the gestation period. LDH_1 fraction is found to decrease from 18% at 16th week to 15% at 40th week. Similarly LDH_5 fraction increases from 8% at 16th week to 16% at full term. The LDH_1/LDH_5 index decreases from 2.3 at 16th week to 0.9 at full term. This is in contrast to adult brain where the LDH_1/LDH_5 ratio is 4.0 and LDH_5 is 6%. The foetal brain does not attain the adult pattern at birth, as in other tissues but some time in postnatal life. Later it remains relatively constant, attaining a plateau (Fig. 1).

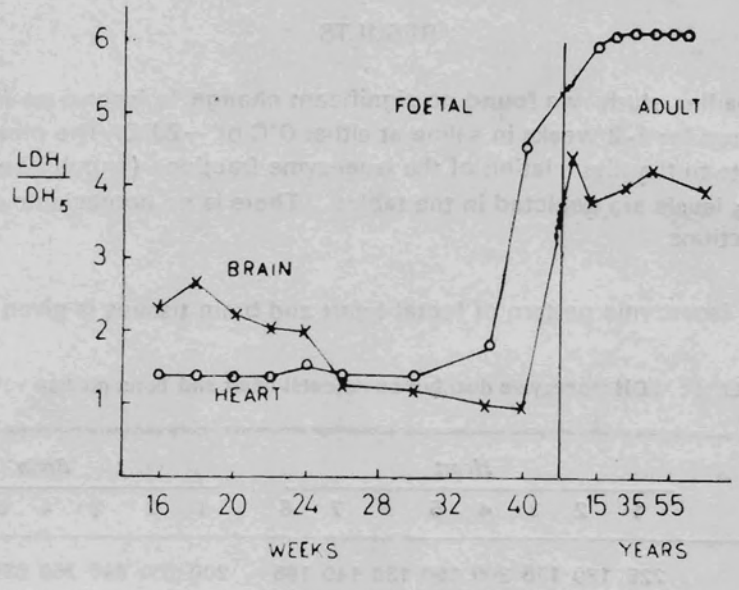

Fig. 1 : LDH_1/LDH_5 ratio in aerobic human foetal tissues at various stages of development.

The LDH isoenzyme pattern of foetal muscle, liver and kidney is given in Table II.

Muscle : It is seen from table II that the pattern changes with age and attains almost adult pattern at birth, that is, the muscle changes from aerobic to anaerobic as the age increases. The full term tissue shows predominant LDH_5 band and low LDH_1/LDH_5 index (0.4). LDH_1 decreases from 17% to 11% from 16 weeks to 40 weeks and LDH_5 increases from 5% to 24%. The ratio decreases from 3.4 to 0.4 (Fig. 2).

Liver : The adult liver pattern consists of predominantly LDH_5 and less LDH_1(Table II). LDH_5 fraction increases from 24% to 42% from 16 to 40 weeks. There is not much change in LDH_1 fraction. The LDH_1/LDH_5 index at 40th week reaches 0.3, almost

TABLE II : LDH isoenzyme distribution in foetal muscle, liver and kidney median values.

LDH isoenzyme	*Muscle*								*Liver*								*Kidney*							
distribution	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8
Total LDH U/L :	200	220	240	300	280	280	280	260	200	260	200	240	320	300	390	250	150	140	120	160	100	184	200	220
LDH_1 %	17	14	11	12	10	10	11	3	13	14	11	12	9	9	11	6	26	28	23	20	21	21	18	20
LDH_5 %	5	13	17	21	20	30	24	48	24	19	22	24	26	44	42	48	7	8	6	9	11	11	15	15
LDH_1/LDH_5	3.4	1.1	0.6	0.5	0.5	0.3	0.4	0.1	0.5	0.7	0.5	0 5	0.3	0.2	0.3	0.1	3.7	3.5	3 8	2.2	2.1	1.9	1.2	1.3

Nos. 1 to 8 under each tissue represent the stages of the foetal tissues in weeks and adult in years and the figures in parenthesis the number of samples as : 1.16 weeks (2). 2.20 weeks (2), 3.24 weeks (2), 4.28 weeks (2), 5.32 weeks (1), 6.36 weeks (1), 7.40 weeks (1) and 8. Adult 5 years to 55 years (5).

nearer to adult liver index. The full term pattern almost resembles the adult one which has 48% of LDH_5.

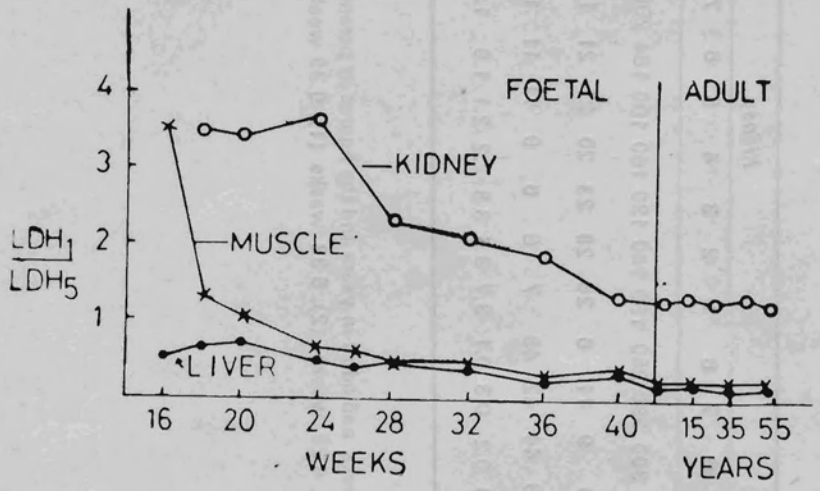

Fig. 2 : LDH_1/LDH_5 ratio in anaerobic human foetal tissues at various stages of development.

Kidney : In kidney, during development LDH_1 fractions decreases from 26% to 18% at full term and the cathodic fractions increases from 7% at 16th week to 15% at 40th week (Table II). The LDH_1/LDH_5 ratio also decreases from 3.7 to 1.2 at full term. At full term the LDH pattern almost resembles the adult pattern.

DISCUSSION

The observation that many enzymes exist in multiple molecular forms and many of the patterns are tissue specific has enhanced the possibilities of understanding the molecular pathology of many diseases.

To evaluate modulations during ontogeny many studies have been conducted on tissues during foetal and neonatal periods. Two enzymes, LDH and MDH, have received much attention in these studies. Many of the reported studies on isoenzyme patterns have been done on animal tissues and extrapolated to human system. Markert (7) showed that most of tissues in mice during development show predominance of cathodic fractions of LDH. Similar observations have been reported by Flexner *et al.* (8) and Bonovita *et al.* (9).

In the preesnt study human foetal tissues were studied from 16 weeks to full term and were compared with control tissues from 5 to 55 years of age.

It is seen that the foetal heart, in early stages of life, shows predominance of LDH_1 and less of LDH_5. As the age increases LDH_5 decreases and LDH_1 in turn increases and at birth atttains almost the adult pattern.

Around this time, the patent ductus and the interatrial foramina closes, and the foetal circulation changes to normal pattern. This change also parallels the higher oxygenated blood of prenatal circulation in contrast to the low oxygenated mixed arterial and venous blood in foetal circulation (10). The adult heart shows predominance of LDH_1 which is characteristic of aerobic nature of the tissue. At full term the cardiac muscle becomes aerobic though in the early stages it is anaerobic in nature. Foetal brain, unlike heart, behaves somewhat in a different way. In the early stages of life LDH_5 which is more when compared to adult brain, increases with age and LDH_1 in turn decreases and at full term both are almost equal. The adult brain shows less of LDH_5 and more of LDH_1. The ratio of LDH_1/LDH_5 is 0.9 at full term when compared to the adult ratio of 4.0. The predominance of LDH_1 in adult brain indicates the aerobic nature of brain. It seems from this study that at full term the foetal brain is still anaerobic in nature. Tissue culture studies on cells from brain of various sources have shown that there is a gradual decrease of the faster moving isoenzymes as the cells age (11). It has also been reported in animal experiments that the adult pattern of brains LDH isoenzyme is attained after 3-4 weeks after birth (12). It seems probable that these happen in human tissue as well. The predominance of LDH_5 in brain at full term suggests the prevalence of anaerobic glycolysis. The aneaerobic glycolysis leads to the production of coenzymes needed for many metabolic pathways which help in building up of tissues. As the development proceeds successive changes like new enzyme and protein formation occurs. Once this is over the glycolysis returns to oxidation system and at this stage brain becomes aerobic. The changes in the LDH isoenzyme profile during ontogeny appear to parallel the development of the ontogeny of muscarinic cholinergic receptors in human frontal cortex (13). The LDH isoenzyme pattern is probably linked with neuronal differentiation and maturation. This feature is best exemplified in the LDH isoenzyme profile and retinal maturation (14). In general, the embryo is resistant to anoxia or hypoglycemia and this persists even in late foetal life (15). It is also noted that the capacity to survive anaerobically is lost at about 3-4 weeks after birth. So it seems the brain LDH isoenzyme pattern in human returns from anaerobic pattern to aerobic pattern after 3-4 weeks after birth.

Since in early stages of life the cerebral blood flow is less with low oxygen con-

tent, the tissue will respire anaerobically and in turn shows a predominance of LDH_5 (16).

The ratio of LDH_1/LDH_5 in a tissue has been used to know the oxygen supply to that region. In this study it is seen that in brain, the index decreases from 2.3 to 0.9 at full term, whereas, heart shows high ratio at full term suggesting no oxygen debt. It could be safely concluded that there is a predominance of anaerobic glycolysis in early stages of foetal life. This study supports this fact in tissues like heart and brain.

The LDH isoenzyme pattern in human skeletal muscle has been shown to contain predominantly the intermediate fractions during early stages of iife which attains the adult pattern at full term (17). In this study we found that the LDH_5 fraction increases from 5% at 16 weeks to 24% at full term and LDH_1 in turn decreases and after birth the pattern almost resembles the adult pattern. In the foetal life, most of the muscles are rich in red fibres containing large amount of myoglobin. At birth they change to white fibres which receive their energy via the anaerobic glycolysis. This shift in the fibre type appears to be modulated by the LDH isoenzyme profile with physiological implications.

Liver, an anaerobic tissue in adult during development shows a similar change. At full term, the LDH_5 fraction attains almost the adult level. Unlike other tissues there is no change in LDH_1 fraction during development. Foetal kidney also shows similar changes during development. The kidney shows a change from anodic pattern at 16 weeks to cathodic pattern. These changes in foetal kidney are attributed to the morphological developments of renal tissue.

In our study on brain tumors (2), it has been observed that most of the malignant brain tumors shows predominance of LDH_5 fractions suggesting increased anaerobic glycolysis in malignancy. This pattern is also seen in foetal brain tissues during development. Hence it seems that in both cases of rapid cell turnover, i.e. in tumors and foetal tissues a premature pattern of enzyme structure reappears.

Considering the observations made during this study and several published reports it is evident that there is a possible developmental mechanism where the adult pattern originates from an initial foetal picture. In general, it appears that the adult pattern consists of predominantly either the faster or slower moving isoenzymes resulting from a gradual shift during foetal and neonatal life.

This data on ontogeny of LDH isoenzyme in human tissues is very important in understanding the disease process of that particular tissue. There is no such data available

in literature on human tissues. This report on human foetal tissues, especially on brain during development is helpful for further studies in understanding the mechanism of oncogenesis as well.

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