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Lactate Dehydrogenase

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ABSTRACT

Lactate dehydrogenise is an important enzyme which is involved in carbohydrate metabolism. At the end of glycol sis , a molecule of glucose is broken in to two molecules of private with two molecules of cofactors (NADH+H+).NAD+ (Nicotinamide Adenine Dinucleotide radical) are involved in different steps for the removal to NADH+H+. The two molecules of pyruvate enter into the mitochondrial aerobic breakdown via kerb's cycle .The reduced enzymes also enter the mitochondrial membrane to get oxidised back to NAD by atmospheric oxygen via electron transport system .if the supply of oxygen is interrupted , or some inhibition in the enzyme system occur..... or the rate of the aerobic breakdown of carbohydrates in response to grater metabolic demand exceeds the oxidative phase, NDA+ available in cytoplasm tends to get exhausted, in order to cope with this ,the NADH molecules formed as a result of glycol tic demand exceeds the oxidised from of NAD+ molecules ,the reactions takes place in place of the enzyme lactate dehydrogenise. Reaction is as follows. Which occurs in both direction (reversible reaction). $CH_3.CO.COO^- + NADH + H^+ \Leftrightarrow CH_3.CHOH.COO^- + NAD^+$ Pyruvate Lactate This reaction is catalysed by lactate dehydrogenises as such , in various sorts of stress conditions including the chemical stress .more lactate dehydrogenises level in the body is expected than in the normal condition. This enzyme is also interesting in showing iso enzyme patterns under different condition .As revealed by gel electrophoresis. it has been shown to exist in five molecular forms of iso enzymes' of locate dehydrogenises are cloud be indentified in human serum , fish and other animals (An as tasi and banister ,1980,Radhian 1989) Kaplan (1963) and market (1955) demonstrated that these forms of two different sub units. These sub units have been designated as A and B by marker (1955) and as M and H BY KAPLAN (1963) .The five verities would ,therefore , be jermed A_4Bo A_3B_1,A_2B_2,A_1B_3,A_0B_4 according to market and according to Kaplan as lactate dehydrogenise 1 to 5 as LDH-1, LDH-2 , LDH-3,LDH-4, and LDH-5,.The LDA-1, most cathodic where as LDH-5,is most anodic. In human serum the normal range of LDH is 70-240 I .U. per litter (king.1960). LDH is widely distributed, being found in all cells in man but it is especially plentiful in cardiac and skeletal musicales, liver, kidney, and red blood cells. An increasing serum lactate dehydrogenises is found in myocardial infarction. An increase activity has also been found in liver disease, particularly I infective hepatitis.

Keywords – Nicotinamide Adenine Dinucleotide Radical, Carbohydrate Metabolism, Infective Hepatitis

INTRODUCTION

Lactate dehydrogenates, like many other enzymes is present in the urine and in daily execration has been reported to be increased to be increased in renal disease (Rosalki and Wilkinson 1959) particularly increases of

carcinoma of the kindly and bladder (wocker and darfman 1962) it has been reported that 15% of a series of 58 patients with malignant neoplasm of urinary tracts had normal execration and conversely that approximately 70% of 63 patients with bening conditions had increased execration of LDH.

Khen et al (1994) , reported that in the water beetle, cybister confuses the sublethal concentration of DDT and EDTA caused the inhabitation the activity of LDH in the insect. However little information , level of glucoseand lactic acid was increased when compared to unexposed control.the LDH activitiy was decreared and the present changes in liver was also reduced and present changes in liver, gill and brain were 70.56,53,66 and 26.74 respectively . significant alteration in content of metablits and enzyme activity under malations texicity seemed to suggest trarsient shift from aerobic analrobic mode of living.

Sah (1990), reported the histochemical determination of LDH activity of muscles of catla catla (Ham).

Quantitative estimation of lactatedehydrogenae

Quantitative estimation of the serum lactatedehydrogenase was done by colorimetric method of king (1959-1965)

Reagents;

1. Glycine buffer:-

7.05gm of glycine and 5.85 g, of sodium chloride were dissolverd in distilled water and made up to a liter.

2. Buffered substrate solution:-

125 ml of glycine buffer and 75ml of 0.1 Nsodium lactate solution.

3. Nicotinamide adenine dinucleotide (NDA) solution:-

10mg of NAD was taken and mixed in 2 ml of distilled water and was kept at o to 4˚ c for longer stability. It was approximately 0.2 ml.

4. 2'4 Dinitrophenyl Hydrazine Reagent:-

200 mg of 2.4 Dinitrophenyl hydrazine was taken and mixed in hot normal Hcl and was made up to a liter with this acid.

5. (NaoH) Sodium hydroxide (o.4N) soloution :-

16mgs of NaoH was taken and dissolved in 100ml distilled water to bprepare 0.4 N sodium hydroxide solution.

6. Standerd sodium pyruvate solution:-

11gms of sodium pyruvate was taken and dissolved in 100ml of buffered substrate solution .it bcantained one micromole of phruvate/ml.

7. Reduced Nicotinamide Adenine Dinucletiod solution:-

1 micromole /ml of buffered substrate was prepared from disodium salt (Moleculer weight-716).

Techniques:

Serum from control and treated fishes was collected in a centrifuge tube as deseribed earlier.

Procedure

1. 1ml of buffered subatrate was pippeted and also µml serum (0.2ml serum was diluted to 0.5 ml of 7 distilled water) into each of two tubes. (First designed as 'blank) tube and were shaken to mixed.
2. and .02 ml of NAD solution was added in second test tube and .12 ml distilled water in 1st (blank) tube were shaken to mixed.
3. Exactly after 15 minute of adding NAD, 1 ml of dinitrophenyl hydrazine reagent was added in both 'blank' and 'test' tubes.
4. Tubes were shaken to mix and left in water boyh for further 15 minutes.
5. Then both tubes were removed from water both and 10ml of 0.4 NaoH was added.

6. Optical density was noted at 440 nm filter with five minutes of adding NaOH soln.							
I.U./liter	0	167	333	500	667	833	1000
ML NADH2 in Substarte	0	0.05	0.1	0.15	0.2	0.25	0.3
ML Pyruvate Solution	0	0.05	0.1	0.15	0.2	0.25	0.3
ML.Buffered Substrate	1.0	0.9	0.8	0.7	0.6	0.5	0.4
ML. NAD Solution	0	0.2	0.2	0.2	0.2	0.2	0.2
ML Water	0.3	0.1	0.1	0.1	0.1	0.1	0.1

(For the standard curve the above tubes were set.)

LACTATE DEHYDROGENASES (LDH)

The lactate dehydrogenase activity in the serum of C. punctatus under control and treatment with copper sulphate , cadmium chloride and mercuric sulphate at various conditions as 24 hrs ,48 hrs and 72 hrs .as revealed by colorimetrically were as follows:-

Lactate dehydrogenates activity in the serum of control fish was 275±8.0 I .U. / Litre.

Effect of CuSo:-

On treatment with copper sulphate, the lactate dehydrogenases activity declined to 125+5.0 I.U./ Liter at 24 hrs.($p<0.001$). Lactate dehydrogenases activity elevate slightly at 48 hrs.to 200+6.0 I.U./liter then declined sharply at 72 hrs.to 150+4.5 I.u./Litre ($p<0.001$)

Effect of Cadmimum Chloride ($Cdcl_2$):

The activity of lactate dehydrogenase in serum was 250+8.0 I. U/ litre ($P<0.001$). The activity was elevated to 320+5.0 I.U./Litre at 48 hrs., ($P<0.001$), then declined at 72 hrs. to 100 +3.0 I.U./litre ($P<0.001$)

Effect of $HgSo_4$:

On the treatment of Mercuric sulphate the activity of Lactate dehydrogenase in serum increased significantly to 300+5.0 I.U./litre at 24 hrs. and increased further to 375+8.0 I.U./litre at 48 hrs. ($P<0.001$). At 72 hrs. the LDH activity declined to 250+6.0 I.U./litre, ($P<0.001$).

Table-1.

Lactate dehydrogenase (LDH) concentration in Serum of Channa punctatus during control and after different periods of treatment with Cooper Sulphate ($Cuso_4$).

Period of tretment	Serum I.U./Litre
Control	275+8.0
24Hrs.	125+5.0***
48 Hrs.	200+6.0***
72 Hrs.	150+4.5***

(Mean+S.D.)

S.D.=Standard deviation

***=$P<0.001$ (Significant)

DISCUSSION

Lactate dehydrogenase constitute a glorious chapter of our heritage on account of its metabolic supremacy as key enzyme that is involved in the anaerobic oxidation of $NADH_2$ to NAD .the reaction takes place in cytosol of cells and hydrogen atoms are recived by pyuvate which in turn is reduced to lactate.

Lactate dehydrogenase activity increased during pregnancy and maximum at full term stage of pregnancy is rattus (solanke et. Al 1996)lactate dehydrogenase activity was observed to be maximum at pro ocstrous stage of oestrous cycle and full term stage of pregnancy in the rattus . a type sub-units and pattern of circulation oestrages which is noticeable at dioestrous may enable the ueres to maintain TCA cycle in a fully nfunctional state this tern enable the ueres to drive the maximum energy from glucose metabolism which assist in catabolism of uterine protein (Golbraith et al 1970).

Khan (1994) reported the decline in LDH activity in immature and female cybister confuses.

It is difficult to understand the significances of the reported alteration of enzyme activity for the enzyme inhibition was rarely reported to be concentration dependents difference in the in vitro and vivo effect have often been reported . for instances, exposure of yellow eel for days to .01 mg/1pcp, decreased the activities of pyruvate kinase and LDH and increasing the activites of hexokinase,glouse 6phosphate dehydrogenase (G-6-PDH),6- phosphoglucoinate dehydrogenase, fumerase and cytochrome oxidase in vitro showed, however ,

inhibition of activity of all the above mentioned enzymes ,indicating that invitro studies on enzyme activity do not necessarly reveal the pestivide indeced stress under nature conditions (Bostron and johansson 1972).

CONCLUSION

The decrease in alkaline phosphatsase concentration was probably due to its decreased need in protein synthesizing machinery .Here, it is worth mentioning that in this experimental fish, channa punctatus heavy metals, copper sulphate, Cadmium chloride and Mecruric suphate were responsible for activating stabiliting the protein synthesizing machinery and as such greater utilization of amino acides was noticed. This is in agreement with the results of Yamashita, 1968, Doriman, 1973, Shakoor et. al, 1978, and Sumpter et.al. 1984, thus it is explictly palpable that the heavy metals help in protein systhesis by two ways: Firstly by activating the greater utilization of amino acides, secondly by increasing the concentration of alkaline phosphatase which activates protein synthesis. This protein sysnthesis leads to the synthesis of many other enzyme involved in many other physiological pathways.

The serum dehydrogenase concentration decreased significantly on treatment with copper sulphate.

On treatment with Cadmium Chloride, the serum lactate dehydrogenase decrease signficantly at 24 hours but decreased significantly at 72 hours on treatment with Mercuric Suphate.

The serum lactate dehydrogenase concentration (LDH) decreased in response to Copper suphate treatment whereas there was an increase in its concentration on treatment with Cadmium chloride and Mercuric suphate. This decrease in LDH concentration is quite in the line of the fingings of Mercury and pocker (1979) who concluded that inactivation of LDH by various heavy metals compounds in an aqueous media arose from enzyme coprecipitation and that the observed enzyme LDH inactivation was not a chemical inhibition.

Thus, it may be inferred that copper suphate is responsible for enzyme coprecipitation. Earlier, Bostrom and Johan sson (1972) had already reported the in activation of LDH in yellow cel by 0.1 mg/litre PCP treatment for 4 days.

A perusal of the above mentioned findings, assumptions and suggestions makes it explicit that the Cooper Suphate, Cadmium Chloride and Mercuric Suphate bring about certain alterations in some biochemical aspects of a fresh water teleost, Chann punctatus.

It is hoped that the present investigation has not only explained some of the unexplored complexities of piscine biochemistry in response of heavy metals, but it will also provide a number of new and fertile areas for further investigations.

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