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LACTIC ACID DEHYDROGENASE ISOENZYME IN THE PERIODIC AMPUTATED RAT INCISOR PULP

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白鼠 前齒 週期的切除時 齒髓內 LDH ISOENZYME에 關한 硏究

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》국문 초록《

白鼠의 前齒를 週期的으로 切除後 齒髓內의 LDH isoenzyme을 電氣泳動法으로 分離하고 그의 活性度를 測定하여 正常과 比較觀察한바 다음과 같은 結論을 얻었다.

1. 正常齒髓內에서 5個의 LDH isoenzyme의 存在를 確認하였다.
2. LDH 活性度는 週期的으로 切除한 齒牙의 齒髓가 正常齒髓에 비해 훨씬 增加하였다.
3. 齒牙를 週期的으로 切除함으로써 齒髓內 LDH isoenzyme의 Pattern을 變化시킬 수가 있다.

INTRODUCTION

There have been considerable interest on the heterogeneity of enzymes catalyzing the same function in tissue(4,7,8,15,16,18,35,40). Among the enzymes that from various species of animal and within different tissues of the same animal exist in multiple molecular form (23),"isozyme", which was termed by Market & Møller (23) to describe the different proteins with similar enzymatic activity and catalyzing same reaction and similar substate specificities, the lactic acid dehydrogenase, LDH, is the most extensively studied. The reasons of it are its central position in metabolism ,where catalyze the reversible reduction of pyruvate to lactate with NADH, as a reducing agent, and their characteristic distribution in various animal and plant tissues.

Meister(24) was the first showed that LDH existed in multiple molecular forms in the same organism. And Neilands (27) demonstrated activity in each of the two electrophoretically distinct protein which was separated by Meister(24) from the crystalline ox-

heart enzyme. Weiland & Phleiderer(28) observed that the most organs contained up to five protein fractions with each LDH activity, whereas several studies(1, 2, 12, 25, 30, 38) indicated that LDH could be separated into more than five distinct forms.

It is well known that LDH exists as five isoenzymes of different composition(5). They are each designed LDH-1, LDH-2, LDH-3, LDH-4, LDH-5, in accordance with their electrophoretic mobility at basic pH(5). Of these, LDH-1 is the most negatively at physiological pH and hence the most fast-moving in electrophoresis. The five isoenzymes are thought to be tetramers, since it may be dissociated by urea or guanidine into four polypeptide subunits of equal size, made up two different kinds of polypeptides (A and B or M and H) synthesized under the control of two different genes(5, 22, 26), then, LDH-1 is H_4, LDH-5 is M_4, while others are hybrid forms; LDH-2 is H_3M, LDH-3 is H_2M_2 and LDH-4 is HM_3(5).

The five isoenzymes behave differently toward increasing pyruvate concentration(21, 32), and a possible relationship between substrate inhibition and metabolic role and subunit composition has been studied by many investigators (5, 18, 31). The LDH isoenzyme patterns reflects the relationship between the capacity of the tissue for aerobic and anaerobic metabolism (25). In animal tissue, it has been known that there are two kinds of patterns of LDH isoenzymes distribution; one predominating in the LDH-l and another predominating in LDH-5, each representing typical H- and M-LDH respectively as observed by many investigators (6, 13, 14, 23, 34, 39). These relative preponderance is explained in terms of aerobic-anaerobic relationship with regard to respective tissue metabolism; that is, H-LDH being abundant in aerobic tissues and M-LDH abundant in anaerobic tissues.

In the mean time, the isoenzyme composition of the serum proved to be of great diagnostic value (3, 36) related to the heart and liver disese, cancer and other disorders (37). In recent, several reports have appeared in the literature regarding the presence of LDH isoenzymes in the saliva(11), teeth (20, 33) and other oral tissues(9). The studies of the LDH isoenzymes were briefly mentioned above.

The purpose of this report is to investigate the relative variations of LDH isoenzymes and their activities in the pulp of rapidly growing four incisors of male rats.

MATERIALS and METHODS

Amputated pulp preparation:

Twenty male rats were divided in two groups;

1. Rats with periodic amputation of the four incisors.
2. Rats with no incisor amputation.

The incisor amputations were performed usually under light ether anesthesia, 2 or 3mm above the gingval border, every two days during two weeks, totaling 6 amputations. And two days after the last amputation, the rats were decapitalized, and both maxillary and mandibular incisors were carefully extracted and the dental pulp were pulled out carefully, pooled in each group. The pulps were throughly rinsed in the ice-cold saline solution and used fresh.

Preparation of pulp tissue homogenate:

The 20%(W/V) homogenates of pulp tissue were prepared in 0.25 M sucrose solution, previously chilled in the refrigerator, by grinding for two 1 minutes periods with an interval of 1 minute in a Potter Elvenhjem type homogenizer made up glass tube and Teflon pestle. The homogenates were centrifuged at 10,000 x g for 30 minutes, and the supernatants were used as the source of tissue enzyme preparation and protein measurement.

Electrophoresis:

Agarose gel electrophoresis was carried out according to Wieme(41), with 0.05 M Veronal buffer, pH 8.6, of ionic strength 0.04. Electrophoresis was run at 15V/cm and 3.5mA/cm at 4°C. However, visualization of enzyme activity was carried out in a modified fashion as follows by means of formazan reaction. Cellulose acetate(Separaphore III) strip was soaked in the substrate mixture containing 10^{-2} M Na-*l*-lactate, 2×10^{-3} M NAD^+, 2×10^{-4} M PMS(phenazine methosulphate) and 1.5×10^{-3} M NBT(nitroblue tetrazolium). The soaked strip was then superimposed on the agarose gel bed, followed by incubation. The incubation with this method was carried out at 37°C for 30 min.. The agarose gel bed underneath and the superimposed cellulose acetate strip were altogether taken into 10% acetic acid for decoloration of background for 10 min.. Densitometric evaluations of cellulose acetate strips were scanned with the Gelman densitometer (Gelman Instrument Co.) and interpreted by means of a planimeter.

Assay of LDH activity:

LDH activity was measured spectrophotometrically using Na-*l*-lactate as substrate by a method adapted from Neilands(28). The method consisted of incubating a mixture containing 180μ mole of glycine-NaOH buffer, 50μ mole of sodium lactate, 2μ mole of NAD^+, and 0.02 ml. of the enzyme sample. The rate of increase of optical density was calculated from reading at 340 mμ, in the Calbiometer(Calbiochem Co.), a UV spectrophotometer, due to the formation of NADH by its extinction coefficient. A unit of the enzyme activity is defined as a μmole of NADH produced during one minute per ml. of homogenate. The protein content of the enzyme solution was determined by the method of Lowry et al. (19).

RESULT and DISCUSSION

For the reduction of diffusions and the stabilization of reaction, this present investigation combined agarose gel as supporting media and cellulose acetate. The result of combination for electrophoretic separation was very satisfactory.

As shown in Fig. 1., it is evident that normal rat incisor pulp contains five distinct isoenzymes of LDH. In the normal rat incisor pulp, the prevailance of LDH-1, LDH-2 are apparent from Fig. 1. and 2. Therefore, it gives the impression that the pulp tissue is capable of highly aerobic metabolism, as indicated by Cahn et al. (5) that tissue cont-

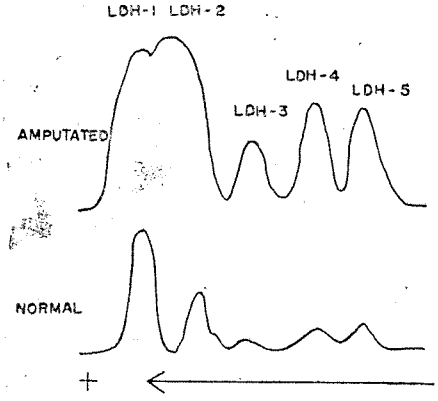

Fig. 1 Typical examples of LDH isoenzymes pattern after densitometric scanning from the amputated and normal rat incisor pulp.

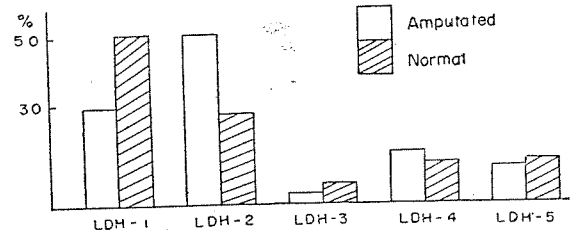

Fig. 2 Comparison of per cent distribution of LDH isoenzymes in the amputated and normal rat incisor pulp.

aining a preponderance of LDH-1 and LDH-2 allow pyruvate to accumulate and activate the citric acid cycle, whereas tissue containing a preponderance of LDH-4 and LDH-5 do not allow pyruvate to accumulate but respire anaerobically. Therefore, in anaerobically metabolic tissue LDH-5 is more prominent, whereas LDH-1 is more prominent in aerobically metabolizing tissue(5). Similar finding and conclusion were recently reported for LDH isoenzyme pattern of rat incisor pulp(20) and developing mouse molar teeth(33). Their observation indicated that aerobic metabolism provide the energy for dentinogenesis. Oehman(29) found that, in replanted young human teeth, the odontoblast showed a much lower survival rate of the pulp. This could be explained by a strictly aerobic metabolism in odontoblasts and a more anaerobic metabolism in other parts of the pulp. The former would be more sensitive to anoxia caused by the interruption of the circulation. Odontoblasts contains predominating LDH-1 isoenzyme band, but no LDH-5 band

Table I Distribution of LDH isoenzymes in the amputated and normal rat incisor pulp

	Zone per cent distribution of LDH isoenzymes				
	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
Amputated	28.1	49.6	1.2	12.0	9.1
Normal	50.4	24.5	3.9	9.7	11.5

was present (17). The presence of strong LDH-5 in this present investigation is correlated to the respiratory studies of bovine molar pulp by Fisher(10) who suggested that prominence of primitive anaerobic metabolic pathways and inherent partially deficient aerobic metabolism is cause of variation in oxygen consumption.

As is evident from Table I. and Fig. 2., the periodic amputation of rat incisor tooth affect the pattern of LDH isoenzymes in the pulp. But it is considerable interest to note that the pattern of LDH isoenzymes in the periodic amputated rat incisor pulp is inversely

Table Ⅱ Comparison of LDH activity in the amputated and normal rat incisor pulp. Data are the mean value obtained from the triple determination of activities.

	Total Activity*	Specific Activity**	% Change of total activity
Amputated	80.07	5.04	415.3%
Normal	19.29	2.56	100.0%

* Units$\times 10^{-2}$/ml. 20% (W/V) pulp tissue homogenate

** Units$\times 10^{-2}$/mg. protein of pulp tissue homogenate

related to that of normal in the LDH-1 and LDH-2 region. It could be occurred by the method of extraction or storage, electrophoretic techniques, staining procedure, variations of different portion of the sample.

According to the Table Ⅱ, total LDH activity of periodically amputated rat incisor pulp is much more great in comparison to the normal. In view of that periodically amputated tooth need much more energy for the continuous growth, can be safely assumed that LDH activity is increased for the metabolic demands in the dentinogenesis under certain control mechanism. It is, therefore, well established fact that the higher the activity of the enzyme, the more rapid is the growth of tissues. During this experiment, there was generally good correlationship between the activity measurement obtained by means of the spectrophotometric determination and the intensity of the bands separated by electrophoresis. The greatest activity in the periodic amputated rat incisor pulp was seen in the LDH-2, and LDH-1 region; the LDH-4 had less activity and very little activity was usually seen in the LDH-3 area. It was also found that specific activity of LDH was increased in the periodic amputated rat incisor pulp compared to the normal, as summarized in Table Ⅱ. Therefore, present investigation clearly demonstrated that LDH activity in rat incisor pulp markedly increased after periodic amputation of incisor tooth.

SUMMARY

In the periodic amputated rat incisor pulp, the distribution of five isoenzymes of lactic acid dehydrogenase was evaluated and the total LDH activity was assayed.

This study has established the followings;

1) It demonstrates the existence of five distinct isoenzymes of LDH, with LDH-1 and LDH-2 predominating, in the rat incisor pulp.
2) The total LDH activity in periodically amputated rat incisor pulp is markedly increased as compared to the normal rat incisor pulp.
3) It is possible that the periodic amputation of tooth effects the pattern of LDH isoenzymes in the pulp, especially LDH-1 and LDH-2 region.

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