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LACTIC ACID DEHYDROGENASE IN THE COBALT^{60} IRRADIATED RAT INCISOR PULP.

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》국문 초록《

백서에 Co^{60} 조사시 전치 치수내 LDH isoenzyme에 관한 연구

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백서에 1000R의 Co^{60}-gamma선을 전신 조사 후 시간에 따른 LDH isoenzyme의 Pattern과 그의 활성도의 변화를 관찰한 바 다음과 같은 결론을 얻었다.

1. 조사후 72시간에 LDH-1 와 LDH-4의 뚜렷한 Pattern을 나타내어, 이는 치수의 energy 대사가 염기성과 호기성 대사과정을 동시에 행함을 의미한다.
2. LDH의 총활성도는 조사후 24시간에 현저히 감소하였으나 72시간 후에는 거의 비조사군에 가까워졌다.

INTRODUCTION

In recent years, biochemical studies of dental pulp have revealed interesting properties regarding energy metabolism. Among the enzymes with related to the energy metabolism of dental pulp, lactate dehydrogenase(LDH, L-lactate:NAD$^+$ oxidoreductase, EC. 1. 1. 1. 27) play a central role in cell metabolism, where catalyze the reversible reduction of pyruvate to lactate.

In the viewpoint of that LDH isoenzyme pattern of a tissue indicates the relation between the capacities for aerobic and anaerobic metabolism, several investigators studied LDH isoenzyme in the dental pulp in regard to the energy metabolism of this tissue. Linde and Ljunggren (1), in the study of the LDH isoenzyme pattern in continuously growing rat incisor pulp, concluded that dentinogenesis would derive its energy from aerobic pathways. But in their study of LDH patterns of human dental pulp, suggested an evidence for the presence of a prominent anaerobic metabolism in this tissue (2). Roberts and Strachan(3) analyzed homogenates of developing mice molar by acrylamide gel electrophoresis. They demonsrtated five LDH isoenzyme bands and with increasing

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age of the tooth there was a decrease in all isoenzymes. In his study with periodically amputated rat incisor tooth Choi(4) suggested that periodic amputation of tooth affect the LDH isoenzyme pattern in pulp, especially in LDH-1 and LDH-2 region, and found that total LDH acivity was markedly increased in the amputated rat incisor pulp.

Concerned with energy metabolism in this tissue, Fisher(5) with the study of respiration of bovine dental pulps suggested that prominence of primitive anaerobic metabolic pathways and inhertially deficient aerobic metabolism is cause of variation in oxygen consumption. And Kozam(6) studied oxidative process of the rat and rabbit incisor pulps. Recently Fisher & Walters(7) and Fisher & Schwabe(8) concerned also with this problems.

In the present paper, the authors intended to investigate the irradiation effect on the LDH isoenyme pattern and their total activities.

MATERIALS AND METHODS

Cobalt^{60} gamma ray irradiation:

At Twenty-four, 48, 72 hours after a single dose of 1000 Roentgen of Cobalt^{60} gamma ray application to the whole body 5 rats for each irradiated group and 10 rats for non-irradiated group were sacrificed by cervical dislocation, and both maxillary and mandibular incisors were carefully extracted and the dental pulp were pulled out, pooled in each group. The pulps were frozen unless treated immediately.

Preparation of pulp tissue homogenate:

The pooled pulp were homogenized in a Potter-Elvenhjem-type homogenizer made up glass tube and Teflon pestle, frozen previously in a deep freezer, with ice-cold 0.25 M sucrose solution to obtain 20% (W/V) tissue homogenate. The homogenate were centrifuged at 10,000 x g for thirty minutes, and the supernatants were used as the source of enzymes.

Electrophoresis:

Agarose gel electrophoresis was carried out according to Wieme(9), with 0.05 M Veronal buffer, pH 8.6, of ionic strength 0.04. The electrophoresis was carried out at 4°C with a voltage of 150 V and a current of 7 mA, and was stopped after 2½ hours when the methylene blue, which was used as a marker, head reached 3 cm from the beginning. Visualization of enzyme activity was carried out in a modified fashion as follows by means of formazan reactions. Cellulose acetate(Separaphore III) strips were 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 $1.5\times^{10-3}$ M NBT(nitroblue tetrazolium). The soaked strips were 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 strips were altogether rinsed in 10% acetic acid for 10 min. The cellulose acetate strips were air dried, and scanned with the Gelman Densitometer(Gelman Instrument Co.) The area underneach peak representing one isoenzyme was calculated with planimeter.

Assay of LDH-activity:

LDH activity was measured by a method adapted from Neilands(10) with the use of the Calbiometer(Calbiochem. Co.), a UV spectrophotometer. Standard glass cuvettes of 1-cm light-path contained 180 μ mole of glycine-NaOH buffer, 50μ mole of sodium lactate, 2μ mole of NAD^+ in a final volume of 2.0ml. The reaction was started by the addition of 0.02ml of enzyme sample. The reagents used in this assay were maintained at 25°C in water bath and the Calbiometer was thermostatically regulated as well. A unit of the enzyme activity is defined as a μ mole of NADH produced during one minute per ml. of homogenate.

Protein determination:

The protein content was determined by the method of Lowry et al. (11), in order to obtain specific activities of the enzyme prepreations. Bovine serum albumin of the Nutritional Biochemicals corp. was used as a standard, determining the N content of it through Kjeldahlometry.

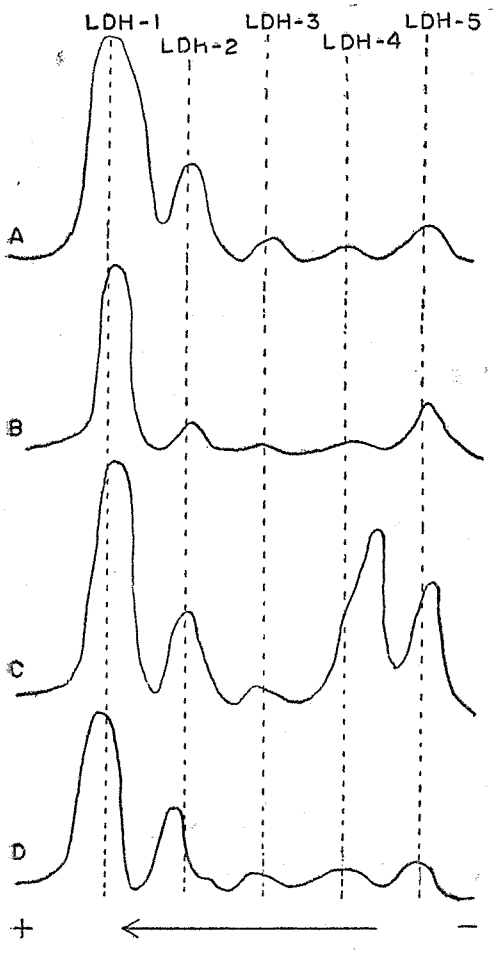
RESULTS

Whole body gamma irradiation was applied to the rats from $Cobalt^{60}$ source in dose of 1000 Roentgen, and its effects on the LDH isoenzyme pattern and its activities in incisor pulp at various intervals after irradiation, were summarized in Table I and II, which visualized in Fig. 1 and 2.

In each irradiated group five isoenzymes of LDH were clearly demonstrable. The five isoenzymes were numbered according to general practice, LDH-1, LDH-2, LDH-3, LDH-4 and LDH-5 from anode to cathode.

As shown in Fig. 1, 2 and Table I, although LDH-1 is most prominent, with LDH-2 almost as strong in all group, the isoenzyme pattern of LDH in Co^{60} irradiated pulp is characterized by a decrease in relative amounts of two fast moving isoenzymes(LDH-1 and LDH-2) as passed the postirradiation time. But it is noticeable at 72 hours postirradiation that LDH-1 is markedly weakened, whereas LDH-4 is become a strong band. A slight variation in patterns between different cellulose acetate strips of the same group was naturally present, but the figures shown are chosen to be representive. As a control, rat serum run stimultaneously. This serum showed the same distance of migration as the LDH bands of the rat incisor pulp.

As tabulated in Table II, the present finding demonstrated that total LDH activity at 24 hours postirradiation was much lower than the non-irradiated, while at 72 hours postirradiation was nearly close to the non-irradiated. Specific activities of LDH of irradiated groups were decreased compared to the non-irradiated, but they were similar between each irradiated group.



A: 24 hours after irradiation
B: 48 hours after irradiation
C: 72 hours after irradiation
D: Non-irradiated

Fig. 1. Typical examples of LDH isoenzymes pattern after densitometric scanning from the irradiated and non-irradiated rat incisor pulp

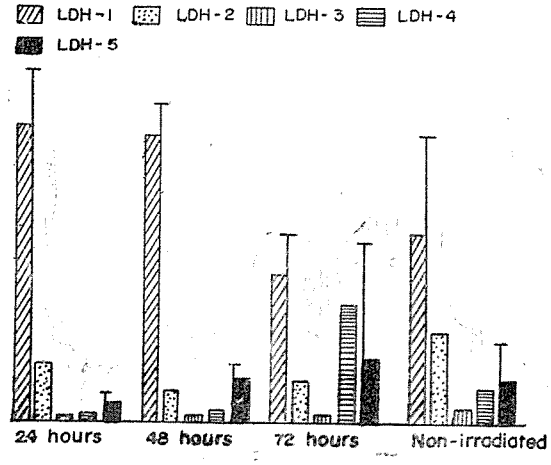


Fig. 2. Comparison of per cent distribution of LDH isoenzymes in each group. (If LDH-1 and LDH-2 and LDH-4 and LDH-5 are pooled, the developmental pattern is even more striking, as can be seen from the thin lines which represent the sum of the figures for LDH-1 and LDH-2 and LDH-4 and LDH-5 respectively).

Table I. Per cent distribution of LDH isoenzymes in the irradiated and non-irradiated rat incisor pulp

Hours after irradition	LDH-1	LDH-2	LDH-3	LDH-4	LDH-5
24	77.0	15.0	1.1	1.9	5.0
48	75.0	8.5	1.7	2.5	12.3
72	39.3	10.7	1.9	30.9	17.2
Non-irradiated	50.4	24.5	3.9	9.7	11.5

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

Hours after irradiation	* Total Activity	** Specific Activity	% Change of Total Activity
24	9.64	1.54	49.9
48	11.73	1.85	60.8
72	16.07	1.77	82.7
Non-irradiated	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

DISCUSSION

The only biochemical changes observed which occur early enough after irradiation, at a low enough radiation dose, and with severe enough consequences to affect the health of the cell are (a) inhibition of DNA synthesis, (b) uncoupling of oxidative phosphorylation, (c) increase of deoxyribonuclease activity, (d) reduction of nuclear synthesis of ATP and (e) apparent deficiency of cytochrome c in mitochondria (12).

As visualized in Fig. 1 and 2, one of the noteworthy findings in our study was the LDH isoenzyme pattern change in the LDH-1 and LDH-2 region at 72 hours postirradiation. There is no reference available to us, dealing with plausible explanation of this peculiar and perplexing changes observed. It is quite clear at present that the H- and M-LDH isoenzymes are under the control of separate genes, and that the hybrids are formed at random when both genes are operating in one cell, in same manner as genetic recombinants (13). Therefore, it is natural that irradiation can affect the pattern of LDH isoenzyme with relation to the biochemical changes mentioned briefly above. Because of unknown level of genetic control, we can not explain the subtle changes in the pattern of LDH isoenzyme by different postirradiation hours and doses used. But it is possible that at 72 hours postirradiation show a LDH isoenzyme pattern with strong LDH-1 and LDH-4 bands, an enzyme capable of cooperation with both aerobic and anaerobic processes.

The well known knowledge of mechanism of radiation effect is that the effects are initiated by ionization of water in living cells ($H_2O - e^- \rightarrow H_2O^+ \rightarrow H^+ + HO\cdot$), producing active oxidizing agent(hydroxyl radical). Beta particle, or electrons removed from molecules are captured by other water molecules($H_2O + e^- \rightarrow H_2O^- \rightarrow OH^- + H\cdot$), producing reducing agent(hydrogen radical). But if oxygen is present this hydrogen radical may form a potent oxidizing agent($H\cdot + O_2 \rightarrow HO\cdot_2$, the hydroperoxyl radical). Hydroxyl radicals may combine to form hydrogen peroxide, which play a small role in radiation effect because of the presence of catalase. These radicals and hydrogenperoxide formed from water can produce many changes in vital molecule indirectly, e.g., hydroxylation, oxidation, reduction, peroxide formation etc., directly, disconformation of molecule, main chain scission, intra- or intermolecular cross linking, or loss of fuctional groups. Any one of these changes could inactivate vital molecules in the living cell. The postulated mecha-

nism of radiation effect at the molecular or submolecular level was summarized above.

If we investigate the total LDH activity change in pulp, upper explanation can be again reasonable. In this study with using 1000 R, total LDH activity of dental pulp was not severly affected, since total LDH activity at 72 hours postirradiation was nearly close to the the non-irradiated as shown in Table II. Therefore, it will be interest to investigate with higher dose or local application to the teeth.

SUMMARY

Whole body gamma irradiation was applied to the rats from Cobalt^{60} source in dose of 1000 R, and its effects on the LDH isoenzyme pattern and its activities in incisor pulp at various intervals after irradiation were investigated.

This investigation established the followings:

1. At 72 hours postirradiation show a LDH isoenzyme pattern with strong LDH-1 and LDH-4 bands, *ie*, an enzyme capable of cooperation with both aerobic and anaerobic metabolic processes.
2. It demonstrated that total LDH activity at 24 hours postirradiation was much lower than the non irradiated, while at 72 hours postirradiation was nearly close to it.

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