Lactate dehydrogenase (LDH) isoenzyme pattern in normal and inflamed human dental pulp

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Enzyme variants may serve an adaptive role in providing the correct vectorial properties for the metabolism of a tissue, or broadening its environmental tolerance range.
To determine if the LDH isoenzyme pattern of human dental pulp changes during inflammation, supernatants from normal and inflamed dental pulp homogenates were separated by polyacrylamide gel electrophoresis.
Inflamed pulps had a higher M subunit content and a markedly increased enzyme activity. These results might reflect adaptive changes at the enzyme level associated with a partial shift towards anaerobic metabolism during inflammation of the pulp.

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Mammalian LDH (L-lactate dehydrogenase, E.C.I.I.I.27) is a tetrameric molecule consisting of two types of subunits (H and M) which upon combination give five possible isoenzymes (LDH-1, H_4 to LDH-5, M_4) (8, 11). These isoenzymes exhibit substrate inhibition, and the degree of inhibition increases with increasing number of H monomers in the molecule (9). Moreover, Kaplan et al. (9) have advanced the hypothesis that the M and H type of the enzyme evolved in order to fulfill certain definitive metabolic roles. The M type is proposed to function more effectively as a pyruvate reductase and the H protein predominantly as a lactate dehydrogenase, indicating that the amount of H subunit reflects the aerobic capacity of the tissue.

The LDH isoenzyme pattern in normal dental pulp has been described (10) and it included both anaerobic and aerobic metabolic capacity. To our knowledge no one has presented information on the LDH isoenzyme pattern in inflamed dental pulp. One reason for this apparent lack of information may be found in the difficulties encountered in the separation of inflamed from healthy pulp tissue. Such a separation is now possible and the present paper describes the different LDH isoenzyme patterns found in inflamed and normal pulp.

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MATERIALS AND METHODS

Six normal pulps were obtained from caries-free teeth extracted for orthodontic reasons. Eight inflamed dental pulps were collected from teeth with deep carious lesions. All teeth were extracted from patients under the age of 20. The pulps were removed immediately after extraction, from longitudinally split teeth, frozen in liquid nitrogen and stored at −85 °C.

The pulps were subjected to serial sectioning at 10 μm on a Jung microtome mounted in a Linde cryostat at −30 °C. Every tenth section was stained with haematoxylin and eosin (HE), and thereafter examined in the light microscope. The pulps from teeth with deep carious lesions showed only minor inflamed areas. To obtain samples from these areas, the normal pulp tissue, identified histologically, was removed dissectionally from frozen specimen using a dissecting microscope (American Optical Co®).

From each pulp about 100 sections were collected in a high speed centrifuge tube containing 250 μl 0.1 M Na_2HPO_4/NaH_2PO_4 buffer pH 7.4. The tubes were incubated at room temperature for 15 min and centrifuged at 0 °C for 1 h at 49000 *g* in a Sorvall RC-2-B centrifuge. The supernatants were used as enzyme source immediately after centrifugation. The isoenzyme patterns were analysed by disc electrophoresis according to Dietz & Lubrano (2) as modified by Dietz et al. (3) and Fritz et al. (4). A temperature-controlled Shandon electrophoresis cell was used for the electrophoresis and a Joyce-Loebl chromoscan densitometer for the quantitation. Total LDH activity was determined by spectrophotometric assessment of rates of oxidation of NADH (Sigma) at a fixed substrate concentration (pyruvate, Sigma) (7). The measuring wavelength was 340 nm. The incubation mixture contained 0.1 M sodium phosphate buffer, pH 7.4, 0.6 nM pyruvate, 4 nM NADH, and 10 μl of the supernatant, in a total volume of 300 μl.

RESULTS

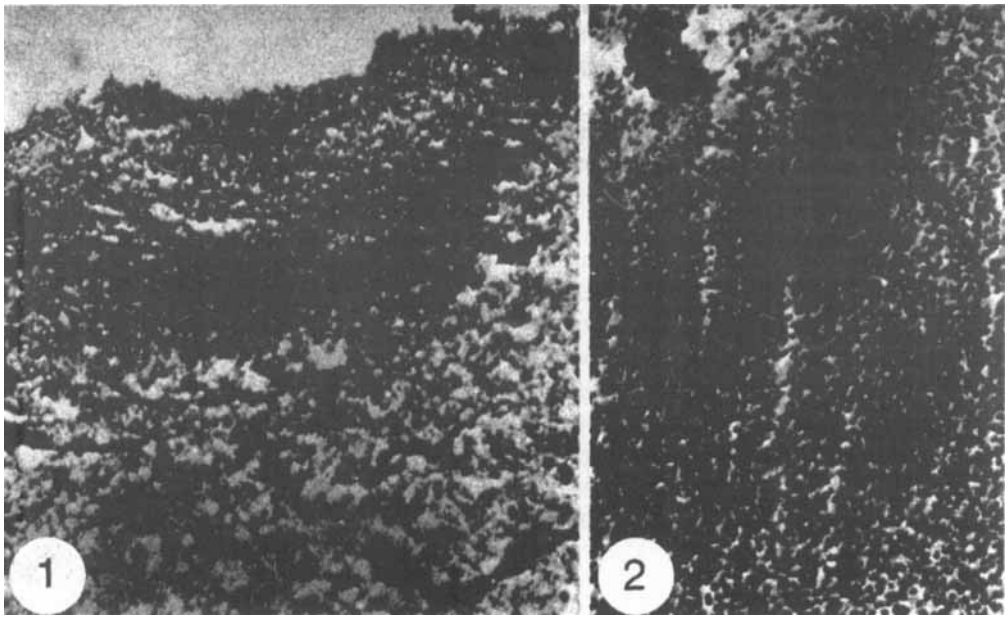
HE staining of sections of pulps from teeth with deep carious lesions revealed only minor areas with chronic inflammatory reactions (Fig. 1). Only samples from such totally inflamed areas were used for the comparative biochemical analysis (Fig. 2). A strikingly higher total LDH activity was determined in inflamed areas, as compared to that found in normal pulps (Table 1). Moreover, the electrophoretic separation of the enzyme exposed great differences in M subunit content of the two tissues (Table 2). LDH 3 invariably was the most prominent band in both tissues, but only inflamed areas contained LDH 5 (Figs. 3 and 4).

Table 1. *Total LDH activity in normal and inflamed areas measured in International units pr. mg protein. Each value represents the mean of six experiments with ± standard deviation*

	$\bar{x}$	sd
NORMAL PULP	51.0	6.0
INFLAMED AREAS	120.5	7.2

Table 2. *Per cent M subunit in normal pulp and inflamed areas. Each value represents the mean of six experiments with ± standard deviation*

	%	sd
NORMAL	35.7	2.1
INFLAMED AREAS	51.8	3.1



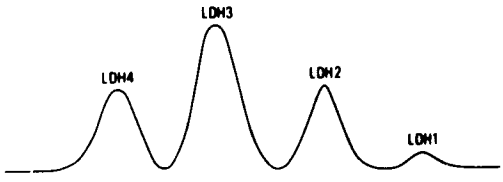


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Fig. 1. Dental pulp with area showing typical response to deep carious lesion (IA) with accumulation of inflammatory cells. X 120

Fig. 2. Area of the dental pulp showing severe inflammation. Used as enzyme source for the biochemical investigations. X 120

Fig. 3. Representative gel and densitometric recording of LDH isoenzyme pattern in normal dental pulp.

Fig. 4. Representative gel and densitometric recording of LDH isoenzyme pattern from inflamed areas of human dental pulp.

DISCUSSION

Frunder (5) found that tissue injuries led to increased anaerobic metabolism. In the present study it has been shown that LDH undergoes changes in the isoenzyme pattern in response to inflammation which may indicate a shift towards anaerobic metabolism. However, different cell types within the same organ may exhibit different isoenzyme patterns (1), and during inflammation increased cellular heterogeneity is observed in pulp tissue. At present the different cell

types from the pulp cannot be separated, and consequently the contribution from the different cell types to the total isoenzyme pattern cannot be established. In vitro experiments have demonstrated that changes in the LDH isoenzyme pattern of lymphocytes can be induced by alteration of the oxygen tension, hypoxia increasing the relative amount of M subunits (6). The increased M subunit content and strikingly higher LDH activity of inflamed tissue as compared to the normal pulp therefore probably reflect a compensatory adaption to increased substrate (pyruvate) levels. This, furthermore, suggests a shortage of oxygen supply to the inflamed tissue, which consequently has to rely to a greater extent on anaerobic metabolism.

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