Biochemical and Histochemical Observations on Isocitrate Dehydrogenase Activity in Early Wound Healing

It has been previously assumed that there is no isocitrate dehydrogenase (ICDH) activity in skin. However, after the development of the fluorimetric method, it has become possible to demonstrate its existence in skin[1]. Literature concerning the activity of ICDH and other oxidoreductases in the healing tissues seems to be scant. Some of these enzymes have been demonstrated histochemically in skin wounds[2,3] and in healing fractures[4]. The aim of this communication is to provide information about the changes in the activity of the NADP-dependent ICDH (L$_s$-isocitrate: NADP oxidoreductase, decarboxylating, EC 1.1.1.42) in an early phase of wound healing in which this enzyme is associated with several essential processes.

In the experimental investigation on rats, circular skin wounds, 3 mm in diameter, were excized in a dorsal area. 3 groups of animals were killed 2, 4, 8, 18, 20, 24, 48, 72, 96, and 120 h after wounding, and a flap of skin surrounding the wound was removed. A control wound of each animal was made 15 min before its death. The skin flaps were fresh frozen with solid carbon dioxide. Sections, 16 µm in thickness and 5 mm in length, were cut in a cryostat at − 20 °C for histochemical and quantitative biochemical determination of ICDH activity. Freezing of the skin flaps and the determination of enzyme activity was performed immediately after having killed the animals.

The biochemical quantitation of ICDH activity was performed by the spectrofluorimetric method as used by CRUICKSHANK et al.[1]. 4 frozen sections, however, were used instead of enzyme solution. The sections were carefully placed in the bottom of 8 × 50 mm test tubes. They were covered with 100 µl of a buffer-substrate solution, consisting of the following ingredients: NADP (1 m*M*), sodium DL-isocitrate (30 m*M*), $MnCl_2$ (0.04 m*M*), bovine serum albumin (0.05%), nicotinamide (20 m*M*), *tris*-HCl buffer (0.08 *M*), pH 8.2. The tubes were incubated at 38 °C for 30 min. The reaction was stopped by pipetting 20 µl aliquots of the incubation mixture into a tube containing 1 ml of a mixture of Na_2-EDTA (1 m*M*) and K_2HPO_4 (0.05 *M*). The solution was activated at 340 nm in the spectrofluorimeter (Hitachi Perkin-Elmer MPF-2A) and the resultant fluorescence was measured at 460 nm. Under these conditions the reaction was linear for 60 min. The ICDH activity remained constant in a frozen state (− 20 °C) for at least 2 h after freezing, and all of the determinations of enzyme activity were performed within this period. The ICDH activity was given as µmoles of the NADPH formed per h and per mg of tissue (wet weight). The protein content, being in the order of 4 µg per 1 section (= 30–40 µg), was determined according to LONG and STAPLES[5] and to SEARSY et al.[6].

The histochemical determination of ICDH activity was performed according to BALOGH et al.[7] in a mixture containing 0.1 *M* veronal buffer (pH 7.4), Nitro-BT as the electron acceptor, sodium DL-isocitrate as the substrate, and NADP as the coenzyme.

As shown in Figure 1, ICDH activity increased from the 2nd h on linearly up to the fifth day. Not before 2 days after injury did the enzyme activity in the wounds reach the level of the so-called control wound. Histochemically, the first increase in ICDH activity could be observed about 8 h after the operation. In the immediate vicinity of the wound edge, a central zone, 200–500 µm in depth, showed decreasing enzyme activity. Surrounding this, a 100–300 µm deep peripheral wound zone exhibited a progressive increase in ICDH activity after 8 h. In 24-hour wounds (Figure 2) the peripheral zone consisted mainly of intensely active mononuclear phagocytes and fibroblasts. 2 days after injury an imminent demarcation of the necrotic central zone was noticed. This process was accompanied by a very intense ICDH activity in the peripheral zone. At that time the preparations had reached such a colour intensity that no further increase could be perceived by eye during the rest of the experimental period. The demarcation was complete 5 days after injury.

The decreasing enzyme activity in the central zone should be regarded as an early, histochemically demonstrable sign of imminent necrosis in this irreversibly injured area[8]. The increase in activity in the peripheral zone represents an enzymatic response to injury. The dehydrogenation product of ICDH is 2-oxoglutarate. This compound is a very important intermediate in amino-carrying reactions (EC sub-group 2.6.1). A sufficient supply of 2-oxoglutarate is thus significant in amino acid metabolism, including protein synthesis. Further, ICDH is indispensable in the operation of the citric cycle, forming an essential part of cellular respiration, i.e. of energy production. Active mitosis in mammalian epidermis, for example, can develop only when a carbohydrate substrate is efficiently oxidized in the citric cycle[8]. The increase in ICDH activity in wounds thus reflects several important processes associated with healing[9].

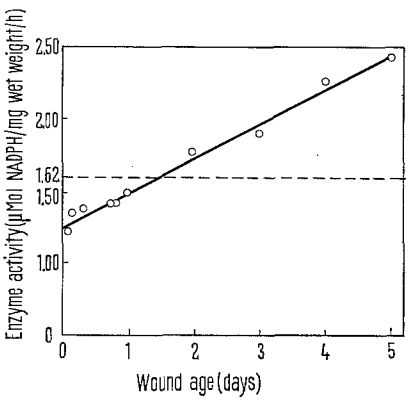

Fig. 1. ICDH activity in early wound healing, determined biochemically. The broken line indicates the level of ICDH activity in the control wounds.

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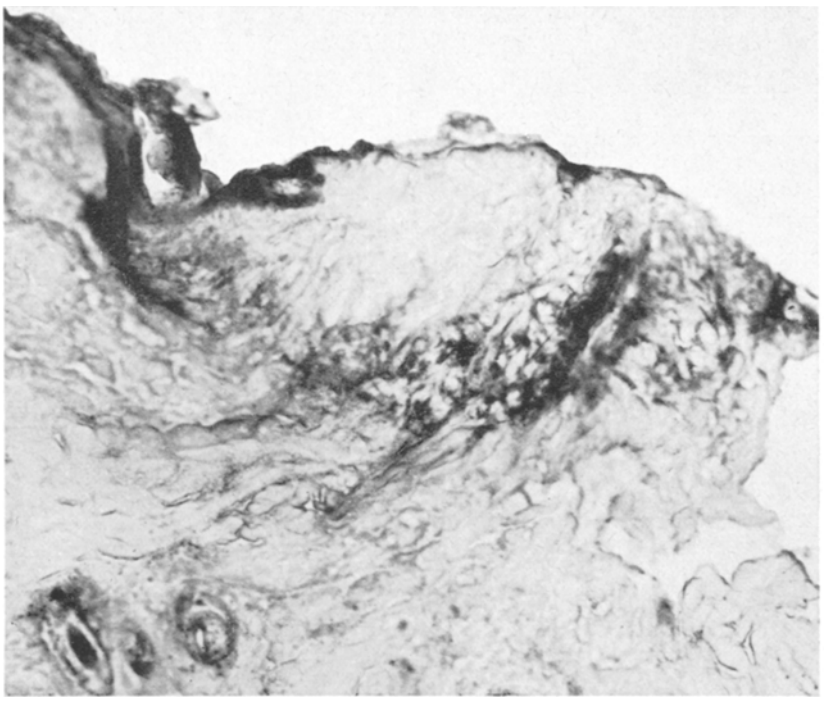

Fig. 2. Histochemical demonstration of ICDH activity in a one-day-old wound. ×100.

Zusammenfassung. Nach einem Absinken während der zwei ersten posttraumatischen Stunden stieg die fluorimetrisch bestimmte Aktivität der NADP-abhängigen Isocitrat-Dehydrogenase bis zu 120 h. Histochemisch erkannte man eine Aktivitätsverminderung in unmittelbarer Nähe der Wundfläche. Die Aktivität nahm zwischen der 8. bis 48. h in der äusseren, die nekrotische Wundnähe umgebenden Zone zu.

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Evolution of a Retinal Specific Lactate Dehydrogenase Isozyme in Teleosts

L-Lactate dehydrogenase (LDH) exists in multiple molecular forms (isozymes[1]) and serves as an excellent model for the study of evolution of proteins[2-7]. The lactate dehydrogenase polypeptide monomers, designated A and B, of vertebrates are encoded in at least 2 genetic loci[8,9]. The random assortment of these 2 subunits generates 5 tetrameric isozymes in higher vertebrates[10], however a smaller number is commonly observed in fish because of restricted assembly[7,11-13].

The tissue and developmental specific synthesis of the LDH isozymes has been attributed to their distinctive kinetic properties, which permit them to operate more efficiently during different conditions of anaerobiosis in the cell[14,15].

A third LDH locus (the E locus) has been postulated to function in the nervous system of teleosts[11,16,17]. The existence of this E locus in teleosts has since been established by genetic, immunochemical, physical, and kinetic comparisons of the teleost LDH isozymes[5-7,13,18,19]. The E gene is activated at the time of retinal differentiation[7,18,20] and is mainly functional within the photoreceptor cells of the neural retina which suggests that the E_4 isozyme plays a unique role in the visual metabolism of teleosts[7,21].

Immunochemical, kinetic, and physical analyses indicate that the LDH E polypeptide is more closely related to the B subunit than to the A subunit[5-7,13,18,19,22]. The E gene probably arose by gene duplication at the B locus

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