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TPN-specific isocitrate dehydrogenase in bovine cornea epithelium

During the course of our investigations on enzyme patterns of the main metabolic pathways in connective tissues[1] enzyme activities were assayed separately in epithelia and connective tissue of bovine cornea. We found an extremely high activity for TPN-specific isocitrate dehydrogenase (EC 1.1.1.42) in cornea epithelium.

Cow's eyes were sectioned out immediately after slaughtering. Samples of both tunica propria and epithelium were homogenized in 50 mM triethanolamine–HCl buffer (pH 7.5), 5 mM EDTA, using a Waring blendor. For determination of enzyme activity the 100 000 × *g* supernatant was used in a optical test under standard conditions[1]. The stepwise extraction along with increasing disintegration of cornea epithelium was carried out as described earlier[2].

The enzyme pattern of cornea epithelium shows an extremely high isocitrate dehydrogenase activity. It exceeds all other enzyme activities assayed in the same tissue (Fig. 1). The maximum isocitrate dehydrogenase activity can be assumed to be twice the measured activity, since Mg^{2+} was used instead of Mn^{2+} for activity determination. In all the tissues of both man and animal that have been investigated so far no comparable isocitrate dehydrogenase activity has ever been encountered (Table I).

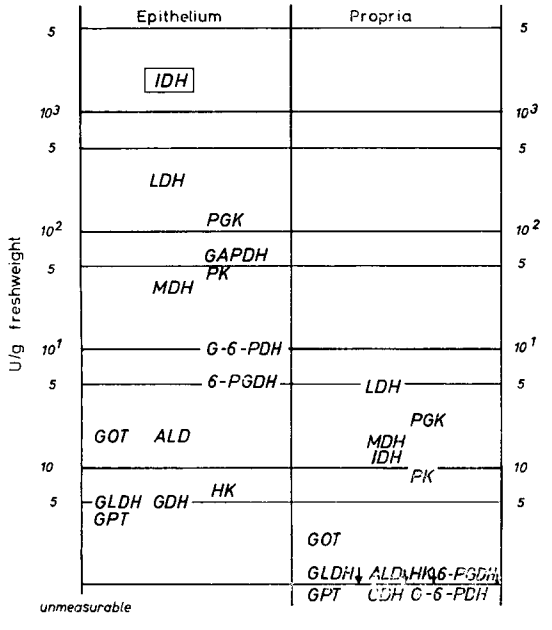

Fig. 1. Enzyme distribution pattern of bovine cornea epithelium and tunica propria. Logarithmic scale. Enzyme activities in international enzyme units (U, the amount of enzyme catalysing the oxidation of 1 μmole substrate per min). Abbreviations used: IDH, isocitrate dehydrogenase; LDH, lactate dehydrogenase; PGK, phosphoglycerate kinase (EC 2.7.2.3); GAPDH, glyceraldehyde phosphate dehydrogenase; PK, pyruvate kinase (EC 2.7.1.40); MDH, malate dehydrogenase; G-6-PDH, glucose-6-phosphate dehydrogenase (EC 1.1.1.49); 6-PGDH, 6-phosphogluconate dehydrogenase (EC 4.2.1.12), GOT, aspartate transaminase (EC 2.6.1.1); ALD, aldolase (EC 4.1.2.7); HK, hexokinase (EC 2.7.1.1); GLDH, glutamate dehydrogenase; GDH, L-α-glycerophosphate dehydrogenase; GPT, alanine transaminase (EC 2.6.1.2).

Furthermore, the isocitrate dehydrogenase activity of cornea epithelium is higher than that found for dehydrogenases from any other known sources, when comparable assay methods were used (Table I). Actually only one other enzyme of the main metabolic pathways exceeds the activity of the cornea epithelium isocitrate dehydrogenase, *viz.* triose phosphate isomerase (EC 5.3.1.1) known from several human and animal tissues.

Stepwise extraction as described (Fig. 2) shows isocitrate dehydrogenase to be a highly soluble enzyme, comparable to lactate dehydrogenase (EC 1.1.1.27). A careful treatment, washing the tissue in isotonic sucrose medium by slight stirring releases about two-thirds of the total isocitrate dehydrogenase activity from the cornea epithelium into the buffer, and a second treatment releases the rest. This suggests that isocitrate dehydrogenase is a cytoplasmatic enzyme. In comparison the glutamate dehydrogenase (EC 1.4.1.3), a mitochondrial enzyme, shows an extraction pattern with the maximum activity in the mitochondrial fraction.

The cytoplasmatic isocitrate dehydrogenase of cornea epithelium is TPN-specific, TPN reacting $2 \cdot 10^4$ times as fast as DPN. However, in that fraction of our extraction sequence which includes mitochondrial material (*cf.* Extract IV, Fig. 2) the corresponding difference is only $2 \cdot 10^3$. This suggests that an additional DPN-specific mitochondrial isocitrate dehydrogenase is present in cornea epithlium,

TABLE I

ENZYME ACTIVITIES OF ISOCITRATE DEHYDROGENASE IN BOVINE TISSUES IN COMPARISON TO TRIOSEPHOSPHATEISOMERASE, MALATE DEHYDROGENASE, LACTATE DEHYDROGENASE, L-α-GLYCEROPHOSPHATE DEHYDROGENASE AND GLYCERALDEHYDE PHOSPHATE DEHYDROGENASE IN DIFFERENT HUMAN AND ANIMAL TISSUES

Tissue	*Enzyme*	*Activity (U*/g fresh wt.)*
Bovine cornea epithelium	Isocitrate dehydrogenase (EC 1.1.1.42)	1190
Bovine heart muscle	Isocitrate dehydrogenase (EC 1.1.1.42)	19
Bovine liver	Isocitrate dehydrogenase (EC 1.1.1.42)	11
Bovine sceletal muscle	Isocitrate dehydrogenase (EC 1.1.1.42)	2
Locust flight muscle[2]	Triose phosphate isomerase (EC 5.3.1.1)	1530
Human heart muscle[11]	Malate dehydrogenase (EC 1.1.1.37)	464
Rat liver[2]	Lactate dehydrogenase (EC 1.1.1.27)	400
Human skeletal muscle[11]	Glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12)	170
Locust flight muscle[2]	L-α-Glycerophosphate dehydrogenase (EC 1.1.1.8)	149

* μmoles substrate/min.

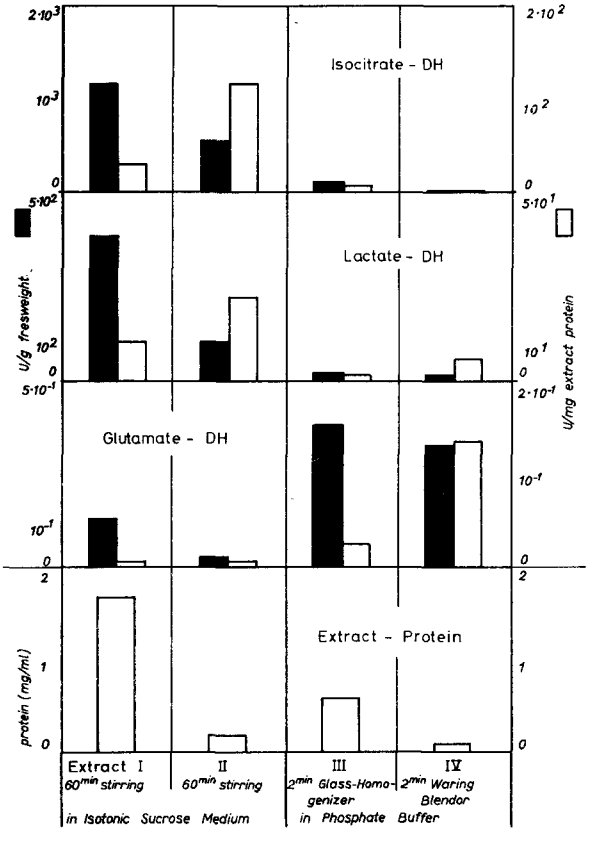

Fig. 2. The enzyme activities of isocitrate dehydrogenase, lactate dehydrogenase and glutamate dehydrogenase at different stages of the stepwise extraction of bovine cornea epithelium.

although with a relatively low activity. In locust flight muscles GOEBELL AND KLINGENBERG[3] were able to show that under the specific metabolic conditions of that tissue the DPN-specific isocitrate dehydrogenase (EC 1.1.1.41) was much more active than the TPN-specific enzyme.

Both tissues represent special types of metabolism. For the cornea epithelium the data presented point to metabolic conditions centering around an unusually high TPN-specific extramitochondrial isocitrate dehydrogenase activity, comparable to the role of L-α-glycerophosphate dehydrogenase (EC 1.1.1.8) activity in locust flight muscle metabolism[4]. However, no final explanation as to the significance of the metabolism of cornea epithelium can be offered so far. Possibly, the high isocitrate dehydrogenase activity could provide an extensive TPNH pool for a number of specific syntheses. Further investigations should reveal the relation of both isocitrate dehydrogenase enzymes to the Krebs cycle and also whether these enzymes take part in other steps of intermediary metabolism as discussed earlier by several authors[5–10].

In conclusion, then, the cornea epithelium because of its extremely high isocitrate dehydrogenase activity—especially of the TPN-specific enzyme—will be valuable for further investigations concerning the role of this enzyme in intermediary metabolism.

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