Occurrence of Ribose Phosphate Isomerase in Bovine Lens

In search of a general mechanism of glucose metabolism occurring in the lens, KINOSHITA et al.[1] studied the carbohydrate breakdown in corneal epithelium. Using glucose labelled with radioactive carbon at position 1 and 6, these investigators showed that glucose metabolism primarily follows the pentose phosphate pathway.

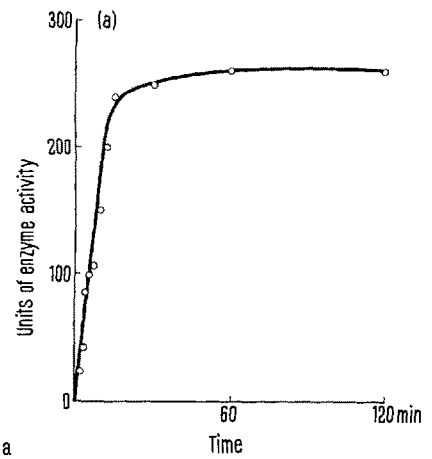
Although the overall glucose metabolism seems to follow the pentose pathway, the individual enzymes participating in the stepwise reaction of glucose oxidation have neither been isolated nor has their existence as separate entities been shown in the lens. We wish to report here the occurrence, localization and kinetics of the reaction of ribose phosphate isomerase in the bovine lens.

Fresh bovine and calf lenses were collected from the slaughter-house just after the death of the animals. The lenses were carefully removed to avoid any damage and quickly frozen in dry ice and acetone. The different parts of the lens were subsequently dissected with the help of a sharp razor blade. The separated parts from 9–10 lenses were pooled, weighed and homogenized in a Dounce homogenizer in 0.1 *M Tris* buffer (pH 7.5) at 0 °C, to give a 20% homogenate.

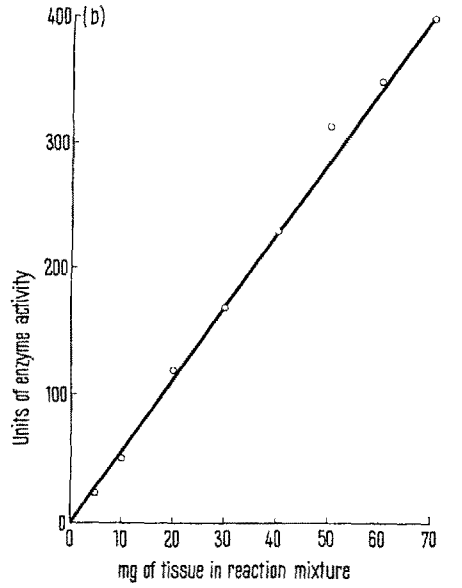
The enzyme was assayed according to its ability to isomerize ribose-5-phosphate to ribulose-5-phosphate, as essentially described by AXELROD and JANG[2] and more recently by DOBROGOSZ and DEMOSS[3]. This procedure employs the cysteine-carbazole reaction of DISCHE and BORENFREUND[4] for ketopentoses. The basic reaction system for the determination of ribose phosphate isomerase consisted of the following: *Tris* buffer (pH 7.5, 0.1 *M*), 0.5 ml; ribose-5-phosphate (sodium salt, 0.003 *M*), 0.5 ml; lens homogenate 0.5 ml. Incubation was done at 37 °C for 10 min. The reaction was stopped by addition of 1.5 ml of 10% cold TCA. The blank is deproteinized before the addition of substrate.

To 1 ml of the TCA extract were added 0.2 ml of 1.5% cysteine-HCl, 6 ml of H_2SO_4-H_2O (2.5:1) and 0.20 ml of 0.12% carbazole in ethanol (absolute) in quick succession (within 30–40 sec). The tubes were vigorously shaken. Colour was allowed to develop at room temperature for exactly 20 min, at which time the absorbancy was determined in a Beckman spectrophotometer (DU-model) at 540 nm. The ribose phosphate isomerase activity was expressed as the micromoles of ribulose-5-phosphate formed from ribose-5-phosphate at 37 °C in 1 h at pH 7.5 by 1 g of wet tissue. In calculating the units of isomerase activity, it was taken into consideration that only 35–40% of ribose-5-phosphate was converted to ribulose-5-phosphate under the conditions of our experiments.

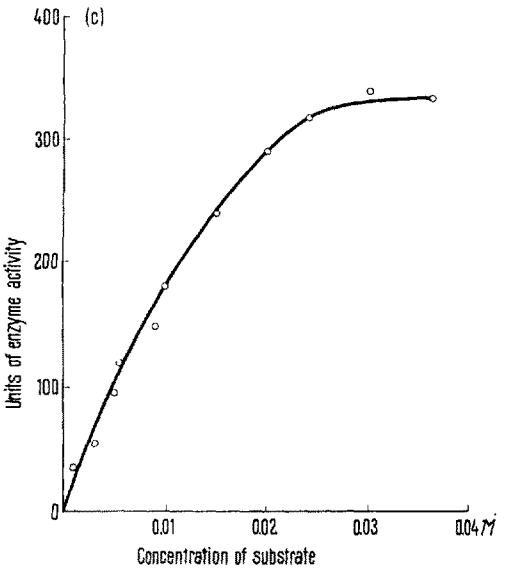
Results showing the effect of varying the incubation periods on the ribose phosphate isomerase activity are illustrated in the Figure (a). The time course of hydrolysis maintains a zero-order reaction up to 15 min, after which



a



b



c

Ribose phosphate isomerase activity of bovine lens homogenate as a function of (a) time, (b) enzyme concentration and (c) substrate concentration. Assay conditions are the same as described in the text. Each result in the Figures represents an average of 5 determinations.

[1] J. H. KINOSHITA, J. MASURAT and M. HELFANT, Science *122*, 72 (1955).
[2] B. AXELROD and R. JANG, J. biol. Chem. *209*, 847 (1954).
[3] W. J. DOBROGOSZ and R. D. DEMOSS, Biochem. biophys. Acta *77*, 629 (1963).
[4] Z. DISCHE and E. BORENFREUND, J. biol. Chem. *192*, 583 (1951).

a plateau is obtained. A linear relationship describing the isomerase activity as a function of enzyme concentration is presented in the Figure (b). A proportional increase in the enzyme activity resulted from a corresponding increase in the substrate concentration up to a concentration of $0.024M$ as indicated in the Figure (c). The Michaelis constant (Km) is calculated at pH 7.6, when the rate of the reaction is found to be proportional to the time until 40% of the substrate undergoes degradation. A Km value of the order $1.0 \cdot 10^{-3}M$ was obtained. With the same substrate, DOBROGOSZ et al.[3] reported Km values of $2.8 \cdot 10^{-3}M$ in *Pediococcus pentosacus*. The Km values (ribose-5-phosphate) of $2.72 \cdot 10^{-3}M$ observed in the enzyme extracts of *E. granulosum* by AGOSIN and ARAVENA[5] also show similarities to that of the Km of ribose phosphate isomerase in the bovine lens.

The data presented in the Table clearly indicate that the activity of ribose phosphate isomerase in the bovine lens homogenate is more (50–60%) than that observed in the homogenate of the calf lens. Epithelium of the lens with cortex and the cortical part next to epithelium exhibited maximum activity. The nuclear part showed very much poorer enzymatic activity than all the other different parts of the lens. It should be recalled here that KINOSHITA et al.[1,6] observed overall glucose oxidation only at the epithelium of the cornea. These results may be considered as an indication of the metabolic activity of the lens.

Localization of ribose phosphate isomerase activity in bovine lens[a]

No. of determinations	Various parts of the lens	Unit range	Average
10	Steer	165–169	166 ± 2.4
9	Calf	110–115	113 ± 2.2
5	Epithelium of the lens and cortex	345–348	346 ± 1.2
5	Cortical part next to epithelium	295–335	316 ± 18.2
5	Lens other than cortical part	165–265	212 ± 87
5	Nuclear part	20– 35	27

[a] Assay conditions are the same as described in the text.

Résumé. La présence de l'enzyme ribose-phosphate isomérase qui transforme le ribose-5-phosphate en ribulose-5-phosphate, a été démontré dans les cristallins de bœuf. Dans les cristallins de bœuf, la concentration de l'enzyme est plus élevé que dans les cristallins de veau. L'enzyme est principalement localisé dans l'épithélium et dans le cortex du cristallin et décroit graduellement jusqu'à ce que la fraction nucléaire présente une activité enzymatique négligeable. Quelques propriétés biochimiques de l'enzyme présent dans l'homogénat de cristallins de bovins, ont été déterminées. La constante de Michaelis (Km) a été calculé comme étant $1.0 \cdot 10^{-3}M$.

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Department of Biochemistry, Faculty of Medicine, Laval University, Quebec (Canada),
4th November 1966.

[5] M. AGOSIN and L. ARAVENA, Enzymologia *22*, 281 (1960).
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Verhalten von Hippocampus-Pyramidenzellen bei retikulärer Reizung

Hochfrequente elektrische Reizung bestimmter Regionen der Formatio reticularis führen im Hippocampus-EEG, abhängig von der Reizintensität, entweder zu einem Thetarhythmus[1] oder, bei hoher Reizintensität, zu einer Unterdrückung des Thetarhythmus[2–4]. Die vorliegenden Untersuchungen wurden unternommen, um festzustellen, inwieweit sich die Aktivität einzelner Hippocampus-Pyramidenzellen (PZ) bei diesen beiden Typen eines Hippocampus-EEG verschieden verhält.

Methodik. An 25 curarisierten Kaninchen wurde die elektrobiologische Tätigkeit des Hippocampus und die Tätigkeit von insgesamt 130 PZ der Areae CA_2 und CA_4 (extrazellulär, mit Stahlmikroelektroden) abgeleitet. Die Reizungen der Formatio reticularis erfolgten mit bipolaren Reizelektroden (Rechteckimpulse von 0,5 msec Dauer, 200/sec, Reizdauer 2 sec). Bezüglich weiterer Einzelheiten der Methodik sei auf die Arbeiten von GREEN et al.[5] bzw. STUMPF[4] verwiesen.

Ergebnisse. Die Tätigkeit der PZ zeigte im allgemeinen bei retikulären Reizungen mit zunehmender Spannung allmähliche Veränderungen, das heisst zunehmende Aktivierung oder Hemmung (Figur 1), jedoch keine sprunghafte Veränderung bei Übergang des Hippocampus-EEG von Thetarhythmus in Unterdrückung. Nur bei solchen PZ, die in Gruppen in Korrelation zu den Thetawellen entluden, hörte dieses Entladungsmuster bei Unterdrückung des Thetarhythmus plötzlich auf (Figur 1 c und d). Von allen untersuchten PZ reagierten auf schwache, einen Thetarhythmus auslösende retikuläre Reizungen 71% mit Hemmung, 13% mit Erregung und 16% blieben unbeeinflusst; für starke, den Thetarhythmus unterdrückende retikuläre Reizungen betrugen diese Werte 82%, 15% und 3%. Aktiviert wurden vor allem jene Zellen, die bei schwachen Reizungen in zu den Thetawellen synchronen Gruppen entluden (Figur 1 c und d).

Bei mehreren PZ trat vereinzelt oder gehäuft eine Inaktivierung der Aktionspotentiale durch Depolarisations-

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