The Effect of Age on Glycolytic and Hexokinase Activities in the Mucosa of Rat Small Intestine

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In previous investigations it was found that glycolytic and hexokinase activities of rat intestinal mucosa decreased during starvation for 36 hr. and, after re-feeding, increased again to the values observed in fed control animals (Srivastava & Hübscher, 1966; Srivastava, Shakespeare & Hübscher, 1968). In this study it is shown that the age of the animal also has an effect on glycolytic and hexokinase activities of rat intestinal mucosa.

The methods employed were those published previously (Srivastava & Hübscher, 1966; Srivastava *et al.* 1968). Mother rats (Wistar strain) were kept on Spillers Autoclaved Laboratory Small Animals Diet (Spillers Ltd., Gainsborough, Lincs.) and the offspring were weaned on to this diet when they were 24 days old. The diet was supplied *ad lib.* to both mothers and offspring. Intestinal homogenates from four rats of the same age were combined (see Table 1). For rats up to 12 days old whole small intestines were used, whereas for rats aged 18 days and older the mucosal scrapings were taken. Total homogenates were assayed for the formation of lactate from glucose and glucose 6-phosphate and

also for hexokinase activity. The results are quoted in terms of specific activity. It is considered that this gives a true measurement of changes in enzyme activity, whereas determination of total activity would reflect the overall growth of the small intestine.

There was a slight increase of glycolytic activity in whole small intestines of rats aged 3–12 days. At day 18 homogenates of mucosal scrapings had a low glycolytic activity, which was of the same order as that of whole intestines from rats aged 12 days. Between days 18 and 28 there was a sharp and statistically significant increase ($P < 0.001$, by the *t* test) in all three activities. Although the offspring were separated from the mother animals only when 24 days old, they were seen to take some rat cake from day 17. It is known that the weaning from milk to solid food is a gradual process (Murphy & Dunn, 1949).

The fact that the specific activity of lactate formation from glucose 6-phosphate showed a larger relative increase than did hexokinase activity, indicates that, in addition to hexokinase

Table 1. *Changes of glycolytic and hexokinase activities*

Each group of rats consisted of four animals from which the homogenates prepared from either the whole small intestine or the intestinal mucosa of the whole length of a small intestine were pooled and then assayed. If more than two groups of rats were investigated the values quoted for glycolytic and hexokinase activity are given as means $\pm$ s.d.; the values quoted for two groups are expressed as means $\pm$ absolute deviation. The numbers quoted in parentheses in the last column refer to the numbers of animals weighed and the weights are expressed as means $\pm$ s.d. N.D., Not determined.

Preparation assayed	Age of rats (days)	No. of groups of rats used	Sex	Lactate formed (μ moles/min./mg. of protein)		Hexokinase activity (μ moles/min./mg. of protein)	Body wt. (g.)
				From glucose	From glucose 6-phosphate		
Homogenates of whole small intestine	3	1	N.D.	1.2	2.0	N.D.	9.1 $\pm$ 1.0 (44)
	6	1	N.D.	1.0	2.1	N.D.	14.7 $\pm$ 1.6 (42)
	9	1	N.D.	1.5	1.8	N.D.	19.8 $\pm$ 1.2 (40)
	12	1	N.D.	2.5	3.3	N.D.	26.8 $\pm$ 1.1 (38)
Homogenates of intestinal mucosa	18	4	♀	2.2 $\pm$ 0.7	2.3 $\pm$ 1.7	36.4 $\pm$ 22.0	38.4 $\pm$ 1.6 (18)
	24	5	♀	5.8 $\pm$ 4.0	8.0 $\pm$ 5.5	44.0 $\pm$ 10.3	61.1 $\pm$ 1.8 (18)
	28	4	♀	14.2 $\pm$ 3.2	16.8 $\pm$ 4.5	97.1 $\pm$ 23.4	80.2 $\pm$ 2.1 (18)
	35	4	♀	11.7 $\pm$ 5.2	18.0 $\pm$ 7.8	94.5 $\pm$ 15.5	116.7 $\pm$ 2.7 (18)
	49	2	♀	9.2 $\pm$ 3.3	15.3 $\pm$ 1.2	80.1 $\pm$ 11.4	168.8 $\pm$ 4.0 (18)
	77	2	♀	10.0 $\pm$ 0.3	12.0 $\pm$ 0.7	78.8 $\pm$ 14.9	N.D.

activity, other enzymes of the glycolytic pathway must have increased in specific activity during the weaning period.

The increase in activity of glycolytic enzymes in rat intestinal mucosa is by no means unique. Other enzymes reported to increase in this tissue during weaning are carboxylesterase (EC 3.1.1.1) (Pelichová, Koldovský, Heringová, Jirsová & Kraml, 1967), β -fructofuranosidase (EC 3.2.1.26) (Doell & Kretschmer, 1964; Yi-Yung Hsia, 1966), α -glucosidase (EC 3.2.1.20) (Yi-Yung Hsia, 1966), leucine aminopeptidase (EC 3.4.1.1) and aminopeptidase (EC 3.4.1.3) (Noack *et al.* 1966). However, there are also enzymic activities that either remain unchanged or decrease in the intestinal mucosa during the weaning period (Koldovský & Chytil, 1965; Lindberg & Owman, 1966; Yi-Yung Hsia, 1966; Pelichová *et al.* 1967), thus lending support to the view that there is not a general increase of all metabolic activities.

The underlying principles involved in the control of the change of enzymic activities are not known. During weaning rats changed from a carbohydrate-poor diet to a carbohydrate-rich one. Rat milk contains between 1.5 and 3.0% (w/v) of lactose (Folley & Greenbaum, 1947; Wrenn, DeLauder & Bitman, 1965; Gul, 1967) and it has been estimated that only 10% of the caloric requirement of suckling rats is met by carbohydrate (Hahn, Koldovský, Melichor & Novak, 1961). In contrast, the Spillers diet employed in this study contained digestible carbohydrate that accounts for 63% of the caloric intake. However, hormonal factors may also play a

role in the glycolytic activity of the intestinal mucosa. It has been shown that the changes of activity of β -fructofuranosidase, β -glucuronidase (EC 3.2.1.31) and β -galactosidase (3.2.1.23) that occur during the weaning period can be partially or completely prevented by adrenalectomy (Koldovský & Chytil, 1965; Koldovský, Jirsová & Heringová, 1965).

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