

# COMPARATIVE STUDIES ON DIFFERENT SMALL INTESTINAL OLIGOSACCHARIDASE ACTIVITIES IN SOME VERTEBRATES AND INVERTEBRATES LIKE MOLLUSCS

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**Summary:** Oligosaccharidase activities of the small intestinal mucosal homogenates were measured in vertebrates viz fish, toad, garden-lizard (calotes), pigeon, rat and some invertebrates viz, mollusca. Maximum activities of the enzymes Lactase, Sucrase and Maltase were found in the mammalian species rat, whereas much less activities were found in the non-mammalian vertebrates among which toad shows the highest values and garden lizard the lowest. Among the invertebrates *Pila globosa* shows higher values of all the enzymes than *Achatina fulica*. The results obtained have been discussed in the lights of phylogeny and diet habits.

**Key words:** intestinal oligosaccharidase vertebrates invertebrates

## INTRODUCTION

The role of intestinal carbohydrase activities were observed previously in human being, but the intestinal carbohydrase activities in invertebrates like Molluscs and vertebrates from fish up to mammals are infrequent in published literatures (1). In some microorganism the lactase is an adaptive enzyme (4). In pig the presence of trehalase, lactase and cellobiase in the proximal region of small intestine and the sucrase, maltase and isomaltase in ileum have been reported (10). It has been observed that  $\beta$ -glucocidase activity could only be demonstrated in the intestine of rats and toads. The intestine of pigeons, finches, turtles and frogs does not react (6).

## MATERIALS AND METHODS

**Chemicals & animals used:** Glucose oxidase (Sigma Chemicals), Horse raddish peroxidase grade D, (Worthington Biochemical Laboratory, Free hold, N. J., U.S.A.), O-Dynasidine, Tris obtained from Sigma Chemical Co., St. Louis, MO, Maleic Acid, Lactose, Sucrose, Maltose, Glucose and Toluene of analytical grade were used.

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The following animals were used at random from the commercial source :

- (a) *Vertebrates*: Fish (*Ophicephalus punctatus*), Toad (*Bufo melanostictus*), Lizard (*Calotes versicolor*), Bird (*Columba livea*) or Pigeon, and Mammal (Albino rat, Charles foster strain).
- (b) *Invertebrates*: Aquatic snail (*Pila globosa*), and land snail (*Achatina fulica*). Only the above two groups of molluscs were used for the present experiment.

**Measurement of oligosaccharidase activity :** Methods described by Dahlqvist (5) was used for our experimental purpose with some necessary modification by Sharma and Majumdar (8), Sharma and Ghosh (9).

The mucosal homogenate obtained from different animals like rat, pigeon, calotes, toad, fish and snails was prepared as described by Sharma and Majumdar (8) and dilution for lactase, sucrase and maltase activity was 1 in 10 with cold maleate buffer at pH 7. 0.1 ml of mucosal homogenate was treated with 0.05 ml of 2% aqueous solution of lactose, sucrose and maltose each and 0.05 ml of maleate buffer and a drop of Toluene was added to each tube. It was incubated at 37°C for 1 hour. After 1 hour the reaction mixture was boiled for 5 minutes and cooled. The volume was made 1 ml by adding distilled water. Then 4 ml of tris glucose oxidase reagent was added and incubated for another hour. When the colour developed, the reaction was stopped by adding one microdrop of 4N HCl and the reading was taken in a spectrophotometer at 420 m $\mu$  and the *mu* of glucose produced after hydrolysis from each sugar was calculated from standard glucose curve. Total hydrolysis of sugars after one hour was calculated in mg by multiplying the value with dilution factor. If X be the reading and d is the dilution factor, the total amount of sugar hydrolysed is X x d x 2 per hour, per ml of original mucosal homogenate preparation. The results which were obtained have been discussed in Table I for comparison.

TABLE I: Small intestinal oligosaccharidase activities in vertebrates and invertebrates.

Mean values expressed in mg of sugar hydrolysed per ml/hour of original mucosal homogenate with  $\pm$  S.D.

No. of Experiments	Enzyme	Vertebrates				Invertebrates		
		Fish	Toad	Calotes	Pigeon	Rat	<i>Pila globosa</i>	<i>Achatina fulica</i>
6	Lactase	0.200 $\pm$ 0.022	0.294 $\pm$ 0.037	0.184 $\pm$ 0.033	0.720 $\pm$ 0.287	4.6 $\pm$ 1.9	0.960 $\pm$ 0.298	0.680 $\pm$ 0.234
6	Sucrase	0.140 $\pm$ 0.031	0.268 $\pm$ 0.031	0.116 $\pm$ 0.042	0.888 $\pm$ 0.298	63.7 $\pm$ 15.3	0.920 $\pm$ 0.231	0.760 $\pm$ 0.268
6	Maltase	0.200 $\pm$ 0.027	0.270 $\pm$ 0.021	0.190 $\pm$ 0.019	0.930 $\pm$ 0.249	171 $\pm$ 60	0.570 $\pm$ 0.156	0.470 $\pm$ 0.145

S.D. = Standard Deviation.

The above table shows the oligosaccharidase activities in different vertebrates and invertebrates. The enzymes lactase, sucrase and maltase are present in all the species of vertebrates and invertebrates. The maximum enzyme activities in vertebrates have been observed in rat, then in pigeon, toad, fish and least in calotes. In invertebrates the enzymes activities are maximum in *Pila globosa* than *Achatina fulica*.



## DISCUSSION

From the above observations it is evident that oligosaccharidases like lactase, sucrase and maltase are present in measurable quantity in the alimentary tract of both vertebrates and invertebrates used in this series, although the degree of their activity showed a wide species variations. Gossrau (6) reported that only the activities of  $\beta$ -glucosidase and lactase could be demonstrated in the intestine of rats and toads, while these were absent in Pigeons, Finches, Turtles and Frogs. The present study in partial disagreement to the above observation could demonstrate the oligosaccharidase activities in several vertebrate and invertebrate species.

The presence of intestinal oligosaccharidases in these species from mullusca to rat may indicate that these require carbohydrates like lactose, sucrose and maltose as their diet either directly from the aquatic and land vegetables or indirectly from animal sources specially in cases of carnivorous species like toad or calotes.

In this series special emphasis have been given to the mucous membrane of the small intestine with regard to disaccharidase activity, because in the opinion of Code(3) disaccharidase activity and consequently absorbtion of disaccharides are found to be miximal in the small intestine. Further, the activity of the enzymes shows variations in the different regions of the gut. Dahlquist (9) noticed that in the pig the proximal region of the small intestine contain trehalase, lactase and cellobiase, whereas sucrase, maltase and isomaltase are mainly located in the ileum. *Auricchio et al.* (2) observed in man low level of sucrase, isomaltase and lactase in the first part of the duodenum, whereas other disaccharidases occur in the jejunum and ileum.

Lactase is an adaptive enzyme (1) and it is found in some microorganisms also. The enzyme activity is induced by lactose related sugars. Parsons (7) reported that the ability of mucosal cells to transfer single hexose units from the mucosal fluid into the vascular bed depends on the presence and orientation of oligosaccharidase molecules in the limiting membrane of the absorbing cells.

It may be assumed in the present study that the presence of carbohydrase activities in the above species is due to their food habit. Formerly, it was believed that in lower invertebrates and vertebrates like snails and pigeon the carbohydrate splitting enzymes are absent from the gastro-intestinal tract. The three enzymes so far studied in this series are very important for hydrolysis of all types of dietary sugars. The presence of maltase activity indicates that there might be other carbohydrate splitting enzymes like amylase, which help in the breakdown of starch into simpler molecules like maltose. Similarly, the presence of sucrase activity indicates that the plants on which they are dependent definitely contain sucrose. The presence of lactase activity in invertebrates like mollusca and vertebrates like fish, amphibia, reptilia and birds like pigeon is really an interesting phenomenon. It is believed that mammals starting from rat upto man take milk from their mother sources just after their birth and that is why milk splitting enzyme like lactase is present in their gastrointestinal tract, but no (functional) correlation for the



presence of lactase in the alimentary tract of the non-mammals like bird and amphibia, reptilia, fish and mollusc can clearly be drawn at the present moment and therefore further studies are needed to explore why and how this enzyme is developed in their gastrointestinal tract. Work is in progress in this laboratory for searching the presence of other enzymes like proteases and lipases for final conclusion.

## REFERENCES

1. Alvariz, A. and J. Sas.  $\beta$  galactocidase changes in the developing intestinal tract of the rat. *Nature*, **190**: 826-827, 1961.
2. Auricchio, S., A. Rubino, R. Tosi, G. Semenza, M. Landholt, H. Kistler and A. Parder. Disaccharidase activity in human intestinal mucosa. *Enzymol. Biol. Clin.*, **3**: 193-208, 1963.
3. Codes, C.F. Sectional Editor. Handbook of Physiology, Sec. 6, Alimentary Canal. Vol. 3, Intestinal absorption. American Physiological Society, Washington D.C. Chap. 69, P. 1323-1347, 1968.
4. Dahlqvist, A. The location of carbohydases in the digestive tract of the pig. *Biochem. J.*, **78**: 280-288, 1961.
5. Dahlqvist, A. Methods for assay of intestinal disaccharidase. *Anal. Biochem.*, **7**: 18-25, 1964.
6. Gossrau, R.  $\beta$ -glucosidase and lactase in the intestine of vertebrates. *Anat. Inst. Univ. Warzburg, Warzburg, W. Germany*, **35**(2): 143-151, 1973.
7. Parsons, D.S. and J.S. Prichard. Hydrolysis of disaccharides during absorption by the perfused small intestine of Amphibia. *Nature*, **208**: 1097, 1965.
8. Sharma, A.K. and A.C. Mazumdar. Studies on the effect of some broad spectrum antibiotics on small intestinal oligosaccharidase activities and absorption of lactose. *The Jap. J. Expt. Med.*, **40**: (4): 221-225, 1970.
9. Sharma, A.K. and A.K. Ghosh. Some observations on small intestinal oligosaccharidase activities in normal and alloxan diabetes, before and after insulin therapy. *The Jap. J. Exp. Med.*, **43**(5): 443-446, 1973.
10. Sheehy, T.W. and P.R. Anderson. Disaccharidase activity in normal and diseased small bowel. *Lancet*, **2**: 1-5, 1965.