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Short Communications

Distribution of lysozyme in guinea pigs: Implications for the function of gastrointestinal lysozyme in herbivores[1]

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Summary. High levels of gastrointestinal lysozyme were present in the stomach of guinea pigs, but not in other portions of the gastrointestinal tract. Because the cecum is the fermentation organ of guinea pigs, these observations call into question the validity of the current hypothesis that the gastrointestinal lysozyme of herbivores functions in the digestion of bacteria from the anterior fermentation organ.
Key words. Lysozyme; guinea pigs; gastrointestinal tract.

Lysozyme (EC 3.2.1.17) is an enzyme that catalyzes the hydrolysis of the beta (1-4)-glycosidic linkages between N-acetylmuramic acid and N-acetylyglucosamine. This substrate is present in the cell walls of bacteria but not in mammalian tissues[3].

The concentration of lysozyme in different tissues varies remarkably among species. Some species have high activity whereas other species have relatively low activity of lysozyme in most tissues. One of the purposes of this study was to determine the lysozyme activity in various tissues of guinea pigs *(Cavia porcellus)*.

Recent studies have suggested that the gastrointestinal lysozyme of herbivores functions as a digestive enzyme acting on cellulolytic bacteria originating from the anterior fermentation organ[4,5]. This hypothesized function for gastrointestinal lysozyme in herbivores has been derived indirectly and is based upon observations of the distribution of lysozyme in a limited number of species. It has been stated that '... only mammals with fore-gut fermentation should have high levels of stomach lysozyme ...'[7]. Thus, in cattle, gastrointestinal lysozyme is present in the abomasum, posterior to the fermentation organ, the rumen[4,6]. In rabbits, the gastrointestinal lysozyme is located in the distal colon posterior to the fermentation organ, the cecum[5]. However, rabbit colonic lysozyme is selectively conveyed to the stomach by the unique coprophagic activity of rabbits termed cecotrophy[5]. Thus, rabbit gastrointestinal lysozyme, although synthesized in the distal colon, is transported to the stomach. An alternative hypothesis would be that gastrointestinal lysozyme of herbivores functions in the stomach independently of acting on bacteria from a fermentation organ. The main purpose of this study was to evaluate these hypotheses. For this purpose, guinea pigs, herbivores, in which the cecum is the fermentation organ and in which cecotrophy does not occur, were selected for use.

Materials and methods. Eight healthy young adult male guinea pigs, weighing between 403 and 445 grams, were used. They were anesthetized with ether, blood was obtained from the heart, and they were killed with an i.p. injection of sodium pentobarbital. The tissues listed in the table were collected from each guinea pig immediately after death. The segments of the gastrointestinal tract were separated according to Cooper and Schiller[8] with the cardia and fundus separated from the body and pylorus of the stomach. All tissues were stored individually at −20 °C until thawed for homogenization and assay. Serum was prepared from the heart blood and frozen. Upon thawing, each tissues was weighed, minced, and mixed with 2.33 ml of homogenization buffer per gram of tissue. The buffer consisted of equal parts of 0.067 M sodium phosphate buffer (pH 7.3) and 1% acetic acid in 95% ethanol. The tissues were homogenized in micro plexiglass tissue homogenizers (Bellco Glass Inc., Vineland, NJ, USA) and centrifuged at 3000 × g for 10 min at 5 °C. The supernatants, less any lipid layer, were collected and recentrifuged. The supernatants were assayed for lysozyme in duplicate by the lysoplate method[9] as previously described[10,11]. The enzymatic activity of the supernatants was determined with chicken egg white lysozyme as the standard and the protein was determined by the method of Lowry et al.[12]. The lysozyme activity in the supernatants was expressed as mean ± SEM chicken egg white lysozyme equivalent μg/mg protein.

Results and discussion. The mean lysozyme activity in each of the tissues of the eight guinea pigs assayed is presented in the table. The nongastrointestinal tissues (bone marrow, lung, spleen and kidney) had lysozyme activities that ranged from 330 to 1008 μg/mg protein. The gastrointestinal lysozyme was localized predominantly in the stomach with approximately equal levels of

Lysozyme activity in tissues of guinea pigs

Tissue	Activity[a] mean ± SEM (n = 8)
Stomach fundus	2205 ± 217
Stomach pylorus	2489 ± 236
Duodenum	8.4 ± 0.8
Jejunum	2.9 ± 0.3
Ileum	2.3 ± 0.2
Cecum	33 ± 6.2
Ascending colon	15.8 ± 3.2
Transverse colon	3.4 ± 0.7
Descending colon	3.6 ± 0.4
Rectum	4.1 ± 0.4
Bone marrow	614 ± 55
Lung	330 ± 51
Spleen	347 ± 76
Kidney	1008 ± 95
Serum	35 ± 5.2[b]

[a]Chicken egg white lysozyme equivalent μg/mg protein; [b]chicken egg white lysozyme equivalent μg/ml.

activity in the fundus and pylorus. The mean activity in the stomach was 71 times that of the next highest gastrointestinal tissue, the cecum, and over 300 times greater than the mean activity of the three segments of the colon.

The activities of lysozyme in the nongastrointestinal tissues of guinea pigs were rather moderate, being greater than the levels in cattle, a species that is relatively lysozyme deficient[6], but substantially less than the levels in rabbits, a species with relatively high lysozyme activity[13,14]. The relatively high activities of lysozyme in the bone marrow, lung and spleen probably represent lysozyme in the leukocytes in these tissues, whereas the high activity in the kidney is a result of lysozyme that has been reabsorbed by tubule cells after being filtered by glomeruli. Evidence has been presented that gastrointestinal lysozyme of herbivores is an isozyme of lysozyme and is the product of a gene that is distinct from the gene that codes for the lysozyme of other tissues[4,15,16]. Associated with this novel isozymic nature of gastrointestinal lysozyme, a unique functional activity has been hypothesized: lysozyme in the gastrointestinal tract digests cellulolytic bacteria that pass into the lysozyme-containing segment from an anterior fermentation organ[4,5,7].

However, because this study has demonstrated that the gastrointestinal lysozyme of guinea pigs is localized in the stomach and because it is known that the fermentation organ in guinea pigs is the cecum, it can be concluded that gastrointestinal lysozyme, at least in guinea pigs, and therefore possibly also in other herbivores, does not function in the digestion of bacteria from the fermentation organ. This conclusion is contradictory to recent hypotheses[4,5,7] regarding the function of gastrointestinal lysozyme in herbivores.

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Seasonal changes in the critical arousal temperature of the marsupial *Sminthopsis crassicaudata* correlate with the thermal transition in mitochondrial respiration[1]

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Summary. During periods of torpor *Sminthopsis crassicaudata*, a dasyurid marsupial, regulated its body temperature above about 16.3 °C in summer and 13.0 °C in winter. Animals with lower body temperatures were unable to arouse. Liver, heart and brain mitochondrial succinate:cytochrome c reductase showed a thermal transition at 16 °C in summer and at 12.5 °C in winter. Thus the lowest regulated body temperature was just above the temperature where changes were detected in mitochondrial respiration.
Key words. Marsupial; torpor; season; critical arousal temperature; mitochondrial respiration; thermal transition.

A marked increase in the Arrhenius activation energy (E_a; a measure of the slope of the log enzyme activity against the reciprocal of the absolute temperature) below a critical temperature (T*) has been described for many mammalian membrane-associated enzymes. For example, in liver and heart mitochondria from homeotherms and/or normothermic (summer active) hibernators, T* is observed at 20–23 °C for succinate oxidase, succinate:cytochrome c reductase, succinate oxidase linked H^+ ejection and mitochondrial Ca^{2+} uptake[2–10]. During the torpid state of heterothermic and hibernating mammals, the lowered body temperature (T_b) is associated with a significant lowering, or even a disappearance of T* for these and several other membrane-associated enzymes, when compared to homeothermic species and normothermic hibernators in summer[2–5,8–10]. In preparation for hibernation various membrane-associated respiratory enzymes of liver mitochondria from ground squirrels exhibit a lowering of T* from 20 to about 12 °C. Furthermore, a constant E_a during the hibernating state suggests that T* is lowered below the minimum T_b of 2 to 5 °C which ground squirrels experience[9,10]. Since homeothermic mammals which exhibit a T* at about 20 °C are unable to survive hypothermia at body temperatures which do not threaten heterothermic species, the lowered T* evident during hibernation/torpor may be an important factor in the ability of many small mammals to enter into, and arouse from, the torpid state. Thus the T* may be a determinant of the 'critical arousal temperature' below which animals