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Biosynthesis of Chitinases by Mammals of the Order Carnivora

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Key Word Index—Carnivora; Mammals; chitin; chitinase; feeding habits.

Abstract—The secretion of chitinases has been examined in six species of Mammals belonging to the order Carnivora. Chitinases were found only in the extracts of the gastric mucosa of two species not adapted to a strictly meat diet [Canidae: dog and fox] while those with exclusive carnivorous habits, [Mustelidae: stoat, ferret, marten; and Felidae: cat], seem not to secrete the enzyme. These observations confirm the existence of a correlation between the ability of a given species to synthetize chitinases in digestive system and the feeding habits of the species.

Introduction

On the basis of studies on the zoological distribution of the chitinolytic enzymatic system, a correlation has been demonstrated between dietary habit of the animal and the secretion of chitinase by some glandular tissues of its digestive tract. These observations were made not only with some invertebrate phyla, specially Arthropoda [1], but also with vertebrates [2].

Among the Vertebrates, most species whose diet contains organisms containing chitin (e.g. insects, fungi) synthetize chitinolytic enzymes in their digestive systems. These enzymes are principally secreted by the gastric mucosa but, in some species, also by the pancreas. These chitinolytic enzymes can be defined as true chitinases devoid of any significant lysozymic activity [3].

In the lower Vertebrates, a correlation between the secretion of chitinases and the nature of the diet is clearly observed in Amphibians and in Reptiles. This correlation is less obvious in Fishes [4].

In the higher Vertebrates so far studied, the capacity of a given species to synthetize chitinases seems also to be related to the nature of the diet. Indeed, birds and mammals so far studied, insectivorous or omnivorous species always secrete chitinases [1, 2, 5]. On the contrary, species which are strongly adapted to a highly specialized diet entirely devoid of chitin, do not secrete chitinases in their digestive system [1, 6]. This has been interpreted as being the result of the loss of the capacity to synthetize the enzymes during evolution, as a consequence of the dietary adaptation [7].

Among the Mammals belonging to the order Carnivora, only one species has been studied, the cat, *Felis domestica* [1]. The gastric mucosa and the pancreas of this species do not contain chitinases. The question arises whether or not the distribution of the biosynthesis of chitinases in other carnivorous mammals which present different degrees of adaptation to a wholly meat diet does confirm the hypothesis of a correlation between the dietary habit and the presence of chitinases in the digestive tract.

The present paper deals with the distribution and the localization of the chitinase biosynthesis in six more or less omnivorous or exclusively carnivorous Mammals of the order Carnivora.

Results and Discussion

The study of the *distribution* of chitinases in digestive tissues shows that the extracts of gastric mucosae of the dog (*Canis domesticus*) and of the fox (*Vulpes vulpes*) contained chitinases (Table 1). Thus, the two species of Canidae studied so far are able to synthetize chitinolytic enzymes. On the contrary, no trace of chitinase activity was observed in the digestive tract of the Mustelidae and the Felidae, i.e. the stoat (*Mustela erminea*), the ferret (*Mustela furo*), the marten (*Martes foina*) and the cat (*Felis domesticus*) (Table 1).

Among the species studied, only the fox and the dog have a very diversified diet. The fox is carnivorous in winter and has a largely vegetarian and insectivorous diet during other seasons [8]. The stoat, the ferret and the

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TABLE 1. DISTRIBUTION AND LOCALIZATION OF THE CHITINOLYTIC ENZYMES BY SIX SPECIES OF CARNIVOROUS MAMMALS

Family	Species	Activity (μg N-AG liberated/hr×g fresh tissue)							
		Mucosae				Pancreas	Liver	Spleen	Kidneys
		Gastric†		Intestinal					
		Cardiac portion	Pyloric portion	Duodenum	Jejunum				
Canidae	*Canis domesticus*	161	252	0	0	0	0	11*	9,5*
	Vulpes vulpes	25*	1239	0	0	0	0	0	0
Mustelidae	*Mustela erminea*	0	0	0	0	0	0	33	0
	Mustela furo	0	0	0	0	0	0	245	16*
	Martes foina	0	0	0	0	0	17*	50	67
Felidae	*Felis domesticus*	0	0	0	0	0	18*	24*	39

* Non-significant values, included show the limits of the method.
† Free from their muscular tissues.

marten, the most typical of the Mustelidae have a strictly meat diet. As far as the cat is concerned, it is well known that the diet is highly specialized, consisting exclusively of meat.

The results, reported in Table 1, agree with the hypothesis of a loss of the capacity to synthetize chitinases by species strongly adapted to a highly specialized meat diet. However, it is worth noticing that the chitinase biosynthesis by the glandular tissue of the digestive tract is restricted to the family Canidae. We may thus ask ourselves, if the synthesis of chitinases in carnivorous mammals is really a property directly related to the dietary habits or is merely related to the systematic position of the different species. More results are needed before one can answer this question.

From the point of view of the *localization* of the chitinases, it appears that in the digestive system they are exclusively present in the gastric mucosa. The intestinal epithelium did not contain any chitinolytic activity. The same situation has been observed in all mammals, so far studied [1, 2, 6]. The pancreas does not seem to secrete chitinase, as far as Carnivora are concerned. The extracts of spleens and kidneys showed a relatively low chitinolytic activity, except for the spleen of the ferret. The existence of chitinolytic activities in such organs is frequent in Vertebrates but very variable from one species to another [4]. Such activities are most likely the result of the presence of lysozymes in these organs [3].

Experimental

Preparation of extracts. The animals were anaesthetized by an injection of Nembutal®. The organs were carefully isolated and washed with a saline solution, dried on filter paper and weighed. The organs were then homogenized in a pestle and mortar with washed sea sand. The obtained suspensions (100–200 mg fresh tissue/ml) were allowed to stand overnight at 4° then centrifuged. The supernatant which contains chitinases was kept at −20°.

Estimation of the chitinase activity. The substrate used was a suspension of "native" chitin, prepared from cuttlefish bones (*Sepia officinalis*) [1]. The chitinolytic activity was measured by the method of [1–9] applied to the study of enzymatic solutions devoid of chitobiase activity [4]. The chitinase activity is expressed in μg of *N*-acetylglucosamine (N-AG) liberated per hr per gm fresh tissue.

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