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CHITINASE: AN ADDITION TO THE LIST OF HYDROLASES IN THE DIGESTIVE TRACT OF VERTEBRATES

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IT was thought for a long time that, in animals, chitinases were present in snails only. The distribution of these enzymes among invertebrates has appeared to be much more extensive. They have been found by Tracey in protozoa[1], in nematodes[2], and in earthworms[3], and by me in arthropods[4–6], cœlenterates[7], polychæte worms[7], etc. The presence of chitinolytic enzymes among vertebrates has, however, never been suspected. The enzymic lysis of chitin is known to be actually performed by two different enzymes, acting consecutively[8–9]: a chitinase (mucopolysaccharidase) and a chitobiase (β-acetylglucosaminidase). This 'chitinolytic system' and these two enzymes have been searched for in the digestive juices and in aqueous extracts of digestive glands and digestive epithelia of some fishes, lizards, birds and mammals.

In order to detect the chitinolytic system (chitinase + chitobiase), the enzymic extracts were incubated with a chitin suspension (from *Sepia* pen) at 37° C. and *p*H 5·2 for 3 hr.; the acetylglucosamine liberated was measured by a method described by Reissig *et al.*[10]. Controls without enzyme or without substrate were run simultaneously.

In order to detect chitinase in enzymic extracts containing little or no chitobiase, the activity of these extracts on chitin has also been investigated after addition of an excess of chitobiase in the enzymic test. A commercial preparation of β-glucosidase (Nutritional Biochemicals Corporation) provided a convenient source of chitobiase, free from chitinase. In these cases, the presence of chitinase was also confirmed by the nephelometric method[11], which does not depend on the presence or absence of chitobiase[9]. In order to measure the chitobiase, the liberation of acetylglucosamine has been followed, using a preparation of 'depolymerized chitin' as substrate[12] (a preparation which contains chitobiose and other small polymers of acetylglucosamine).

Table 1. CHITINOLYTIC ENZYMES IN DIGESTIVE JUICES OF SOME VERTEBRATES

Species	Digestive juices or contents	Activity of chitinolytic system: acetylglucosamine liberated, μgm./hr./ml.
Carassius auratus L.	Anterior intestine	113
	Posterior intestine	814
Gasterosteus aculeatus L.	Whole digestive contents	872
Lacerta viridis Laur.	Gastric juice	1,200
Testudo hermanni J. F. Gmelin	Gastric juice	0
	Intestinal contents	0
Passer domesticus L.	Gastric juice	180
Turdus merula L.	Gastric juice	1,550
Oryctolagus cuniculus L.	Gastric contents	0
	Intestinal contents	2·5*
	Cæcal contents	0

* Non-significant result.

Table 2. DISTRIBUTION AND LOCALIZATION OF CHITINASE AND CHITOBIASE IN DIGESTIVE GLANDS AND TISSUES OF SOME VERTEBRATES

Species	Organs	Activity = acetylglucosamine liberated, μgm./hr./gm. fresh tissues		
		Chitinolytic system*	Chitinase†	Chitobiase‡
Carassius auratus L.	Mucosa anterior intestine	43–88	490	440
	Mucosa posterior intestine	288	—	72
	Pancreas (+ Liver)	—	67	62
Lacerta viridis Laur.	Gastric mucosa	1437–1570	8,064	250–740
	Intestine	0–3·3§	—	45
	Pancreas	810–1350	13,120	0–160
Testudo hermanni J. M. Gmelin	Gastric mucosa	0	0	45
	Intestinal mucosa	0	0	57
Turdus merula L.	Mucosa of glandular stomach	340	3,114	89
	Intestine (first third)	26	144	56
	Pancreas	13§	13§	0
Rhinolophus ferrum-equinum Schreb.	Gastric mucosa	347	5,280	71
	Intestine	0	12§	23
	Pancreas	0	0	29
Oryctolagus cuniculus L.	Gastric mucosa	0	0	0
	Intestine	0	0	70
	Pancreas	0	7·8§	72

* Liberation of acetylglucosamine from chitin, by the enzymic extract.
† Liberation of acetylglucosamine from chitin, by the enzymic extract after addition of an excess of chitobiase.
‡ Liberation of acetylglucosamine from 'depolymerized chitin', a preparation containing chitobiose and other small polymers of acetylglucosamine.
§ Non-significant results.

The results are summarized in Tables 1 and 2. Chitinolytic enzymes have been found in the digestive tract of six species of vertebrates, among the eight species so far investigated, the digestive juices and tissues of turtle and rabbit being entirely devoid of chitinases. In all these eight species, muscles, kidney and liver extracts were found to be free from chitinases, the presence of chitobiase only being evident in the livers of *Lacerta* and *Testudo*.

The following tentative conclusions may be drawn from these results:

(1) The chitinolytic enzymes have been found not only in the digestive contents, but also in glands and

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in carefully washed gastric or intestinal mucosa. Such localization does not support the hypothesis of a bacterial origin.

(2) All the species where chitinase has been found are insectivorous, or eat chitin-covered preys, at least occasionally. In contrast, the turtle and the rabbit, which do not possess any trace of chitinase secretion, are typical phytophagous animals. This observation, if confirmed for other species, would represent the only clear-cut case, with that of lactase, of a correlation between diet and digestive enzymes among vertebrates.

(3) The site of chitinase secretion seems to show an evolution towards a higher specialization of tissues: in the goldfish, the secretion of chitinase is a property of the whole epithelium and of pancreatic islets. This faculty is restricted to the gastric mucosa and the pancreas in *Lacerta* : in bats, the gastric mucosa alone has this property.

In contrast, the secretion of chitobiase, although often very weak, occurs in the majority of tissues, even in the turtle and in the rabbit.

(4) The cases of the pancreas of *Lacerta* and of the gastric mucosa of *Turdus* and of *Rhinolophus* are the first known examples of tissues being able to synthesize large amounts of chitinase, but secreting only very small amounts of chitobiase.

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