CHITIN DIGESTION IN NESTLING LEACH'S STORM PETRELS, OCEANODROMA LEUCORHOA

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Chitin in crustacean exoskeletons represents a substantial source of energy for marine predators. Estimates of krill biomass, for example, range from 80 to 500 million metric tons, corresponding with 16 to 100 million metric tons of chitin, the major skeletal component. For this reason, chitin digestion in fish has long been studied (eg. Okutani Bulletin of the Misaki Marine Biological Institute, Kyoto University. 10:1-47, 1966; Fange et al. Marine Biology 36:277-282, 1976; Rehbein et al. Comp. Biochem. Physiol. 85A:545-551, 1986; Seiderer et al. Mar. Ecol. Prog. Ser. 35:15-23, 1987). Most forms of chitin are tough, highly hydrophobic, insoluble in most ordinary solvents, and relatively inert to biodegradation. The ability to degrade chitin involves the action of two enzymes, chitinase (E.C. 3.2.1.14) and chitobiase (β-N-acetyl-D-glucosaminidase, E.C. 3.2.1.30) (Jeuniaux Nature 192:135, 1961). Chitinase hydrolyses chitin to the repeating subunit chitobiose, the β-1,4 linked dimer of N-acetyl-D-glucosamine. Chitobiase hydrolyses the dimer to N-acetyl-D-glucosamine.

Seabirds such as Leach's storm petrel which feed on crustaceans in the North Atlantic would benefit greatly from the ability to tap the considerable energy source represented by the exoskeletons of their prey. Although a study of microbial and vertebrate chitin degradation by crabeater seals and Adelie penguins is currently under way (Staley Antarctic Journal of the U.S. 21(3):5, 1986), I am not aware of any published results indicating levels of chitinase activity in seabird guts. Our study reports the results of chitinase assays on stomach contents and aspirates from Leach's storm petrel chicks. We also report on a preliminary chitin balance study, providing apparent digestibilities of chitin for Leach's storm petrel. We also demonstrate in this species the capacity to absorb the hydrolysis products of chitin.

Chitinase activity determinations using radioactive chitin (Cabib Meth. Enzymol. 161: 424-430) were performed at 42 ^{o}C with shaking. The incubation mixture contained, in a total volume of 100 µl, 0.05 M buffer (depending on the pH: sodium phosphate, pH 6.3, sodium acetate, pH 4.1, KCl/HCl pH 1.0 and 2.0), 3.6 mg/ml of [^{3}H] chitin (0.5µCi/mg) and extract. After incubation, 0.3 ml of 10% (w/v) trichloroacetic acid was added and the suspensions were centrifuged for 5 min at 200 g. With a micropipet, 200 µl of the supernatant fluid were carefully removed and transferred to a scintillation vial for radioactivity determination. Protein content of the samples was determined using the method of Bradford

(1976) and final enzyme activity values were expressed as µmoles chitobiose (mg protein)$^{-1}$ (h)$^{-1}$.

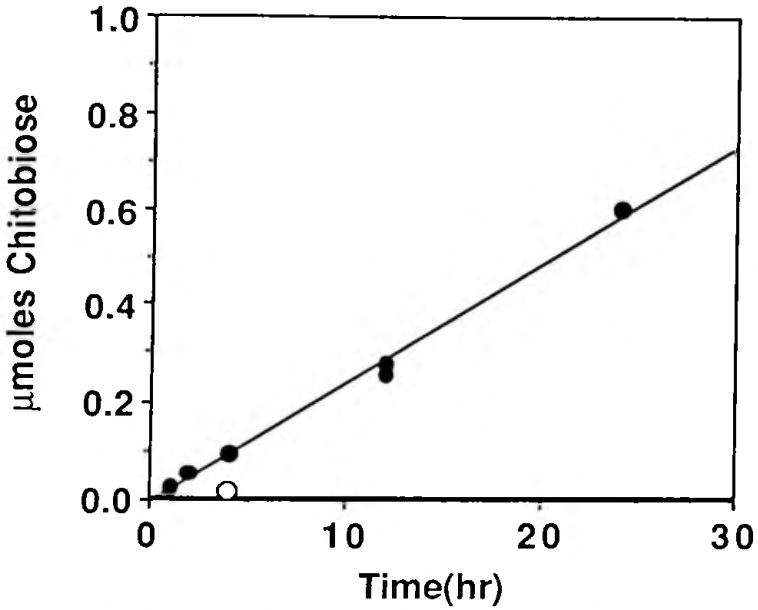

Figure 1. Chitinolysis by proventricular aspirates from Leach's storm petrel chicks. A tritiated chitin suspension was incubated with 1 µg of secreted proventricular protein at 42 °C in a HCL/KCl buffer, pH 2.00. The open symbol represents the activity observed for an aspirate heated to 100 °C prior to incubation. Each point represents the mean value of three replicates. The standard deviation of each mean is contained within the width of the symbol. The observed specific activity was 245 µmoles chitobiose mg^{-1} hr^{-1} (R^2 = 0.994).

Chitinase has been demonstrated in insectivorous birds but is absent in two graminivores, the pigeon Columba palumbus and parrot Psittacus erithacus (Jeuniaux, '61;63; Jeuniaux and Cornelius '78). The main source of chitinase in these species is the mucosa of the proventriculus and in the eight species examined activities ranged from 1350-61,650 mg NAG liberated h^{-1} g^{-1} fresh weight The greatest level was in the starling Sturnus vulgaris, the lowest in the chicken. Jeuniaux (1963) found lower levels of chitinase in the lumen contents of the gizzard and intestine and even lower levels in the intestinal mucosa. These gastric chitinases have an optimum pH of 4.7-5.4 and retain considerable activity at lower pH but above pH 6 activity declines sharply. The preliminary characterization of the proventricular chitinase of Leach's storm petrel chicks shows a lower pH optimum and a higher specific activity than observed with other birds.

Chitobiase, which I have not characterized in Leach's storm petrel, has a predominantly cavital distribution in the five species examined by Jeuniaux (1963). Jeuniaux and Cornelius

(1978) conclude, owing to low chitobiase concentration in all vertebrate species they examined, that the metabolic utilization of chitin hydrolyltic products by vertebrates is uncertain. There is, however, no direct evidence to support this conclusion.

Chitin apparent digestibilities were determined by feeding chicks meals containing known quantities of chitin. Twelve chicks were fasted 48 h before receiving a meal of 6-8% bird body mass (wet) of krill. The birds were fed this meal for two weeks, and all faeces voided since feeding collected separately for each bird. Faeces and samples of food were dried at 45°C for five days, and then weighed. The chitin content of food and faeces samples was determined using the method for crude fiber determination in animal feeds described by Horwitz et al. (1975). Apparent digestibilities of chitin for individual birds were calculated as percentages using the formula:

$$((\text{chitin in} - \text{chitin out})/\ \text{chitin in}) \times 100,$$

where "chitin in" and "chitin out" are total dry masses (g) of chitin in the food and the faeces, respectively. Apparent chitin digestibilites of 35 ± 12.0 % (N =12) were observed with Leach's storm petrel chicks.

Absorption efficiencies were performed using homogenized krill meals warmed to 37 °C and administered with a disposable 5 cm^3 syringe attached to a 10 cm length of polyethylene tubing inserted into the esophagous of Leach's storm petrel chicks (Ages = 45-60 days). All chicks took the feeding without regurgitation. Each bird was fed a meal containing 0.2 mmoles of N-acetyl glucosamine or glucosamine (equivalent to the total amount contained as chitin in the meal), 10 µCi of [^{3}H]-N-acetyl glucosamine or [^{3}H]-glucosamine and 5 µCi [^{14}C]-PEG-4000 with 50 mg of unlabeled PEG-4000 as carrier. After ingestion, each chick was placed on a polyethylene mesh (1/4") platform suspended in a 2 gallon Bain Marie polyethylene container (Cole-Palmer) to collect excreta. Containers were kept in the dark and maintained at 14 ± 3 °C to simulate the nest environment as much as possible. Accumulated excreta in each container were extracted with 50 cc of distilled water and homogenized with a Polytron homogenizer to uniform composition. Absorption of [^{3}H]-N-acetyl glucosamine or [^{3}H]-glucosamine was calculated from the ratio of [^{3}H] to [^{14}C] in the daily fecal collection by the formula:

$$\%\ \text{absorbed} = 1 - [^3\text{H}/^{14}\text{C in test meal per } ^3\text{H}/^{14}\text{C in daily fecal collection}]) \times 100$$

As apparent from the data presented in Figure 2, Leach's storm petrel chicks have limited absorption capacity for the amino sugars of glucose, the hydrolysis products of chitin.

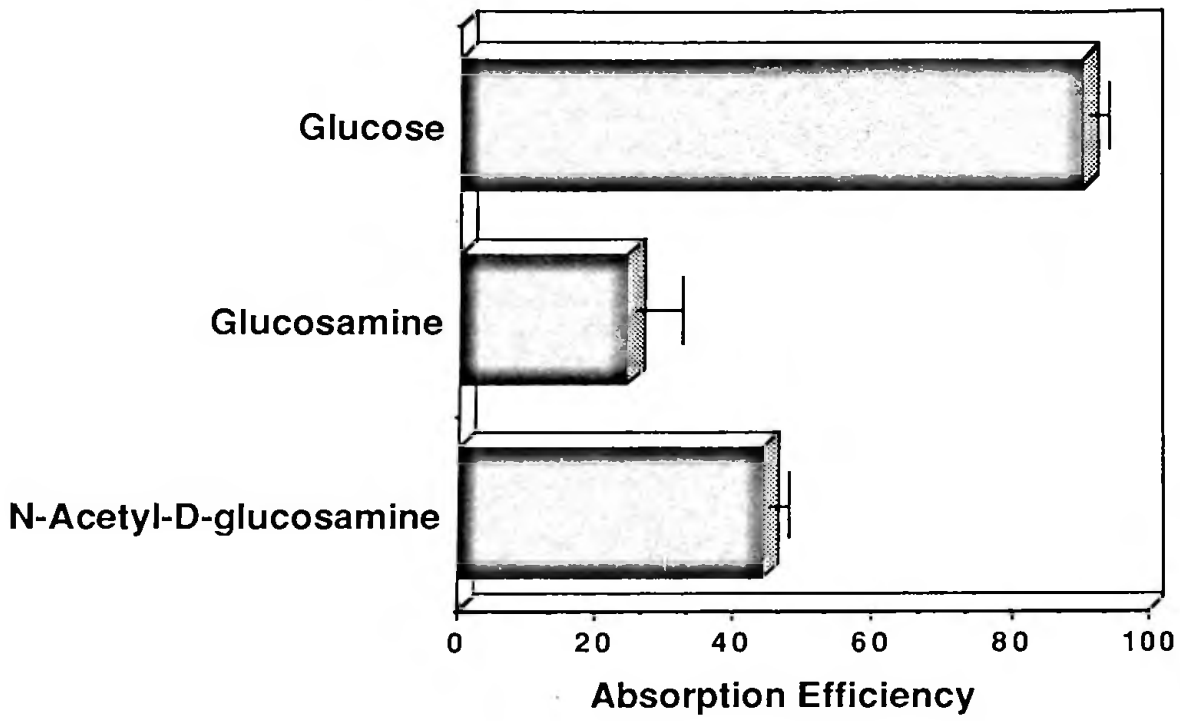

Figure 2. Absorption efficiency of glucose and its amino sugar derivatives by Leach's storm petrel chicks.

In conclusion, we find that chicks of Leach's storm-petrels have the capablity of assimilating a substantial portion of ingested chitin. Whether the chicks do indeed accrue substantial energy and carbon from the carapaces of ingested crustaceans will have to await future studies utilizing radiocarbon labeled chitin.

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