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Symbiotic Cellulose Degradation in Green Turtles, *Chelonia mydas* L.

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A postgastric, fermentative breakdown of structural plant tissue was demonstrated for green turtles. About 90% cellulose was hydrolyzed. Bacterial and protozoan numbers compared with those of the rumen.

Herbivory in mammals is associated with symbiotic microbial degradation of structural carbohydrates by bacterial symbionts found in a specialized part of the digestive tube, either in front of the stomach (pregastric fermentation, e.g., in ruminants) or in a cecum (postgastric fermentation, e.g., in perissodactyls). In both cases, fermenting bacteria break down carbohydrate substrates to volatile fatty acids which are absorbed and utilized by the host, itself incapable of producing enzymes which can hydrolyze the bulk material of the food. Symbiotic cellulose degradation is now established in many groups of mammals and in some birds (2, 6, 7, 9, 10). Here we give evidence of a postgastric symbiotic degradation of structural plant compounds in a reptile: the marine turtle, *Chelonia mydas* L., which belongs among the few consumers of sea grasses (otherwise restricted to dugongs, manatees, and a few species of waterfowl, fish, and sea urchins; see 8). The ability of captive green turtles to break down cellulose has previously been demonstrated by Karen Bjorndal (personal communication).

During a cruise with *Alpha Helix* in the Caribean Sea in October 1977, a live female green turtle (170 kg; carapace length, 105 cm) was purchased from fishermen at Miskito Cays off Nicaragua. The animal was kept on board alive, lying on its back for 36 h. It was then anesthetized with chloroform and killed by cutting the carotid arteries. The ventral carapace was removed and the intestinal tract was dissected out. For measurements of pH and Eh of the intestinal contents electrodes were inserted through small incisions into the gut wall.

To demonstrate cellulolytic and amylolytic activity in the gut contents, 10-ml samples were ground with sand in 1 M phosphate buffer (pH 6.8) and filtered through nuclepore filters (0.45 μm). The filtrate was made up to 20 ml with buffer and incubated with crystalline cellulose or potato starch and a drop of xylene at 32°C. In addition, controls without substrate or without the crude enzyme extract were incubated. After 19 and 32 h, samples were tested for reducing sugar (11).

To demonstrate the microbial degradation of structural plant material, 1-ml samples of intestinal contents were incubated in 15 ml of anoxic seawater and a spatula (balances could not be used on board) of homogenously ^{14}C-labeled barley hay (particle size, about 0.5 mm; specific activity, 33 μCi g^{-1}; total carbohydrate, 65%). The containers were closed with stoppers, avoiding a gas phase, and incubated for 41 h at 32°C. Thereafter the samples were acidified with HCl, and CO_2 was collected in a CO_2 absorbant. They were then centrifuged (20,000 rpm for 30 min), and the supernatant was filtered through nuclepore filters (0.45 μm). The radioactivity of all fractions, CO_2, filtrate, filters, and precipitate (after dissolving in concentrated NaOH) was measured by liquid scintillation counting and corrected for quenching with an internal standard. Protozoa were quantified microscopically on board on fresh samples.

The following observations were carried out on formaldehyde-fixed material (bacterial counts) or oven-dried (110°C) samples. Bacteria were counted (5). Organic carbon, ash, Si, total carbohydrate, cellulose, and protein were quantified (3, 12).

The total length of the intestinal tract was 18.5 m. The esophagus measured 10 cm from the anterior incision; it was followed by a well-defined, 30-cm-long stomach. The different intestinal sections posterior to the stomach (small intestine, cecum, and colon; see 4) were ill defined, and we did not observe the ileocolic valve. Presumably the small intestine corresponds to the first 4 m and the hindgut corresponds to the

last 10 m. The ratio between the total intestinal length and body length of the green turtle exceed that recorded for other reptiles (4) in accordance with the herbivorous habit of the adult animal.

The intestine contained 13.3 liters of material. With the exception of a short section of the small intestine, which contained unidentified animal tissue, the gut exclusively contained remains of seagrasses, dominantly or exclusively *Syringodium filiforme,* which grew abundantly in the capture area. Until about 7 m from the stomach the intestinal content contained much fluid; thereafter, it became more densely packed and drier. After about 4 m microscopic observations showed a progressive degradation of the plant tissue.

The pH of the stomach was low (4.5 to 4.9), indicating that ingested food is first subjected to acid digestion. Within the first 25 to 100 cm of the small intestine, the pH increased to values around 6.8. From 5 to 6 m from the stomach the pH fell again (to 5.5 to 6.0), presumably in conjunction with the production of fatty acids. In the colon the pH slowly increased again to values around 6. The contents of the esophagus and the stomach were oxidizing or moderately reducing (Eh, +280 and −30 mV, respectively). In the small intestine the Eh fell, to reach very low values at 4 to 6 m (−350 mV), indicating strictly anoxic conditions. The low redox potential of the cecum is typical of values reported for the cow rumen (6, 7).

The sections after the small intestine contain bacteria as well as a protozoan fauna. The latter consists of a hitherto undescribed trichostome ciliate and at least three species of unidentified zooflagellates, two of which belong to the genera *Trigonomonas* and *Karotomorpha.* Direct counts of bacteria with fluorescence microscopy are minimum values since it was not possible to detach all bacteria from particles. High concentrations of bacteria appear from about 4 m after the stomach (about 10^{10} ml^{-1}). Thereafter they show a minimum (6×10^9 ml^{-1}) and increase again to 1.7×10^{10} ml^{-1}. Through the colon their numbers then slowly decrease. The minimum density of bacteria corresponds to the maximum density of protozoa (Fig. 1). Concentrations of bacteria and protozoa compare well with those of the cow rumen (6, 7). Direct observations of sea grass particles revealed a dense cover of bacteria along the fibers of the more or less degraded plant material.

The content of the small intestine (samples 75 cm from the stomach) showed a weak amylase but no cellulase activity. Significant enzymatic hydrolysis of both substrates could be detected in samples from 5 and 10.5 m from the stomach.

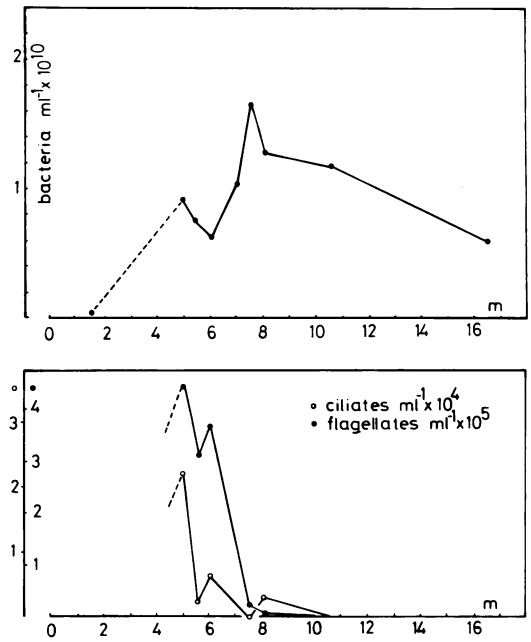

FIG. 1. *Counts of bacteria and protozoa from the intestinal content. (Abscissa) Distance from the beginning of the small intestine. Very few bacteria and no protozoa were found in the small intestine (0 to 5 m).*

The incubation with ^{14}C-labeled hay indicated a degradation of about 35, 41, 42, and 21% of the material (in terms of carbon atoms) in samples from 5.5, 6, 7, and 8 m from the stomach, respectively. Of the carbon of the degraded material, 70 to 80% was found in solution (presumably as volatile fatty acids), material remaining on the filters (assumed mainly to be bacteria) constituted 17 to 18%, and CO_2 constituted 3 to 12%. Any CH_4 produced during the incubation would have been lost with the fractionation methods used. The proportions of the end products of the degradation (volatile fatty acids–bacterial cells–CO_2) are roughly in agreement with that found in the rumen (6, 7). The dilution of the gut content with seawater and the primitive fractionation procedure used on the ship make a quantitative estimate of the degradation rate questionable, but the experiment does qualitatively demonstrate the microbial degradation of structural plant material.

In the stomach and the anterior part of the small intestine, organic carbon, total carbohydrate, cellulose, and protein constituted 25 to 34, 24 to 27, 12 to 16, and 3 to 6% of the dry weight, respectively, in accordance with the dominating role of vascular plant tissue in the diet. In the colon, the corresponding figures were 23, 9 to 11,

3 to 4, and 5 to 10%. The substrate-silica ratios for the different components are shown in Fig. 2. Comparisons between the values for the stomach and the posterior colon show that organic material, total carbohydrate, and cellulose are broken down with efficiencies of about 78, 92, and 95% respectively. The high efficiency of cellulose breakdown compares with the upper range of efficiencies measured in ruminants and in the dugong (6, 7, 10).

Estimates of protein content varied according to the analytical method used (Folin [Lowry] or fluorescamine). Results suggest that protein is hydrolyzed in the stomach and the resulting amino acids are absorbed in the small intestine. Additional protein seems to be synthesized after about 7 m, presumably by bacteria with ammonia as their nitrogen source, and some of this protein is hydrolyzed in the posterior colon. The observed increase in protein-silica ratio corresponds to values of 2 or 10 mg of protein per g (dry weight), not inconsistent with a production of 1×10^{10} to 2×10^{10} bacteria per ml of intestinal contents. However, the protein distribution does not quite parallel that of the bacteria.

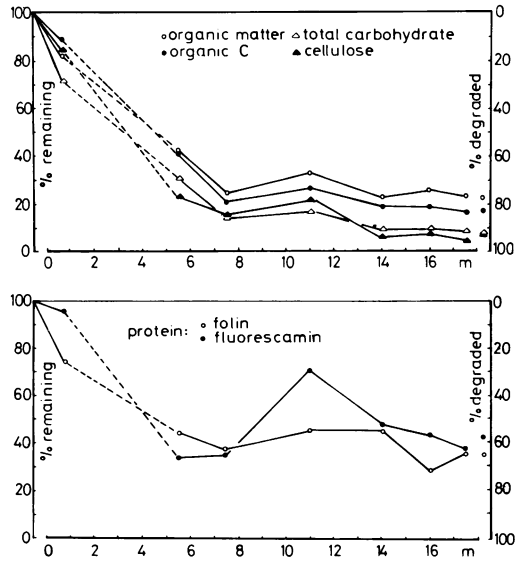

FIG. 2. *Ratios between different constituents of the intestinal content and Si in different portions of the gut. The ratios for the stomach content have arbitrarily been set to 100. (Abscissa) Distance from beginning of the small intestine.*

Estimates of the quantitative uptake of organic carbon are very unreliable. During the period the animal was kept alive it produced fecal material corresponding to 11% of the intestinal contents, indicating an intestinal turnover time of 13 days or an ingestion of somewhat less than 1 kg of sea grass (wet weight) per day. Of this material some 100 to 150 g of organic carbon is assimilated, of which 20 to 25% derives from cellulose. However, that the animal was lying on its back and not allowed to feed during the 36 h may have resulted in a considerable decrease in the defecation rate and a corresponding underestimate of the intestinal turnover.

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