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# Aristotle revisited: The function of pyloric caeca in fish

(intestine/nutrient uptake/digestion/adaptation)

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ABSTRACT **The function of the pyloric caeca of fish has been uncertain since their detailed description in 345 B.C. by Aristotle. He suggested three hypotheses about their function: "to store up the food," "putrify it up," and "concoct it" (i.e., storage, fermentation, and digestion). Our results for trout, cod, largemouth bass, and striped bass support the third but not the first or second of Aristotle's theories. In all four species, the caeca prove to be a major site of sugar, amino acid, and dipeptide uptake, contributing more uptake than the entire remaining alimentary tract in trout and cod. Caecal brush-border membranes contain hydrolytic enzymes. X-ray plates taken at various times after trout had ingested radioopaque marker, and observations of trout fed blue dye plus glass beads of graded sizes, show that caeca fill and empty of food with the same time course as proximal intestine. Thus, whereas the caeca of mammals and birds serve as fermentation chambers, fish caeca are an adaptation to increase gut surface area.**

Along the proximal intestine of many fish species are blind diverticula termed pyloric caeca. Over 2000 years ago, Aristotle (1) described them in detail, recognized their distinction from the distally placed intestinal caeca of birds and mammals, and speculated that their function was "to store up the food as it might be in additional cellars and there putrify it up and concoct it." Modern speculations about function (2–4) have also focused on food storage, fermentation ("putrify it up"), and digestion ("concoct it"). Despite this long history in the scientific literature, the functions of the caeca have remained unclear. Following Collie's recent finding (5) of nutrient uptake by caeca of coho salmon, we have now determined the contributions of the caeca to the gut's total surface area and to its total absorptive capacity for sugar and amino acids in four fish species. We also measured particle sieving and food residence times in caeca of one species, to assess their proposed fermentative and storage roles. The caeca prove to be an absorptive site of hitherto unrecognized major significance.

## MATERIALS AND METHODS

**Fish Species and Their Caecal Morphology.** The four fish species chosen were cod, rainbow trout, largemouth bass, and striped bass, which differ nearly 40-fold in mean number of caeca (222, 56, 25, and 6, respectively). However, these species are very similar in mean diameter (1.2, 1.9, 1.2, and 2.1 mm, respectively), length (2.5, 1.9, 2.5, and 2.0 cm), and thickness (0.8, 0.7, 0.8, and 1.0 mm) of a caecum and also in mean relative length of the intestine apart from the caeca (intestinal length/fork length: 0.72, 0.49, 0.76, 0.46; fork length is the distance from the snout to the fork of the tail). Thus, differences in number of caeca are the main reason why these species differ 15-fold in mean relative length of the whole gut (= intestine + caeca; gut length/fork length, 10.3, 3.9, 2.7, and 0.67, respectively). The trout, largemouth bass, and striped bass were maintained in the laboratory in a semiclosed, recirculating fresh-water system, whereas the cod were held in a flow-through marine system. The trout and largemouth bass were fed a commercial trout chow (Silvercup, Murray Elevators, Utah) and tadpoles, respectively, whereas the wild-caught cod and striped bass were not fed and were used within 2 days of their capture.

**Physiological Measurements.** To establish the rate at which caeca fill and empty of food, we fed gelatin capsules containing equal parts by weight of powdered commercial trout feed and the radioopaque marker $BaSO_4$. At various times after ingestion, we lightly anesthetized a fish with tricaine methanesulfonate (MS-222) and removed it from the water for 10–20 sec to prepare an x-ray plate.

On dissection of recently fed fish, we noticed food particles in the intestine, but the caecal contents appeared fluid. To determine the upper size limit of particles that can enter the caeca, we fed blue gelatin capsules containing equal parts by volume of powdered trout feed and a mixture of glass beads ranging in size from 10 to 1500 $\mu$m (Sigma) to three trout. Five hours later, at a time when the x-ray studies indicated maximal filling of the caeca, we killed the fish, excised the intestine and caeca, and recorded sizes of beads in the different regions.

To detect membrane-bound hydrolytic enzymes, we used a calcium precipitation technique (6) for preparation of brush-border membrane vesicles from guts of four trout. Hydrolysis rates (nmol of substrate hydrolyzed per min, g of tissue, measured as in ref. 7) were determined for the three dissaccharides maltose, sucrose, and trehalose.

The capacities of the caeca and intestine to absorb the products of enzymatic hydrolysis were measured by an *in vitro* method, the everted sleeve technique (8–11). Briefly, we everted and cut cylindrical sleeves 1 cm long from the intestine and ceaca, tied a sleeve over a grooved solid rod of a diameter chosen to fit the sleeve lumen snugly, and measured solute uptakes across the brush-border membrane. Tissues thus mounted were preincubated for 5 min in Ringer's solution at the experimental temperature (20°C) before being suspended over a stirring bar rotating at 1200 rpm (to minimize unstirred layer effects) in solutions containing a sugar or amino acid at 25 mM plus the $^{14}C$ tracer of the same nutrient and L-[$^3$H]glucose or polyethylene glycol (PEG). Solution composition (in mM) was 117 NaCl, 5.8 KCl, 25 $NaHCO_3$, 1.2 $NaH_2PO_4$, 2.5 $CaCl_2$, 1.2 $MgSO_4$ (pH 7.4; gassed with 95% $O_2$/5% $CO_2$).

After a 4-min incubation the sleeve was removed from the rod, weighed wet, and solubilized, and radioactivity was counted in a dual-channel scintillation counter to determine nutrient uptake into the tissue. L-[$^3$H]Glucose served to correct measured D-[$^{14}$C]glucose uptakes for glucose in adherent fluid and for passive uptake (thereby yielding active D-glucose uptake), whereas [$^3$H]PEG served to correct mea-

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sured $^{14}C$-labeled amino acid uptakes for amino acid in the adherent fluid (8).

## RESULTS

**Food Transit.** As illustrated in Fig. 1, some feed with radioopaque marker entered the caeca and proximal intestine from the stomach by 2 hr. Most of the caeca (as well as the proximal intestine) contained marker by 5 hr. By 15 hr, the stomach, proximal intestine, and caeca were evacuated, and marker was confined to distal intestine. Thus, food exchange between caeca and proximal intestine is rapid, and the caeca do not serve to store food, as their small volume also implies. These short residence times of food in the caeca, combined with their small volume, lack of a resident bacterial population (12), lack of an entrance valve, and generally greater development in carnivorous than herbivorous fish, also make a role in fermenting plant fiber unlikely for the pyloric caeca. (Fermentation of fiber is the major role of mammalian and avian caeca, which are best developed in herbivores.)

**Particle Sieving.** All sizes of beads were found in the intestine, but caeca that had filled (as proved by the presence of blue dye) contained no beads larger than 150 μm and mostly beads of 100 μm or smaller. Thus, large particles cannot enter the caeca, probably because their lumina are partly obstructed by villi.

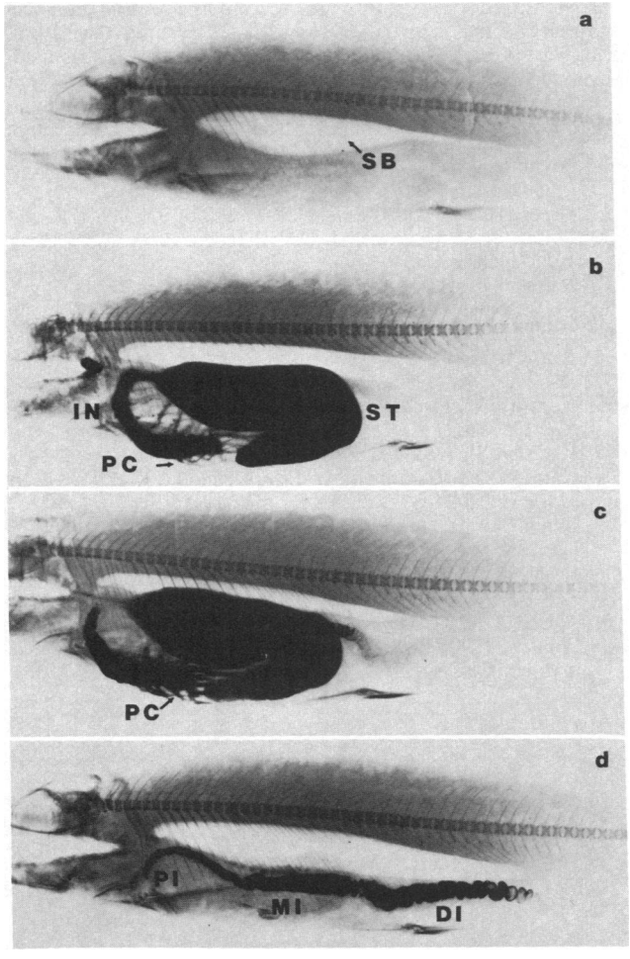


FIG. 1. X-ray photographs of a trout before (*a*) and at 2 (*b*), 5 (*c*), and 17 (*d*) hr after ingestion of radioopaque marker. Note that the caeca fill and then empty with a time course similar to that for proximal intestine. DI, distal intestine; IN, intestine; MI, middle intestine; PC, pyloric caeca; PI, proximal intestine; SB, swim bladder; ST, stomach. (Magnification, ×0.4.)

**Disaccharidase Activities.** Rates of hydrolysis by brush-border membranes of trout caeca and proximal intestine, respectively, were 240 and 190, 91 and 61, and 21 and 24 nmol of substrate hydrolyzed per min, g of tissue for the disaccharides trehalose, maltose, and sucrose, respectively—that is, enzyme activities of the caeca and proximal intestine are similar. The activity sequence among the three disaccharidases reflects the relative amounts of their substrates in trout's natural diets or in the laboratory ration that we provided. Levels of membrane-bound dipeptidase activity in the caeca and adjacent intestine of trout are also similar (13). Thus, caeca are capable of enzymatic hydrolysis.

**Nutrient Uptake Capacities.** We measured uptakes of D-glucose and L-proline in all four fish species and uptakes of nine amino acids (named in Fig. 2) plus the dipeptide carnosine in trout. Uptakes per mg of tissue in the caeca and in proximal intestine agreed within a factor of 2 for all fish species and all solutes, except that the factor was 2.1–2.3 for glucose in largemouth bass and for glycine and phenylalanine in trout. As for the direction of these small differences, uptake in the caeca exceeded that in proximal intestine in 16 of 18 comparisons of the same solute in the same species—probably because a higher proportion of tissue weight in caeca than intestine consists of absorptive mucosa rather than muscle.

Proline and glucose uptakes usually decreased severalfold from proximally to distally along the intestine in all four species. In trout we compared uptakes in proximal and distal sleeves of individual caeca and obtained ratios not differing significantly from 1.0, both for proline and for glucose. Thus, the caeca are longitudinally homogenous in uptake and are most similar to proximal intestine, the region of intestine to which they are attached.

To evaluate the caeca's contribution to the total uptake capacity of the gut, we calculated integrated uptake capacities for each gut region (caeca plus proximal, middle, and distal intestine) by calculating uptakes per cm of sleeve length and multiplying by the total length of the region (10). Fig. 2 depicts, for each fish species and each solute, the proportion of the gut's total uptake capacity that arises from the caeca. Also shown for comparison are the contributions of the caeca to the total surface area of the gut for the four species. For each species and each solute, the caeca usually contribute in similar proportion to that solute's uptake and to gut area. (The proportions agree within a factor of 1.2 in every case except for glucose in largemouth bass, for which the caeca's contribution to uptake is somewhat out of proportion to their area.) However, since the four species differ greatly in number of caeca, the caeca's contribution to gut area (hence to uptakes) varies from 70% in trout and 69% in cod to 42% in largemouth bass and only 12% in striped bass, which has the fewest caeca. In tuna, the caeca's contribution to surface area is even higher, probably >90%.

## CONCLUSIONS

Aristotle's descriptions of food ingestion and caecal anatomy in many fish species were detailed and accurate. His three hypotheses about caecal function were also reasonable ones for their time. The advent of x-ray and radioactive tracer techniques unavailable to Aristotle now argue against two of his hypotheses. The pyloric caeca are neither storage organs ("additional cellars . . . to store up the food") nor fermentation chambers for plant fiber ("there putrify it up"). However, Aristotle's third hypothesis is supported: the caeca do "concoct" food (i.e., digest it enzymatically and absorb it). Specifically, the caeca serve as an adaptation to increase the surface area and hence the nutrient uptake capacity of fish

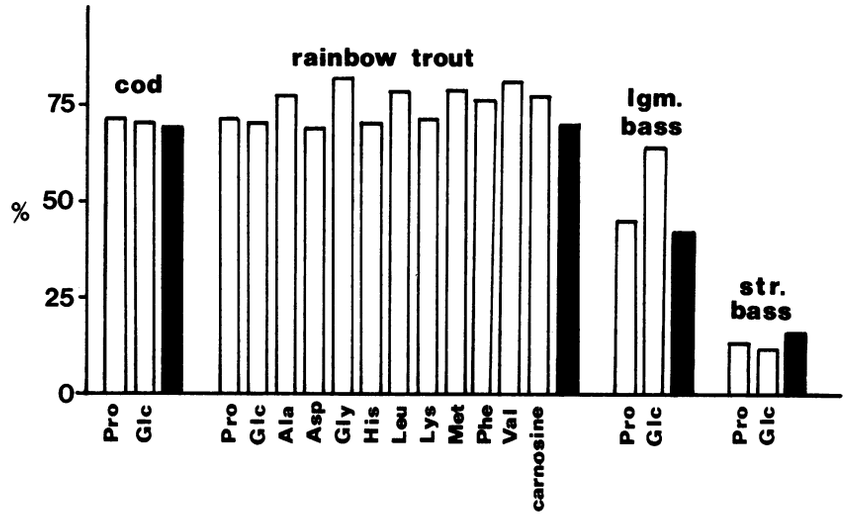


FIG. 2. Percentages that the caeca contribute to the gut's integrated uptake capacity for the named solute (open bars) or to the gut's surface area (filled bars) in the named fish species. "Gut" = intestine plus caeca. Note that the percentages for uptake capacity and area are similar in a given species and that these percentages decrease in the sequence cod ≈ trout > largemouth (lgm.) bass > striped (str.) bass, due to species differences in number of caeca.

gut. In trout and cod they account for more uptake capacity than all remaining regions of the gut combined.

Recall that our four fish species are similar in intestinal area but very different in caecal area and that many other fish species have no caeca at all. One might therefore expect to find corresponding species differences in nutrient extraction efficiency. In fact, fish species differ negligibly in extraction efficiency when compared while eating the same high-quality diet (14). This suggests that species with few or no caeca compensate by achieving similar quantities of absorptive tissue in some different manner. Mucosal thickening is evidently a major alternative adaptation. Among our four species, striped bass have by far the fewest caeca but the thickest gut (11). We also measured (11) intestinal thicknesses and gut lengths relative to fork lengths in four fish species lacking caeca: channel catfish, 1.3 mm and 1.6, respectively; grass carp, 1.2 mm and 1.9; carp, 0.9 mm and 2.0; tilapia, 0.5 mm and 5.8. The sequence of thickness is the inverse of the sequence of length—that is, among species without caeca a thicker intestine compensates for a short intestine. As a result of this compensation, when we compare fish of similar body weight, all species that we studied have gut weights agreeing within a factor of 2, even though relative gut length varies 14-fold, gut thickness varies 6-fold, and number of caeca varies from 0 to 222. Thus, long intestines, thick intestines, and development of caeca are alternative adaptations for achieving a similar mass of absorptive tissue in fish.

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