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Oxalate Degradation by Microbes of the Large Bowel of Herbivores: The Effect of Dietary Oxalate

Abstract. *Rates of oxalate degradation by microbes in gastrointestinal contents from rabbits, guinea pigs, swine, and a horse increased after addition of oxalate to diets. A similar response was previously observed with ruminal microbes from cattle and sheep. Bacteria that utilize oxalate for growth appear to be selected by increased levels of dietary oxalate.*

Increased rates of oxalate degradation by ruminal microbes account for ruminant adaptation to high levels of dietary oxalate (*1*). Adapted ruminants tolerate quantities of oxalate that would be lethal to nonadapted animals (*2*). The experiments reported here show that high levels of dietary oxalate also induce increases in rates of oxalate degradation by microbes in the large bowel of various nonruminant herbivores.

Low levels of oxalic acid are found in many plants that are important in the diets of man and other animals (*3*). Problems due to ingested oxalate arise mainly when: (i) foods containing much higher concentrations of oxalate abruptly become major components of the diet, or (ii) when gastrointestinal function is altered and a significantly increased proportion of the dietary oxalate is absorbed. Nephrolithiasis resulting from increased oxalate absorption is a frequent and serious complication of inflammatory small bowel disease or resection of the terminal ileum (*4*).

Degradation of oxalate by gastrointestinal microbes from humans (*5*), swine (*6*), and certain rodents (*7*) has been shown, but degradation rates have not been measured and specific organisms responsible for this activity have not been identified. Although oxalate degradation by aerobic bacteria has been extensively studied (*3*), these organisms are unlikely to be important in an anaerobic ecosystem such as the rumen or large bowel. An anaerobic bacterium that degrades oxalate to CO_2 and formate was, however, recently isolated from rumen contents (*8*). This obligate anaerobe (now called strain OxB because it has not yet been placed taxonomically) is apparently unable to use any substrate other than oxalate for growth. This property could explain how increased amounts of dietary oxalate select for this organism and result in ruminal populations that degrade oxalate at greatly increased rates.

Laboratory animals were given free access to gradually increasing amounts of oxalate, the normal laboratory diet being replaced with ground *Halogeton glomeratus* (halogeton) plant material. The halogeton contained 14.7 percent oxalate, most of which was present as the soluble sodium salt (*3*). After 2 days with 5 percent of the diet as halogeton and 2 days with 10 percent halogeton, the halogeton was fed at a 20 percent level so that oxalate constituted 2.9 percent of the diet. The animals were killed 7 days after the start of halogeton feeding. Oxalate degradation rates were then estimated as described (*1*) by measurement of $^{14}CO_2$ produced in vitro during short-term incubation of the gut contents with ^{14}C-labeled oxalate.

Mean rates of oxalate degradation by microbes in cecal contents from rabbits and guinea pigs fed diets that contained halogeton were significantly ($P \leq .001$) greater than rates with cecal contents from animals fed the normal diets (Table 1). With cecal contents from white laboratory rats, however, oxalate degradation rates were very low in samples from animals fed either halogeton or the control diet, and conclusive evidence for selection of oxalate-degrading microbes in the rat cecum was not obtained.

When a pig (48 kg) with a surgically implanted cecal cannula was fed a diet containing 10 percent and later 20 percent halogeton, the rates of oxalate deg-

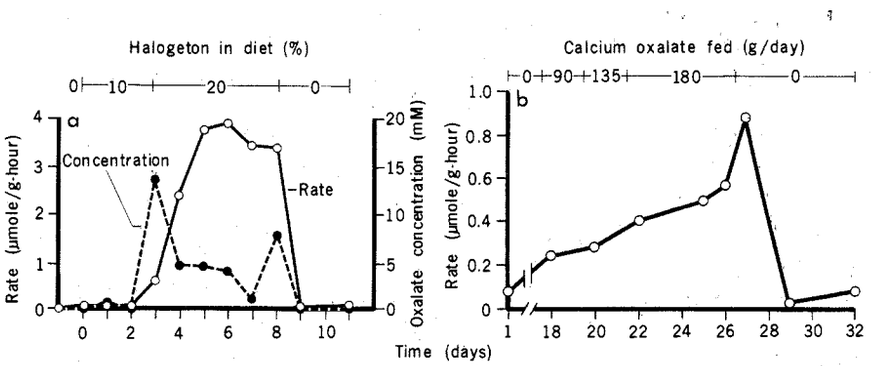

Fig. 1. Rates of oxalate degradation (solid lines) by microbes in (a) cecal contents from a pig and (b) rectal contents from a horse. Degradation rates were measured as in Table 1; except with pig cecal contents, ^{14}C-labeled oxalate was incubated with a filtrate that passed through muslin cloth. Soluble oxalate, measured by gas chromatography of the dibutyl ester (*1*), was not detected in rectal contents from the horse.

Table 1. Mean rates of oxalate degradation by microbes in cecal contents from laboratory animals. Tests for rabbits and guinea pigs were with cecal contents from individual animals (three on each diet), whereas with rats, cecal contents from two animals were pooled for each test (six animals on each diet). The reaction mixtures, which were incubated (in duplicate) under CO_2 for 1 hour at 37°C, consisted of 1.8 ml of a 1:4 dilution of cecal contents in an anaerobic dilution solution (*10*) plus 0.2 ml of 0.1*M* ^{14}C-oxalate (0.02 μCi/μmole). One milliliter of 1*N* NaOH was injected through the rubber stopper to stop each reaction. $^{14}CO_3^{2-}$ was measured by liquid scintillation counting after diffusion (*1*) into Carbo-sorb (Packard Instrument Co.). Controls included tubes stopped at 0 minute. Student's *t*-test was used to determine confidence intervals (logarithmic transformation) on the ratios of rates of oxalate degradation in halogeton diet to those in control diets.

Animal	Rate of oxalate degradation		95 percent confidence interval on ratio halogeton/control
	Control diet (μmole/g-hour)	Halogeton diet (μmole/g-hour)	
Rabbits	0.48	5.8	7.4 to 20.1
Guinea pigs	0.68	7.2	4.6 to 24.4
Rats	0.02	0.08	0.45 to 250

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radation by microbes in cecal contents increased (Fig. 1a). When halogeton was omitted from the diet, oxalate degradation rates again returned to pre-halogeton feeding levels. Similar results were obtained in another experiment with a different pig. In the latter experiment, rates of oxalate degradation by samples taken from the rectum were also measured. These rates were consistently higher than rates measured with samples taken concurrently from the cecum.

Rates of oxalate degradation by microbes in rectal contents from a horse (225 kg) increased after calcium oxalate was added to the diet, and then returned to low levels when calcium oxalate feeding was stopped (Fig. 1b). The highest rates measured were less than those in cecal samples from swine, rabbits, or guinea pigs. Oxalate degradation rates also increased in a similar experiment when halogeton rather than calcium oxalate was fed. In that experiment, halogeton intake was not well regulated because of problems associated with its palatability, and the data are not reported here.

Both CO_2 and formate are produced by oxalate decarboxylase (E.C. 4.1.1.2) from several species (*3*) and by strain OxB (*8*). When ^{14}C-labeled formate (10 m*M*) was incubated with gastrointestinal contents from each species studied here, rates of $^{14}CO_2$ production were much greater than oxalate degradation rates. Thus, ^{14}C from oxalate would not accumulate in formate, and we believe our measurements of $^{14}CO_2$ production from oxalate are reliable estimates of the potential or capacity for oxalate degradation by these populations.

Our measurements of oxalate degradation rates suggest that oxalate-degrading microbes are normally present in the large bowel of rabbits, guinea pigs, horses, and swine; and that concentrations of these oxalate degraders increase in response to the increased availability of oxalate. These results are similar to those obtained when increased dietary oxalate caused increased rates of oxalate degradation by ruminal microbes from sheep and cattle (*1*). It is likely that oxalate-utilizing bacteria are present in the gastrointestinal tracts of many animals.

Our inability to demonstrate selection of oxalate-degrading bacteria in the white rat agrees with results of Shirley and Schmidt-Nielsen (*7*). They found that significant quantities of ^{14}C from dietary ^{14}C-labeled oxalate were excreted as $^{14}CO_2$ by pack rats (*Neotoma albigula*), sand rats (*Psammomys obesus*), and hamsters (*Mesocricetus auratus*), but that this was not true with rats (*Rattus norvegicus*). The paucity of microbes with oxalate-degrading capacity in the intestine of the laboratory rat may be due to limited contact with other herbivores and a deficiency of oxalate (the substrate for the organisms) in the rat diet.

An isolate obtained by enrichment culture from cecal contents of an oxalate-adapted pig (*9*) appears to be identical to strain OxB obtained from the rumen. We propose that these bacteria or similar organisms are widely distributed and are more likely to be the agents of oxalate degradation in the large bowel than are any of the known bacteria that degrade oxalate under aerobic conditions.

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Dolphin Vocalization Mechanisms

Abstract. *Although humans have difficulty whistling when in a habitat that is more than 20 meters underwater, dolphins can make certain sounds at great depths through a related mechanism. Other dolphin sounds, such as clicks and complex buzzes, are produced by vibrations of the tissue of the nasal plugs, apparently without the use of the larynx; in these instances, the air sacs act as reservoirs. This was determined from studies of* Tursiops truncatus *and* Delphinus delphis *with harmless ultrasonic beams projected noninvasively to determine movements of the air sacs.*

About 340 B.C., Aristotle wrote that dolphins produce squeaks and moans (*1*). Since then various observers have described dolphin sounds as blats, bleats, chirps, clicks, creaks, pulses, quacks, racs, rasps, squeals, squawks, wails, and whistles (*2*). Considerable speculation exists about how small-toothed whales make sounds, such as simultaneous but independent whistles and clicks, without vocal cords and without blowing bubbles. Various mechanisms have been proposed (*3*), but a consensus has not been reached.

We projected into the heads of phonating dolphins narrow beams of low-intensity ultrasound at a frequency too high for them to hear; when aimed to reflect from moving surfaces of air spaces in the head, the sound returned was modified in frequency as measured by Doppler shifts. We thus determined which structures do and do not vibrate or otherwise move during sound production and modulation. We found, for example, that there are two general types of sounds, distinguished by whether or not tissue vibrations are involved in their generation, and that in dolphins the larynx does not seem to be involved in sound formation. Our procedures were noninvasive, harmless, did not disturb the animals, and could be done in or out of the water, thus leaving the acoustic situation normal.

Recently, the heads of dolphins have been explored by x-ray movies (*3*) and by pulsed ultrasonic imaging (*4*). Both imaging techniques involve periodic observations that are separated by insensitive intervals, and this intermittent "sampling" limits the rapidity of motion or the frequency that can be followed. Even a simple Doppler motion detector can follow the vibration of human vocal cords (*5*). To monitor motion in our studies, we used a modified commercial fetal heart monitor and its probe (*6*) as an ultrasonic source and receiver in some instances, and a special Doppler direction-resolving instrument (*7*) in other instances. The steady input of ultrasound at a frequency of 2 MHz is many times higher than the highest dolphin frequency; the subjects do not hear it directly but individual cycles of their sound movements can be recorded. The 2-MHz frequency produces a wavelength in soft tissue of 0.75 μm, and movements smaller than 10 μm can be observed in test

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