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10. Clark (*8*) showed experimentally that postautotomy tail movement propels tails through leaf litter and suggested that the tail may therefore move away from a predator and be retrieved by its owner. However, in more than 50 snake-lizard encounters that resulted in tail autotomy, we did not observe a snake to loose its grip on a tail. In addition, many lizard species with active autotomized tails, such as most eublepharine and gekkonine lizards, occur in relatively open, litter-free habitats.
11. C. M. Pond [*Annu. Rev. Ecol. Syst.* **9**, 519 (1978)] suggested that autotomized tail movement might mimic snake movement, and, presumably, frighten predators. However, many predators that encounter autotomized tails are themselves snakes or predators of snakes.
12. Most accounts simply suggest that autotomized tail movement distracts a predator [for example, (*9*); H. B. Cott, *Adaptive Coloration in Animals* (Methuen, London, 1944), pp. 160–161; A. Bellairs, *The Life of Reptiles* (Universe Books, New York, 1969)]. However, there have apparently not been experimental studies of this proposal nor has a mechanism been reported.
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17. Adults of both species were collected in the spring of 1980 in Dallas and Harris counties, Texas. Only specimens with original (nonregenerated) tails were used in experiments.
18. Thrash rates: *S. lateralis* > 300 per minute; *A. carolinensis* < 50 per minute. Mean frequency of tail break: *S. lateralis*, 64 percent (N = 31); *A. carolinensis*, 38 percent (N = 32).
19. Autotomy was induced by gripping tails at the basal caudal fracture plane (*2*) with forceps. In thrashing tail trials, lizards and their autotomized tails were placed in front of the cat immediately after autotomy. In exhausted tail trials, tails were allowed to thrash to exhaustion (> 5 minutes), after which lizards and their autotomized tails were placed in front of the cat.
20. In thrashing tail trials, intact tails were presented to snakes, base first; snakes attacked tails, after which autotomy and thrashing occurred. In exhausted tail trials, autotomy was induced with forceps. After tails had thrashed to exhaustion, they were presented with forceps, base first to snakes. Thrashing and exhausted tails were not significantly different in mass, length, or maximum diameter. *Lampropeltis triangulum* is a natural predator of *S. lateralis* [F. N. Blanchard, *U.S. Natl. Mus. Bull.* **114**, 1 (1921)]. Specimens (three subadults) were obtained from a commercial dealer.
21. For thrashing tails, subdue time (S_t) is the time from autotomy to manipulation of the tail in the snake's jaws. For exhausted tails, it is the time from attack to the tail to manipulation. Ingestion time (I_t) is the time from manipulation to completion of ingestion. Total handling time (H_t) = S_t + I_t. All times were measured to the nearest 0.01 second with an electronic stopwatch.
22. To establish resting lactate content of intact tails, we maintained lizards at rest for 24 hours, froze them in liquid nitrogen, removed tails, and measured lactate concentration. To establish lactate content after autotomy and thrashing, we induced autotomy at the basal caudal fracture plane, allowed tails to thrash for 60 seconds, froze them in liquid nitrogen, and measured lactate concentration. Data from our experiments with predators handling autotomized tails suggest that 60 seconds is the most ecologically important time span after autotomy; in most trials the predator had begun tail manipulation before 60 seconds had expired. However, *S. lateralis* tails may thrash for 4 to 5 minutes. Thus, our data may in fact underestimate total lactate production of an autotomized tail. Tails were homogenized in five to eight times their mass in 0.6N perchloric acid in a tissue grinder. Samples were centrifuged for 10 minutes at 3000 rev/min. The supernatant was removed and filtered with a syringe filter. Samples were analyzed for lactate content with an enzymatic test kit (Single Vial Lactate, Bio-Dynamics/bmc Co.) and a spectrophotometer. All samples were read at 340 nm.
23. Unless otherwise noted, we used the Mann-Whitney U test [S. Sigel, *Nonparametric Statistics for the Behavioral Sciences* (McGraw-Hill, New York, 1956), pp. 116–127.
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28. To estimate lactate concentration of intact tails after escape behavior, we chased lizards through leaf litter (*S. lateralis*) and among wooden perches (*A. carolinensis*) for 60 seconds, froze them in liquid nitrogen, removed tails, and measured lactate content (*22*).
29. Tail lactate content will depend at least partly on the proportion of the tail that is muscle versus skeletal and fat tissue. Thus, a higher lactate concentration in the autotomized tail of one species might reflect proportionally more muscle tissue in that tail [R. W. Putnam, *Physiol. Zool.* **52**, 509 (1979)].
30. See L. J. Vitt and J. D. Congdon [*Am. Nat.* **112**, 595 (1978)] for some examples.
31. We thank C. L. Simmons, H. W. Greene, M. E. Feder, A. F. Bennett, and G. W. Ferguson for comments on the study and the manuscript, and H. E. Rawlins, Jr., R. E. Gatten, J. B. Murphy, D. Barker, B. Tryon, R. G. Coghill, J. Norwood, and T. Faulkner for assistance during the study. Supported in part by grants to B.E.D. from the Theodore Roosevelt Memorial Fund (American Museum of Natural History), the Gaige Fund (American Society of Ichthyologists and Herpetologists), and Sigma Xi, and to L.C.F. from the Faculty Research Fund. Funds for manuscript preparation were provided by the Department of Biology, Texas A&M University.

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A Bean α-Amylase Inhibitor Formulation (Starch Blocker) Is Ineffective in Man

Abstract. *A commercial α-amylase inhibitor with potent inhibitory activity in vitro was used in a randomized double-blind, cross-over clinical trial in six nonobese, healthy adult males. In these subjects, this inhibitor had no effect on the response of blood glucose, insulin, or breath hydrogen to a standardized starch meal. It is concluded that this formulation has no effect on starch digestion in humans.*

A large number of α-amylase inhibitor formulations (starch blockers) derived from kidney bean have recently become available to the general public as dietary supplements. Advertisements for these products claim that they decrease starch digestion and absorption, resulting in weight loss. In June 1982, consumption of these starch blockers in the United States was estimated to be 10 million tablets per week (*1*). We have studied the effects of one of several apparently similar commercial inhibitor formulations on starch digestion and absorption in humans.

In vitro, α-amylase inhibitors prevent hydrolysis of the α-1,4-glycosidic linkages of starch by noncompetitive binding to the enzyme; however, their effect in humans is unknown (*2*). Marshall and Lauda isolated and characterized the inhibitor derived from kidney beans and developed an in vitro assay for inhibitor activity (*3*). Other reports have described similar inhibitors derived from other plant sources (*4*). These inhibitors appear to be glycoproteins specific for mammalian amylase of either salivary or pancreatic origin. They appear to have no other physiologic activity, for example, trypsin inhibition or hemagglutination.

Effective inhibition of starch digestion in vivo would diminish glucose formation and absorption by the small intestine and increase the amount of undigested starch reaching the colon. We tested the effect of the inhibitor on glucose formation and absorption by measuring changes in the concentrations of glucose and insulin in the serum of human volunteers after they had consumed a starch meal. We simultaneously measured breath hydrogen levels that would rise if greater than 6 to 10 g of unabsorbed carbohydrate reached the colon (*5*). If α-amylase inhibitors were effective, we would expect a reduced increase in glucose and insulin and elevated breath hydrogen production.

We obtained α-amylase inhibitors from four commercial sources. The inhibitors were assayed (*6*) for inhibitory activity in vitro by the method of Marshall and Lauda (*3*). We selected the most active of these commercially available inhibitors for study and used two tablets containing 16,666 units of total activity. This dose was compared to a calcium phosphate placebo which exhibited less than 5 percent of the inhibitor's activity and was physically indistinguishable from the inhibitor (*7*).

We studied six, healthy, nonsmoking

Table 1. Areas under the curves (*12, 13*) for serum glucose, insulin, and breath hydrogen. N.S., nonsignificant.

Subject	Glucose (mg/dl-min)		Insulin (U-min)		Breath hydrogen (mm-min)	
	Placebo	Inhibitor	Placebo	Inhibitor	Placebo	Inhibitor
1	22406	20771	7578	4431	2320	730
2	25804	23355	3696	2850	1360	1735
3	23243	23183	3651	4125	5825	5500
4	19721	25020	2840	3713	790	4700
5	21431	23936	2615	4763	775	6490
6	21311	21660	2396	2519	1370	1415
Paired *t*-test	$P > .50$ N.S.		$P > .90$ N.S.		$P > .20$ N.S.	

males (age range, 23 to 32 years) who were within 15 percent of their ideal body weight and currently taking no medication. They had no history of gastrointestinal problems, food allergies, or unusual dietary habits and had not varied more than 10 percent in their body weight within the last year. Oral glucose tolerance and thyroid (triiodothyronine and thyroxine concentrations) tests were normal in all the subjects. Informed consent was obtained from all the subjects before the study.

Blood glucose concentrations were determined by the glucose oxidase method (*8*), immunoreactive insulin concentrations by a radioimmunoassay kit from Corning Medical and Scientific (*9*), and breath hydrogen by gas chromatography with a Quintron Microlyzer (model 12; Quintron Instrument Co.), standardized with a known H_2 concentration every 30 minutes.

The study was initiated between 7 and 8 a.m. after a 10-hour overnight fast. Baseline breath hydrogen samples and blood samples for determination of glucose and insulin concentrations were taken prior to the meal. Each subject consumed a test meal, representing a high starch dietary intake, consisting of 270 g of baked potato (pulp only) and 230 ml of Libby's tomato juice (for flavoring). The α-amylase inhibitor or placebo was crushed to a powder and homogenized with the meal in a Waring blender just prior to consumption (*10*). Each meal contained 6.2 g of protein, 0.5 g of fat, and 57.7 g of carbohydrate (predominantly starch, 41.2 g, and oligosaccharide glucose equivalents, 8.0 g) and was generally consumed within 15 minutes (*11*). Postprandial blood samples were taken at 15, 30, 60, 90, 120, 150, 180, and 240 minutes. Breath hydrogen samples were taken every 15 minutes for 6 hours after meal consumption. If hydrogen levels appeared to rise during the last hour, samples were taken for an additional hour in order to detect any late peak.

Each subject consumed two test meals, 7 days apart. Random use of placebo or active inhibitor allowed each subject to serve as his own control. No gastrointestinal complaints were noted, except for a minor case of heartburn. All six subjects completed the study.

We plotted the concentrations of blood glucose and insulin against time for each individual and compared the respective areas under the curves thus obtained (*12*) for inhibitor and placebo. We found no significant differences using the paired *t*-test (Table 1). Comparison of the means for each corresponding time point also showed no significant differences between inhibitor and placebo (Fig. 1). Breath hydrogen results were similarly analyzed (*13*) and revealed no significant inhibitory effect (Fig. 1 and Table 1), suggesting that the inhibitor did not result in detectable amounts of undigested starch reaching the colon. This is consistent with the absence of increased flatus and diarrhea in our subjects.

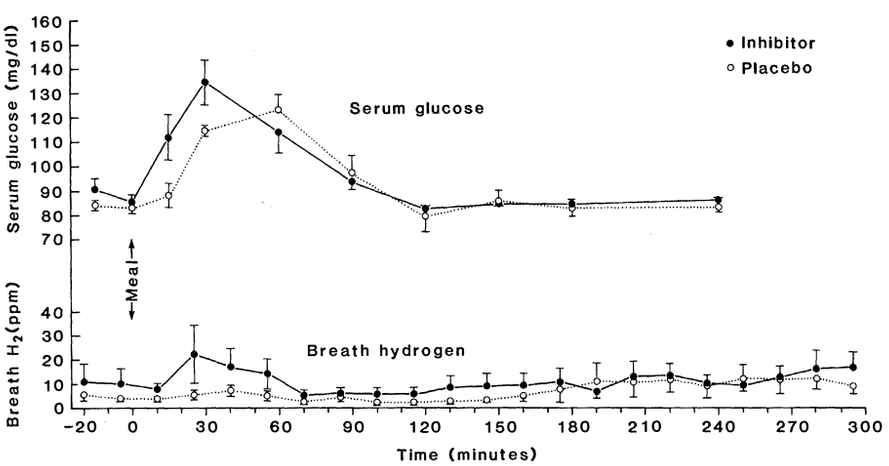

Fig. 1. Serum glucose and breath hydrogen levels after a starch meal. Points represent the means (± standard error) of six subjects. Student's *t*-test revealed no statistically significant differences between the means ($P < .05$).

The discrepancy between the in vivo and in vitro activity of the inhibitor suggests the persistence of intraluminal amylase activity despite administration of twice the commercially recommended dose of an inhibitor with potent in vitro activity. The absence of an effect in vivo might be the result of inactivation of the protein inhibitor by gastric acid and pepsin or pancreatic proteases. Also, intraluminal conditions are clearly not favorable for optimal inhibition; for example, in vitro the *p*H optimum for inhibition is 5.5 and an incubation period of 20 minutes is required (*3*). Finally, because amylase is secreted in such great excess to that required, persistence of only 4 percent of normal levels is adequate to hydrolyze a starch meal (*14*).

We conclude that formulations of the kidney bean–derived α-amylase inhibitor that are now available do not alter the digestion of cooked starch in humans.

Note added in proof: Since this manuscript was submitted, Bo-Linn *et al.* (*15*) reached a similar conclusion by demonstrating that fecal calorie excretion was not increased in normal subjects given starch-blocker tablets with a high starch meal.

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4. J. R. Powers and J. R. Whitaker [*J. Food Biochem.* **1**, 217 and 239 (1977)] isolated a similar inhibitor from navy beans, and other investigators have found such inhibitors in wheat [E. Keen and R. M. Sanstedt, *J. Am. Chem. Soc.* **65**, 1247 (1943)] and other sources [B. B. Elliott and A. C. Leopold, *Physiol. Plant. Pathol.* **6**, 65 (1953)].
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12. The area under the curve was obtained by converting the trapezoidal areas into rectangles and then summed.
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Chromosome Localization of Highly Repetitive Human DNA's and Amplified Ribosomal DNA with Restriction Enzymes

Abstract. *Restriction endonucleases cut and partially removed DNA throughout fixed air-dried human metaphase chromosomes. Some enzymes produced a G-banding pattern; some revealed the presence of multiple chromosome-specific classes of highly repetitive DNA in C-band heterochromatin. Enzymes that produced the informative C-band patterns had recognition sequences that were four or five, but not six, base pairs long and did not contain a cytosine-guanine doublet. In both rat and human chromosomes, regions containing amplified ribosomal RNA genes were specifically removed by the restriction endonuclease Msp I.*

The DNA in metaphase chromosomes fixed in methanol and acetic acid and immobilized on glass slides is susceptible to attack by nucleases. Deoxyribonuclease I and micrococcal nuclease cleaved such DNA into fragments just a few base pairs (bp) long (*1*); this extracted the DNA and abolished Giemsa staining (*2*). If the chromosomes were exposed to the GC-specific (G, guanine; C, cytosine) ligand chromomycin A3 prior to and during deoxyribonuclease digestion, the GC-rich DNA was protected while the remaining DNA was cut into tiny fragments and extracted, leaving an R-band pattern (*3*). Type II restriction endonucleases should provide more interesting probes because each enzyme cuts only within a specific recognition sequence 4 to 6 bp long, or longer, and produces longer fragments of DNA (*4*). Restriction endonuclease Hae III, which cuts at GGCC, produced a chromomere pattern of Giemsa staining on muntjac chromosomes fixed in methanol and acetic acid; Eco RI, which cuts in the much less abundant GAATTC (A, adenine; T, thymine) sequence, had no detectable effect on these chromosomes (*5*).

The present study was undertaken to characterize the effects of various restriction endonucleases on human chromosomes, especially on the highly repetitive, but complex and heterogeneous, human satellite DNA's (*6*), which are concentrated in the heterochromatic C-band regions (*7, 8*). In addition, we examined the effect of Msp I on the somewhat less repetitive amplified 18*S* plus 28*S* ribosomal RNA genes (rDNA), which contain abundant Msp I sites (*9*).

Digestion with Alu I, Hae III, or Mbo I, enzymes with recognition sequences 4 bp long, led to a marked decrease in Giemsa staining in localized regions of human chromosomes, producing G-band or modified C-band patterns (*10*) (Fig. 1). Four other enzymes that recognize 4-bp sequences, namely, Cfo I, Hha I, Hpa II, and Msp I, had no effect, probably because each contains in its recognition sequence the CG dinucleotide (*11*). Three enzymes with recognition sequences 5 bp long, Dde I, Eco RII, and Hinf I, also produced marked diminution of Giemsa staining and modified C-band patterns, whereas Ava II had no detectable effect. Enzymes with recognition sequences 6 bp long, or longer (*12*), generally produced very little effect beyond the G-band-like pattern sometimes present in the controls exposed to the reaction mixture minus the enzyme.

The chromosome banding patterns observed after restriction endonuclease digestion were highly specific for each enzyme (Fig. 1). Treatment with Alu I (Fig. 1a), Dde I, or Eco RII left intact the major C bands (those on chromosomes 1, 9, 16, and, in male cells, the Y). Treatment with Mbo I (Fig. 1b) removed most of the C bands on chromosomes 1, 16, and the Y (not illustrated), but not that on chromosome 9. These enzymes also had different effects on the minor C bands present on other chromosomes, which could be identified by the pale G bands that remained on most of them after enzyme treatment. For example, Eco RII had no effect on the C bands on chromosomes 11 and 12; Mbo I (Fig. 1b) removed the C band from 11 but not 12, and Alu I (Fig. 1a) or Dde I removed the C bands from both 11 and 12. Hae III abolished staining of the GC-rich R bands, producing a more pronounced G-band pattern than the other enzymes; it had no effect on the major C bands (Fig. 1c). Hinf I diminished staining of every chromosome region (Fig. 1d). It abolished Giemsa staining of all C bands except those on chromosomes 3 and 4 (identified by faint residual G-band patterns) and left large gaps in place of the major C bands. DNA in the entire human chromosome complement was available for reaction with restriction endonucleases, since Giemsa staining could be eliminated from every region of every chromosome. No variation in effect was observed between individuals in this small sample; differences between homologs (for example, the chromosomes 9 in Fig. 1, a to c) reflected differences in the amount of C-band material.