The three genes we have targeted in these experiments are likely to be important in mouse development and physiology, but their functions remain undefined genetically. The adipsin gene encodes a serine protease with complement factor D activity and has links to systemic energy balance and obesity (*15, 24*); the aP2 gene product is an adipocyte-specific fatty acid binding protein (*25–27*) whose precise role in physiology is unknown. The c-*fos* proto-oncogene is involved in the regulation of gene transcription (*28, 29*), and disruption of this gene may have numerous effects on cell function. The analysis of the phenotypic effect of disruption of these three genes should lead to a greater understanding of their function in the organism.

It is probable that many factors are involved in determining the frequency at which detectable homologous recombination occurs at a given genetic locus (*2, 3*). Our data, however, demonstrate that it is feasible to target genes not expressed in ES cells in order to study their function in mouse development and physiology.

Table 1. Rates of homologous recombination after double drug selection. Electroporations were performed with the linearized vector described in Fig. 2. The data are pooled from several transfections. ES cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 15% fetal bovine serum (FBS) and 0.1 m*M* 2-mercaptoethanol on gamma-irradiated (4000 rads) Sto feeder layers made G418-resistant by transfection with the vector pSV2neo. Cells (2×10^7) in 1 ml of medium were electroporated at 750 V/cm in DMEM and FBS with vector DNA at 1 n*M*. These cells were plated out on G418-resistant feeder layers. The number of viable cells 24 hours after electroporation was always between 40 to 60%. At 36 hours after transfection, G418 (100 μg/ml) and gancyclovir (2 μ*M*) were added. After selection for 10 days, drug-resistant colonies were picked and expanded on Sto feeder layers without further drug selection. The asterisk indicates that one control plate for each transfection was selected with G418 alone and was plated at 1/20 the density of doubly selected plates (5×10^5 cells on a 150-cm^2 plate versus 2×10^7 cells on a 150-cm^2 plate), and the number of G418-resistant colonies for the entire transfection was extrapolated from this number. Clones listed as homologous recombinants were confirmed as such by digestion with two or more restriction enzymes and Southern blotting, as shown for the representative clones in Fig. 3.

DNA transfected	Cells electroporated	No. G418* resistant	No. G418 and gancyclovir resistant	Homologous recombinants
c-*fos* vector	4×10^7	2×10^4	16	1
Adipsin vector	10^8	5×10^4	22	2
aP2 vector	8×10^7	4×10^4	142	12

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Foregut Fermentation in the Hoatzin, a Neotropical Leaf-Eating Bird

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The only known case of an avian digestive system with active foregut fermentation is reported for the hoatzin (*Opisthocomus hoazin*), one of the world's few obligate folivorous (leaf-eating) birds. Hoatzins are one of the smallest endotherms with this form of digestion. Foregut fermentation in a flying bird may be explained by increased digestive efficiency by selection of highly fermentable and extremely patchy resources, coupled with microbial nutritional products and secondary compound detoxification. This unexpected digestive system gives a new perspective to the understanding of size limitations of vertebrate herbivores and to the evolution of foregut fermentation.

FOREGUT MICROBIAL FERMENTATION as a means of digesting fibrous plant matter has been reported in mammals such as ruminants, monkeys, sloths, and macropodid marsupials (*1*). Although a few bird species display hindgut fermentation (*2*), there are no documented cases of extensive foregut fermentation structures or associated digestive physiology in the entire class Aves. We now report a well-developed ruminant-like digestive system in a neotropical folivorous bird, the hoatzin, *Opisthocomus hoazin*. This is the first report of this digestive system outside the mammals, and opens new insights into the evolution of foregut fermentation.

The hoatzin is a 750-g cuculiform bird that ranges from the Guianas to Brazil and inhabits riverine swamps, gallery forests, and oxbow lakes (*3, 4*). Early descriptions suggested that the crop of this species has replaced the gizzard and proventriculus as the primary site of digestion (*5*). None of these authors documented or suggested foregut fermentation, although some noted that the characteristic odor of the bird was similar to fresh cow manure (*6*).

At our study site (*7*), more than 80% of the hoatzin's diet is composed of green leaves. Although the birds fed on the leaves of 52 species of plants in 25 families, 90% of the diet is composed of only 17 plant spe-

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cies. Hoatzins tend to prefer new growth of their food plants (*8*). These preferred portions both within a plant species and across a season are significantly higher in water content and nutritive value than nonpreferred portions (*9*).

The high mass-specific energetic demands of a small, flying endothermic herbivore must be met by a high rate of energy assimilation. We studied the hoatzin's possible morphological and physiological adaptations to increase digestive efficiency, including (i) high dietary selectivity, (ii) high relative gut capacity, (iii) efficient reduction of particle size, and (iv) selective passage rates of fermentable substrates. Additionally, we measured direct and indirect (in vitro) digestibilities by hoatzins (*10*).

The hoatzin's crop and esophagus are the main fermentation gut structures, and some additional fermentation occurs at the paired ceca (Fig. 1). At these sites, neutral *p*H supports bacterial concentrations within the range of ruminants (*11*). Microbial fermentation by-products, such as volatile fatty acids (VFA), are an important energy source for foregut fermenting vertebrates. Within the hoatzin's crop and esophagus, VFA are present in high concentrations, suggesting that VFA metabolism accounts for a significant proportion of the total energy requirements of the hoatzin (Table 1). The specialized gut is capable of a significant food particle size reduction at the crop and caudal esophagus (*12*). Particle size is likely reduced by cornified epithelial ridges of the internal ventral surface of the highly muscular crop. This mechanism is functionally similar to a rumination process, with the advantage that "chewing" and fermentation occur at the same site. The hoatzin gut is also capable of selective particle retention, an important trait for efficient fermentation. Mean retention times for 1-mm^2, 8-mm^2, and 3-mm^3 plastic markers (tape and beads) administered orally at a single pulse dose were 19.5, 34.8, and 43.0 hours, respectively (*13*). These retention times are among the longest recorded for a bird and enough to maintain a stable population of bacterial gut symbionts (*14*).

Cell wall fractions of the digesta were 34% as low at the lower intestine as at the anterior crop, suggesting active fiber digestion. Indeed, digestibility trials with captive hoatzins on a high-quality experimental diet showed 34.4% cell wall digestibility (*15*). We also compared in vitro fermentation by bacterial extracts of hoatzin's crop and cow's rumen, and no significant differences in digestibility rate or total cell wall in vitro digestibilities were found (*16*).

In the hoatzin, the functional conversion of the crop and esophagus into fermentation structures has caused considerable anatomical and behavioral modifications. The anterior sternum is much reduced to allow room for the voluminous fermentation structures. This considerably reduces the area for flight muscle attachment on the sternal carina (*5*). As a consequence, hoatzins are poor flyers, and young require 60 to 70 days to fly (*4*). The anatomical and developmental costs of folivory and foregut fermentation may be an evolutionary reason for the development of functional wing claws and the unusual predator-escape mechanisms of young hoatzins (*17*).

Although foregut fermentation in a 750-g endotherm is not predicted by current theories of herbivore digestive strategies (*18*), our combined data demonstrate the presence of a well-developed foregut fermentation system in the hoatzin. Various anatomical, behavioral, and physiological adaptations in hoatzins seem to overcome the size limitations found in mammals with foregut fermentation. Although herbivory establishes severe energetic and physical restrictions on flying organisms (*19*), flying allows increased food selectivity, adding a new dimension to foregut fermentation. A flying arboreal folivore, such as the hoatzin, can exploit extremely patchy and temporally dynamic resources that otherwise would not be accessible to other herbivorous vertebrates. The extreme selectivity of a high-quality leafy diet maximizes fermentation rate and digestive efficiency. Additional nutritional benefits of foregut fermentation, such as microbial production of essential vitamins and amino acids and detoxification of plant secondary compounds, may explain the presence of this unusual digestive system in the hoatzin. However, given the abundance of leaves as a resource base, it is not obvious why hoatzins are the only living bird with foregut fermentation. Their highly specialized digestive strategy may have arisen from an ancestral nonobligate folivore because of an evolutionary trade-off between detoxification of plant chemical defenses and enhanced use of cell wall as a nutritional resource. Finally, the hoatzin digestive system gives a new perspective to the evolution of foregut fermentation in mammals and other vertebrates.

Table 1. Average relative weight of the full gut, *p*H, total concentration of VFA and proportions of VFA in various segments of the gut of four adult hoatzins compared to other foregut fermenting animals (*18*). Standard deviation in parentheses. VFA concentrations in the small intestine were very low.

Segment	Percent weight of total gut	*p*H	Total VFA concentration (m*M*)	Molar percentages of VFA			
				Acetic	Propionic	Butyric	Isobutyric
Hoatzin							
Crop	60.6 (2.8)	6.3 (0.4)	114.5	67.6	15.3	6.5	10.7
Esophagus	13.9 (2.3)	6.7 (0.3)	70.3	68.9	14.9	8.5	7.7
Proventriculus	1.7 (0.2)	2.1 (0.6)					
Gizzard	2.4 (0.3)						
Small intestine	13.0 (2.9)	5.7 (1.2)					
Cecum	2.2 (0.5)	7.5 (0.1)	94.7	77.4	9.0	0.0	13.6
Large intestine	4.5 (2.7)						
Sheep rumen		6–7	94	50	29	9	
Cow rumen		6–7	137	64	22	12	
Colobine monkeys		5.5–7	103–219	50–70	17–28	10–14	

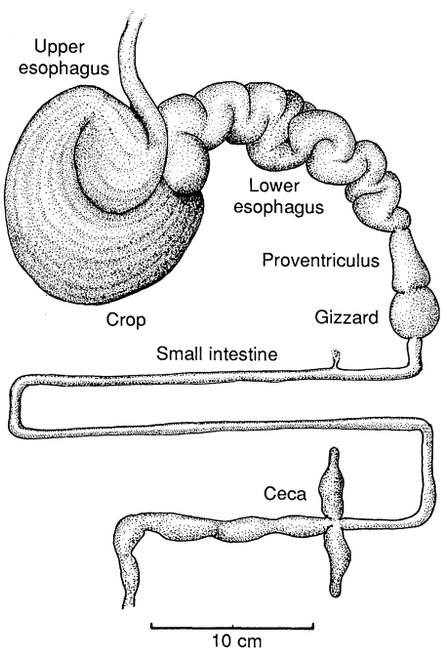

Fig. 1. The unique form and function of the hoatzin's gut is similar to that of mammals where foregut fermentation supplies a high percentage of the animal's energy requirements (*18*). The contents of the enlarged muscular crop and lower esophagus weigh up to 17.7% of the total adult mass. The interior lining of the crop has deep ridges that increase absorptive area. Moreover, the greatly sacculated esophagus effectively delays the passage rate of particles to the lower gut. The paired ceca may be important sites for vitamin microbial production as well as nitrogen, ion, and water recycling and absorption.

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7. Hato Masaguaral (67°35′W, 8°34′N), is located along a tributary of the Guárico River, in the central plains (llanos) of Guárico state, Venezuela.
8. Of a total of 51,704 bird-minutes of observation at the study site, parts of plants eaten by hoatzins were: buds, shoots, and new leaves, 67.8%; mature leaves, 12.6%; flowers, 10.4%; fruits, 8.1%; and unidentified leaves, 1.1%. Green leaves of *Zanthoxylum culantrillo* (Rutaceae), *Acacia articulata* (Fabaceae), *Lonchocarpus cruciarubieres* (Fabaceae), and *Guazuma ulmifolia* (Sterculiaceae) make up 56% of what was eaten in the observed foraging time during the breeding season.
9. Eaten portions are significantly higher in water content and crude protein ($P < 0.001$) and lower in total cell wall, lignin, cellulose, and hemicellulose ($P < 0.01$) than uneaten portions (two-tailed Mann-Whitney U test). No significant differences were seen for cutin and soluble phenolics. Average nutritional value of the natural diet is (in percentage dry matter) 73% water content, 78% organic matter (OM), 46% neutral detergent fiber (NDF), 32% acid detergent fiber (ADF), 17% cellulose, 13% hemicellulose, 10% lignin, and 18% total nitrogen (N) [nutritional analysis followed H. K. Goering and P. J. VanSoest, *Agriculture Handbook 379* (U.S. Department of Agriculture, Washington, DC, 1970)]. Soluble phenolics were measured by acetone extraction.
10. Quantitative descriptions of the digestive morphology and foregut fermentation of hoatzins were based on ten individuals collected between 1983 and 1988. The *p*H of gut contents was measured at the time of collection. Digesta from potential fermentation sites was preserved in sulfuric acid solution. Concentrations of volatile fatty acids (VFA) were measured by gas chromatography [A. Wilkie, M. Goto, F. M. Bordeaux, P. H. Smith, *Biomass* **11**, 135 (1986)]. Segments of the gut were isolated and weighed to examine relative capacity when full and emptied of their contents. Digesta from all segments of the gut was collected for particle size analysis and comparative nutritional analysis.
11. Microflora was mostly composed of gram-negative rods with an average concentration of 1.10×10^9 per milliliter ($n = 6$, SD = 0.03×10^9). No protozoans were found.
12. Mean particle size was measured with a computerized particle analysis video system with a camera mounted on a microscope. Mean particle size was significantly different between the upper esophagus and the lower esophagus before the entrance of the proventriculus [290 μm (SD = 119) and 158 μm (SD = 36), respectively]. Two-tailed Mann-Whitney U test ($P = 0.03$, $n = 5$).
13. Mean retention times were calculated as $t = \Sigma m_i t_i / \Sigma m_i$, where m_i is the amount of marker excreted per unit dry matter at the ith defecation at time t after dosing ($n = 3$, SD = 2.9, 7.2, and 5.7). We used commercial flagging tape as the 1- and 8-mm^2 markers and plastic beads as the 3-mm^3 markers. All markers had a specific gravity of 1.01. Particles are selectively retained in the narrow passages between the anterior and posterior chambers of the crop and the extensive sacculation of the caudal esophagus.
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15. Offered and excreted fractions were quantitatively recovered and expressed in percentage of dry matter (%DM at 105°C). The nutritional composition of an experimental diet of romaine lettuce, ground alfalfa pellets, and soybean protein concentrate was 20% DM, 19% NDF, 18% ADF, 11% cellulose, 25% N, and 81% OM. Captive hoatzins ($n = 4$) maintained a constant body mass during and after the experiment. Average intake rate was 44.3 g(DM)/day (SD = 8.07). Apparent digestibilities for dietary fractions ([1 − (DM excreted/DM ingested)] × 100) were: 77.1 ADF, 59.5 cellulose, and 71.4 OM. In vitro OM digestibility of the experimental diet by cow ruminal inoculum was 77.6. Apparent digestibilities may be underestimated, since no separation of urinary and fecal products was possible.
16. Samples of 100 mg of dried ground young alfalfa (39.5% cell wall) were incubated with 10 ml of inoculum for 3, 6, 12, 24, 48, and 72 hours in 20 ml screw-cap Hungate tubes under anaerobic and isothermic conditions. The inoculum consisted of a 1:5 (w:w) dilution of hoatzin crop content (or cow rumen content) in a standard buffering solution (McDougall's artificial saliva). Rates of in vitro cell wall digestibility were the slope of regression curves of the natural logarithm (ln) transformation of percentage digestibilities on hours of fermentation. For cow rumen $y = 0.0096x + 3.647$ ($n = 13$, slope SE = 0.0035) and for hoatzin crop extract $y = 0.0076x + 3.94$ ($n = 12$, slope SE = 0.0011). In vitro cell wall digestibilities after a 72-hour incubation were 80.2 and 79.9 (SD = 19.32 and 8.37; $n = 5$ and 3) for cow and hoatzin, respectively.
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20. We dedicate this work to the memory of R. Parra, who passed away in February 1988. His ideas and enthusiasm will always be with us. Funding was provided by the New York Zoological Society, Universidad Central de Venezuela, Consejo Nacional de Ciencia y Tecnología de Venezuela (CONICIT), Organization of American States (OAS), and the Smithsonian Institution. S.D.S. thanks E. Dolensek for his guidance in early phases of the study. We thank O. Parra and the personnel of the Laboratory of Forage Analyses at UCV Maracay, T. Blohm for permitting our work at Masaguaral, and many field assistants. K. A. Bjorndal, D. J. Levey, C. Martinez del Rio, and B. K. McNab made valuable editorial comments.

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Neutrophil Mac-1 and MEL-14 Adhesion Proteins Inversely Regulated by Chemotactic Factors

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The neutrophil Mac-1 and gp100^{MEL-14} adhesion proteins are involved in neutrophil extravasation during inflammation. Both the expression and activity of Mac-1 are greatly increased after neutrophil activation. In contrast, neutrophils shed gp100^{MEL-14} from the cell surface within 4 minutes after activation with chemotactic factors or phorbol esters, releasing a 96-kilodalton fragment of the antigen into the supernatant. Immunohistology showed that gp100^{MEL-14} was downregulated on neutrophils that had extravasated into inflamed tissue. The gp100^{MEL-14} adhesion protein may participate in the binding of unactivated neutrophils to the endothelium; rapid shedding of gp100^{MEL-14} may prevent extravasation into and damage of normal tissues by activated neutrophils.

CIRCULATING AND BONE MARROW neutrophils provide a front line of defense that can be rapidly mobilized and activated against infectious agents. The first step in extravasation involves adhesive interactions of neutrophils with the vascular endothelium, which must be regulated to allow localization of neutrophils only to inflammatory sites. Similarly, activation of neutrophils must be limited to those cells at the inflammatory site, so that damage to normal tissues is minimized. The leukocyte integrins, LFA-1, Mac-1, and p150,95 [collectively referred to as the CD18 complex (*1*)], are essential in the neutrophil extravasation process, as demonstrated experimentally (*2, 3*) and from the study of humans with a genetic deficiency of CD18 expression (*4*). Optimal CD18 function requires cell activation (*4–7*), which is also accompanied by rapid mobilization of an intracellular pool of Mac-1 and p150,95 to the cell surface (*8*). A second class of neutrophil adhesion protein, gp100^{MEL-14}, has been implicated in neutrophil extravasation (*9, 10*). Neutrophil gp100^{MEL-14} is related to lymphocyte gp90^{MEL-14}, which mediates adhesion to the specialized high endothelial venules (HEV) of peripheral lymph nodes (*11*). The MEL-14 monoclonal antibody (MAb) prevents murine neu-

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