Identification of the regions of secondary structure—and the choice of portions of the protein to be included in the drawing and of the sites to be labeled—are under control of the user through input data.

The program is written entirely in standard FORTRAN IV. The version that produced the line drawings in Figs. 1 and 2 has run on the IBM 370/165 and 168 and on the DEC VAX 11/780. These systems are available to many potential users.

We constructed the program by application of well-known techniques [for example, see (*6*)]. Orientations of α-helices are found by superposition of the backbone coordinates onto a standard helix oriented along the *z*-axis. For ribbon diagrams and strands of β-sheet, the orientation of each peptide plane is determined. For curved sheets, the midline of the arrow is computed by a spline fit to the centers of gravity of successive peptides, and the orientation of the normal to the arrow is calculated by spline fits to the direction cosines of the normals to the successive peptide groups. Whenever the program draws a (potentially) opaque structure, it saves the coordinates of the boundaries of one or more polygonal "windows" for use in a subsequent hidden-line removal step.

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Transfer of Fermentative Microbes Between Generations in a Herbivorous Lizard

Abstract. Iguana iguana *is herbivorous throughout life and utilizes a microbial fermentation system in the elaborated hindgut to break down plant cell walls. Iguanas reared in captivity grew more slowly than wild hatchlings and failed to develop the same complex populations of fermentative microbes. Captive hatchlings fed fresh fecal material from an adult iguana acquired the complex microflora and grew as rapidly as wild hatchlings. In the field, hatchlings actively associated with adults during the first weeks of life and obtained the complex microflora during this time. Acquisition of the fermentative microflora by neonatal iguanas apparently requires direct contact with older conspecifics.*

Most herbivorous mammals satisfy part of their energy requirements through the fermentative activity of specialized bacteria and protozoans in some portion of the digestive tract. These complex populations of microbes break down plant cell wall materials, which are otherwise indigestible to the herbivore, and release wastes, including certain volatile fatty acids, which the herbivore absorbs and utilizes for energy via the Krebs cycle (*1*). Young herbivorous mammals acquire their fermentative microflora through contact with the mother, usually by consuming fecal material. Protozoans and some bacteria can be transmitted to neonatal ruminants only by very close contact, such as by the consumption of feces or rumen eructate (*2*). Neonates artificially reared in isolation from older conspecifics fail to acquire all the normal species of microbes and may exhibit impaired growth and other abnormalities (*2*), presumably because their fermentative capacity and therefore nutrient intake are impaired.

Common iguanas (*Iguana iguana*) are unusual among lizards in that they are herbivorous throughout life (*3*). Iguanas depend on a microbial fermentation system in the elaborated hindgut (*4*) for as much as 30 percent of their daily energy requirements (*5*). Unlike their mammalian counterparts, neonatal iguanas have no direct contact with their mothers and thus lack this obvious mechanism for obtaining the specialized populations of hindgut microbes. In the Gatun Lake area of Panama, female iguanas travel to nesting sites in February, excavate nests, deposit their eggs, and then return to their home ranges, which may be as far as several kilometers from the nesting site (*6*). The hatchlings emerge in May, leave the nest area, and often settle in habitats quite distant from any adult iguanas (*7, 8*). Even in those areas where both adults and young are found, such as the outer edge of the forest around Gatun Lake, iguanas of different ages typically occupy different habitats; adults dwell in the forest canopy (15 to 30 m high) and hatchlings in low vegetation (1 to 4 m). I investigated how iguanas—reptiles that lack parental care—obtain the fermentative microflora, and how important the common microbe populations are for normal growth and utilization of plant food.

During 1979 and 1980, I hatched and reared iguanas in captivity (*9*) and found that they always grew more slowly than hatchlings in the wild. In addition, they failed to develop the same hindgut microflora (*10*). Although it is generally impossible to identify species of bacteria or to infer their biochemical function on the basis of appearance, many protozoans and some bacterial forms are morphologically unique and can be identified by simple microscopic examination. I found two such morphologically distinct microbes only in the hindguts of wild hatchlings. The first, a bacterial complex or sarcina (*Lampropedia merismopedioides*), also occurs in the hindguts of a variety of herbivorous reptiles, as well as in the rumen of cattle and sheep (*2, 11*). The second, a large ciliate protozoan, is probably an undescribed species (*12*), but resembles the holotrichs of the rumen (*2*). The occurrence of the *Lampropedia* and the protozoan exclusively in the hindguts of wild hatchlings suggested that these animals were exposed to additional sources of fermentative microbes, possibly by direct contact with other iguanas.

In 1981, I conducted experiments to locate sources of the morphologically distinctive microbes and to determine, under controlled conditions, whether possession of the more complex microflora was associated with improved growth rate and digestive efficiency. I used the ciliate protozoan as a marker, without assuming any useful function of the microbe itself. Because protozoans have the most stringent requirements for transmission (*2*), conditions that allow passage of the recognizable protozoan between iguanas should also permit transfer of the important, but visually unrecognizable, bacterial species.

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Newly hatched iguanas consume appreciable amounts of soil within the nest chamber and also lick the eggshells and each other (*13*). Hatchlings could obtain fermentative microbes in this manner if their mother contaminated the nest chamber soil, or the eggs, with her feces. I examined over 20 iguana nests between 1979 and 1981 without detecting any fecal material. Soil lining the nest chamber did not contain higher concentrations of bacteria than nearby soil did (*14*). Also, iguanas that had hatched and eaten soil in a natural nest failed to develop *Lampropedia* or protozoa (*10*) after being caged outdoors for 3 to 6 weeks (*15*) and grew no faster than iguanas hatched and reared in captivity (Table 1). These results indicate that maternal contamination of the nest is not a significant source of fermentative microbes.

Another potential source of microflora was the air or surfaces of the outdoor environment. Fermentative microbes might be obtained from contamination by other iguanas or from naturally occurring populations on plants. I reared naturally hatched iguanas and iguanas hatched in captivity in outdoor cages located where adult iguanas were relatively abundant (*15*). After 3 to 5 weeks, these groups of hatchlings had not developed the complex microflora and grew no faster than controls reared in captivity (Table 1). These results suggest that general environmental contamination is not sufficient for acquisition of the normal hindgut microflora.

A logical source of fermentative microbes was direct contact with other hatchlings. Iguanas in their first weeks of life are unusually gregarious for lizards, often moving and sleeping in groups of two to six or more (*7*, *8*). I caged three 20-day-old hatchlings reared in captivity with a 1-month-old wild hatchling for 2 weeks. All three of the captive hatchlings developed populations of the ciliate protozoan, which strongly suggested that transmission of the complete microflora had occurred (Table 1). This shows that hatchling iguanas can transfer fermentative microbes among themselves, although it does not explain how they obtain them initially.

Hatchling iguanas, like herbivorous mammals, could obtain their fermentative microflora by consuming fresh feces from an adult. I reared two groups of hatchlings as before (*9*), but fed the hatchlings in one group fresh fecal material from a caged adult iguana—about 10 mg daily from the third to the twentieth day of life. At 20 days of age, 12 of the 13 feces-fed hatchlings had ciliated protozoans in the hindgut, while none of the 12 controls did (*16*). The feces-fed group grew significantly faster than the control group and at a rate similar to the average rate for wild hatchlings in Panama (Table 1) (*13*). In a digestion trial comparing 5-week-old iguanas from the two groups (*17*), the feces-fed hatchlings assimilated their food significantly better than the controls did (*18*).

Table 1. Acquisition of hindgut microbes and growth rates of hatchling iguanas under experimental conditions.

Treatment	*N*	Protozoa present after 3 weeks	Mean growth rate from 0 to 5 weeks (mm/day)	Mann-Whitney *U* test for growth rate*
		Caged outdoors		
Hatched in natural nests	6	−	0.16	$U = 15$, $P = .467$
Hatched in captivity	8	−	0.18	$U = 26$, $P = .525$
		Caged indoors		
Controls	12	−	0.16	
Caged with wild hatchling	3	+		
Fed feces	13	+	0.22†	$U = 7$, $P = .033$
		Free outdoors; hatched in natural nests		
Wild hatchlings (1974 to 1979)	12	+	0.23†	$U = 22$, $P = .025$

*Values of growth rate were compared with growth rates of controls. †Significantly different from control values.

Table 2. Position of hatchling iguanas as a function of time after emergence.

Age (weeks)	Number of sightings			
	Perch height		Distance from nearest adult	
	Forest canopy (15 to 30 m)	Low vegetation (0 to 5 m)	Less than expected	More than expected
0 to 3	33	4	29	6
4 to 6	0	16	3	13
	$\chi^2 = 38.18$, $P < .001$		$\chi^2 = 19.07$, $P < .01$	

These experiments demonstrate that a hatchling iguana must consume fresh fecal material from an adult in order to acquire the hindgut microbes that best promote food utilization and growth. I also investigated whether newly hatched iguanas in nature actually have close enough contact with adults to obtain the microbes in this manner. In 1981, I searched for hatchlings in the vegetation along the margin of Gatun Lake (*19*), beginning before hatching had started and continuing for the next 2 months. The first hatchling iguanas I found were perched high in the forest canopy, usually 1 to 5 m from adult iguanas. Hatchlings and adults perched in a variety of tree species, and many trees of the same species had no iguanas in them. In the first 3 weeks of the hatching season, a significant portion of the hatchlings observed were in the forest canopy, whereas 4 to 6 weeks after hatching, most hatchlings were in low vegetation (Table 2). Furthermore, during the first 3 weeks, hatchling iguanas were positioned significantly closer to adults than they would have been if they were distributed randomly through the vegetation (*20*). Four to 6 weeks after hatching time, the hatchlings were distributed randomly with respect to adults.

These results demonstrate that newly hatched iguanas are able to contact adult iguanas and leave their customary habitats to do so. Although I did not directly observe interactions between hatchling and adult iguanas high in the forest canopy, I identified fecal material in the stomachs or small intestines of three of nine wild hatchlings examined during 1981. Hatchlings of terrestrial herbivorous lizard species have been observed consuming feces from adults (*21*).

In conclusion, hatchling iguanas must form temporary associations with members of a previous generation, in locations removed from both their hatching sites and their home habitats, in order to acquire their fermentative microflora. Later, the composition of the hindgut microflora may be adjusted through contact with other hatchlings. Acquisition of fermentative microbes may be as important a determinant of the unusual gregariousness of hatchling iguanas as detection of predators (*7*, *8*). Burghardt (*7*) has likened the apparent social organization of hatchling iguanas to the herding behavior of dinosaurs. My work suggests an additional comparison between mod-

ern and ancient herbivorous reptiles, especially if, as seems likely, many large herbivorous dinosaurs also depended on microbial fermentation systems. In the absence of parental care, each new generation must actively contact a previous one for acquisition of fermentative microbes. Attraction of young animals to older conspecifics for this purpose could represent the mechanism for origin of multigenerational dinosaur social groups, whose fossilized footprints have recently come to light (*22*).

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10. Weighed samples of the hindgut contents were preserved with 1 ml of 3 percent Formalin buffered to *p*H 7 and examined at a magnification of ×150 for the presence of protozoa. Concentrations of microbes were determined from a 1:100 dilution of the same solution examined in a bacterial counting chamber at ×600.
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15. Outdoor cages were constructed by surrounding individual tree branches with nylon mesh fabric. They were located in a region of the lakeshore where adult iguanas were commonly seen feeding or sleeping. Food was provided as in (*9*).
16. Fisher's exact probability test, $P < .005$.
17. Hatchlings were caged individually and fed weighed amounts of *Tetragonia expansa* leaves daily. Feces were collected daily, dried, and weighed. Gross digestive efficiency [GDE = (food eaten − feces produced)/(food eaten) = (food retained)/(food consumed)] was estimated from dry weight of food eaten and feces produced daily.
18. For feces-fed hatchlings, GDE = 0.83 ($N = 4$); for controls, GDE = 0.79 ($N = 6$). Median test, $P = .024$.
19. Iguanas were sighted on their sleeping perches at night by means of a 200,000-candlepower hand-held light; they were observed from a boat passing along the lakeshore. Age, sex, perch height, and tree species were recorded, and location was plotted on an outline map for each iguana sighted. Distances between individuals were estimated for iguanas closer together than 10 m; greater distances were measured on the maps.
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Autoantibodies to Insulin Receptor Spontaneously Develop as Anti-Idiotypes in Mice Immunized with Insulin

Abstract. *Mice immunized with insulin developed antibodies to both insulin and the insulin receptor. The antibodies to insulin receptor displaced labeled insulin from insulin receptors and mimicked the actions of insulin in stimulating the oxidation of glucose and its incorporation into lipids, and in inhibiting lipolysis. The antibodies to insulin receptor could be blocked by or bound to the antibodies to insulin, and therefore were identified as anti-idiotypes. Thus, immunization against a hormone may activate spontaneously an idiotype–anti-idiotype network resulting in antibodies to the hormone receptor.*

Several diseases of man can be related to the action of autoantibodies binding to receptors on the surface of normal cells of the individual. For example, patients with Graves' disease have antibodies that abnormally stimulate the thyroid gland by activating receptors for thyroid-stimulating hormone (TSH) (*1*). These antibodies to TSH receptor cause hypersecretion of thyroid hormones and hyperthyroidism. Myasthenia gravis is caused by antibodies that bind to and block the receptor for acetylcholine at the neuromuscular junction (*2*). Another instance of autoimmunity to receptor is exemplified by those patients with antibodies to the insulin receptor who suffer from severe insulin-resistant diabetes (*3*). The pathophysiology of autoimmunity to hormone receptors is not well understood. How might an individual's immune system be triggered to produce antibodies that bind to seemingly normal membrane components?

One explanation for receptor autoimmunity is that it arises in the course of anti-idiotypic regulation of the immune response. Animals may be immunized against the variable regions of specific antibody molecules (idiotypes) and so produce anti-idiotypes, antibodies directed against idiotypes (*4*). Some anti-idiotypes recognize the combining site of the idiotype (*5*). Thus, either antigen or anti-idiotypes may bind to the combining sites of idiotypic antibodies. Jerne (*6*) proposed that the immune system may be regulated by a network in which antigen induces production of idiotypes which in turn induce anti-idiotypes that can feedback to shut off or modify the original idiotypic response. Antibodies to idiotypes can readily be prepared by immunizing animals against purified idiotypes; anti-idiotypes so produced have been shown to influence immune reactions (*7*). However, the spontaneous generation of anti-idiotypes in response to immunization against an antigen has seldom been detected (*8*), and therefore it has been difficult to demonstrate a physiological function for the hypothetical idiotype–anti-idiotype network.

Sege and Peterson (*9*) proposed that a hormone could be used as an antigen to stimulate idiotypic antibodies that recognize the hormone. Such antibodies might be used to induce anti-idiotypes that sterically fit the combining sites of the idiotypes. Thus, the anti-idiotypes could have a three-dimensional configuration similar to that of the hormone antigen, and such anti-idiotypes might be able to bind to the hormone receptor. Accordingly, Sege and Peterson immunized rabbits against rat antibodies to insulin and obtained anti-idiotypic antibodies that bound to rat insulin receptor.

We explored the possibility that animals immunized against insulin might spontaneously develop antibodies to their own insulin antibodies, and that some of these anti-idiotypes might interact with the insulin receptor. Our approach was to immunize mice against bovine or porcine insulins and to assay samples of their serum for the appearance of two factors: antibodies to insulin

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