Table 1. Gross evidence of growth at 5 days in primary and challege grafts. Significance at $P \leq .05$ (by χ^2 method).

	Total (No.)	Growth (No.)	Rejection rate (%)
Primary	113	83	26.6
Challenge	35	19	46

mals were killed revealed depletion of lymphocytes in the Malphigian follicles to be most marked in animals 7 days after transplantation of challenge grafts. Spleens of these animals were larger as well.

The successful growth of these transplants was subject to a selective process partially affected by experimental technique and partially by the hosts' responses to the graft. With technique as a constant, the selective process eliminated almost twice as many challenge grafts as primary grafts (Table 1). Only the most vigorous challenge grafts were used for microscopic study. Therefore, the smaller values for these grafts (compared to primary grafts) in Figs. 1 and 2 are all the more noteworthy.

These studies indicate clear differences between primary and challenge grafts of trophoblast when judged by such criteria as gross success rate, size of the graft sites, number of viable cells at different stages, and host containment. The only difference between the test and control groups was that the test animals were previously exposed to a pure trophoblast colony which was absorbed. Therefore, prior exposure to trophoblast inhibits subsequent growth of this tissue, a phenomenon similar to the "second set" response demonstrating immunologic sensitization of a host to a prior graft.

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Ruminant-Like Digestion of the Langur Monkey

Abstract. *The adaptation of langur monkeys to a laboratory environment has made possible a detailed investigation of their digestive physiology. The diverticular form of the langur stomach permits a bacterial fermentation of the leafy diet, which results in important contributions to the nutrition of these primates. The demonstration of a ruminant-like digestion in langurs extends the known taxonomic distribution of this digestive adaptation.*

Old World monkeys of the subfamily Colobinae differ from all other primates in the large size and anatomical complexity of the stomach. These differences are related to a diet consisting mainly of leaves, hence the name "leaf-eaters," commonly used to describe these primates. Members of the Colobinae are found in large numbers in a broad belt across Africa and throughout southern Asia. Those in the latter area are commonly known as langurs.

Gastric contents of the langur constitute a high proportion of the animal's body weight and are normally maintained at a *p*H of 5.0 to 6.7. This range of *p*H permits an active fermentation of ingesta by the large numbers of anaerobic bacteria present. Cellulose-digesting bacteria occur in high numbers. Fermentation products are volatile short-chain fatty acids, ammonia, carbon dioxide, methane, and small amounts of hydrogen. The acids are produced in sufficient quantities to make major contributions to the nutritional economy of the langur monkey.

Considerable literature has accumulated on the peculiarities of the gastric anatomy of the Colobinae (*1*). A superficial resemblance of the stomach to the rumen of herbivorous animals has been noted, but a number of authors (*2–4*) have stated that rumination does not occur. Although rumination is an obvious characteristic of ruminants, recent work has placed greater emphasis on the fermentative processes occurring in the rumen. A major question with regard to the Colobinae is whether such a microbial fermentation of ingesta does occur. Drawert *et al.* (*5*) analyzed samples of gastric contents obtained from colobus monkeys in Africa and found high concentrations of short-chain volatile fatty acids, similar in concentration and character to the fermentation end products found in rumen contents.

We experienced the universal problems in adapting members of the Colobinae to conditions of captivity (*6*). On arrival from Asian sources, *Presbytis entellus* and *Presbytis cristatus*, although seemingly starved, showed little interest in the various kinds of leafy and other foods obtainable commercially. Successful adaptation followed the discovery that fresh alfalfa, available locally for most of the year, was eaten eagerly by both species. When high intake of alfalfa was established, the diet could be expanded to include yams, green beans, and a cereal preparation, which were previously rejected.

The stomach of the silvered leaf monkey, *P. cristatus*, has features common to gastric apparatus of other Colobinae (*1, 7*). The greatly distended and sacculated portion (saccus gastricus), corresponding to the fundus, is followed by a tubular portion (tubus gastricus), which leads to a third or pyloric segment (Fig. 1). An examination of the internal structure in both species revealed an esophageal groove (canalis gastricus; *Magenstrasse*) which appears to be characteristic of ruminant-like animals (*8*). This structure may allow ingested liquids to pass directly from the esophagus to the middle compartment of the stomach (*7*).

With colobus monkeys, Kuhn (*2*) obtained values for gastric contents of 11.5 to 20.6 percent of total body weight. In a specimen of *P. cristatus*, with a terminal body weight of 5.4 kg, the gastric contents weighed 938 g, 17 percent of body weight. The *p*H of gastric contents withdrawn by stomach tube from 50 animals ranged from 5.0 to 6.7, permitting a bacterial fermentation of the leafy foods ingested. The large capacity of the stomach ensures a delayed flow of digesta essential for extensive fermentation.

High numbers of bacteria, but no characteristic protozoal flora, were found in gastric contents of both langurs. As determined with the anaerobic culture techniques of Hungate (*9*), viable anaerobic bacteria ranged from 7×10^{11} to 1×10^{12} per gram of dry matter. These counts were consistently higher than the values normally reported for rumen contents. The flora was complex, with the ratio of strict anaerobes to aerobes in the range 100:1 to 1000:1. As langurs thrive on a leafy diet it is significant that large numbers of cellulose-digesting bacteria (8×10^7 to 4×10^8 per gram dry matter) were present in gastric contents. Two forms were isolated, a Gram-

positive coccus and a Gram-negative rod, belonging to the genus *Bacteroides*. These organisms are similar in number and kind to the major cellulose-digesting bacteria isolated from bovine rumen contents. In the langur, fermentation results in a gas mixture of methane, carbon dioxide, and small amounts of hydrogen. A search for methanogenic bacteria resulted in the isolation of *Methanobacterium ruminantium* (*10*) at high dilutions of stomach contents (4×10^9 organisms per gram of dry matter).

Stomach contents of both species of langurs contained volative fatty acids (VFA's) in concentrations and molecular proportions essentially similar to those found in ruminants (Table 1). In other animals these fermentation products are absorbed. The gastric concentrations of these acids therefore represent an equilibrium between their rate of production and their absorption or passage out of the upper stomach. Drawert *et al.* (*5*) found in *Procolobus verus* that the VFA concentration of the contents decreased from 230 mmole/liter in the tubus gastricus to 24 mmole/liter in the pyloric region, an indication that absorption occurred. A constant VFA concentration in langur stomach contents was demonstrated by analyzation of gastric samples taken successively from a specimen of *P. cristatus*. Fresh alfalfa was fed at zero time, and samples of gastric contents were withdrawn by stomach tube. Within 2 hours of feeding, the VFA concentration attained a high concentration which was maintained relatively constant throughout the 6.5 hours of the experiment (Fig. 2). The molecular proportions of the individual acids also remained constant. At 1 hour the molar ratio of acetic to propionic to butyric to *iso*-valeric to valeric acids was 56:24:13:1:7; at 6.5 hours it was 52:25:15:2:6. The ammonia concentration was also maintained at a high level, indicative of active bacterial proteolysis. The magnitude of the ammonia values was presumably related to the known high protein content of the young leafy portions of the alfalfa. The constancy of the gastric *p*H confirmed that langurs possess an excellent mechanism for controlling, within narrow limits, the *p*H of their stomach contents. In this respect, it is interesting that Ayer (*3*) has described the salivary glands of *P. entellus* as being very large in relation to the size of the animal.

A high rate of VFA production was found in fresh samples of gastric contents incubated anaerobically in vitro. Furthermore, the composition of the acid mixture produced in vitro was essentially identical with the composition of the VFA's in stomach contents, an indication that fermentation in vivo was not significantly disturbed. With the zero-time rate method of Carroll and Hungate (*11*), VFA's were found to be produced, at different times of day, at rates ranging from 423 to 526 μmole per gram of dry matter per hour. With the indirect method of El-Shazly and Hungate (*12*), rates ranging from 422 to 528 μmole per gram of dry matter per hour were obtained. Samples obtained before the morning feeding, 10.5 hours after the previous feeding, also produced VFA's within these limits. Thus, in the langur stomach microbial fermentation and VFA production is a continuous process. These fermentation rates are high in relation to rates obtained with domestic ruminants. Increased fermentation rate in small ruminants appears to be a mechanism for yielding relatively higher amounts of fermentation products (*13*).

Our data may be used to calculate the extent to which VFA's contribute to the langur's energy requirements. On the assumption that stomach contents average 12 percent of the body weight, with 15 percent dry matter, the contents of a 4.5-kg langur would amount to 81 g of dry matter. A VFA molar composition of acetic to propionic to butyric to valeric acids of 56:24:13:7 and an average fermentation rate of 460 μmole per gram of dry matter per hour, would result in a daily energy equivalent of 283 kcal, based on the heats of combustion of the individual acids. Maintenance energy requirement is proportional to the three-fourths power of the body weight of an animal. Calculated from Kleiber's figures (*14*) for other animals, the maintenance energy for a 4.5-kg langur would be 218 kcal. This comparison indicates that the langur gastric fermentation is a major factor in the energy metabolism of these animals.

The high numbers of cellulose-digesting bacteria in langur stomach contents suggest that structural carbohydrates may serve as an important energy source. However, regardless of the substrate used, it is clear that the microbial fermentation plays a major role in the langur digestion. The bacterial biosynthetic capacities may also benefit the vitamin and nitrogen economy of the host.

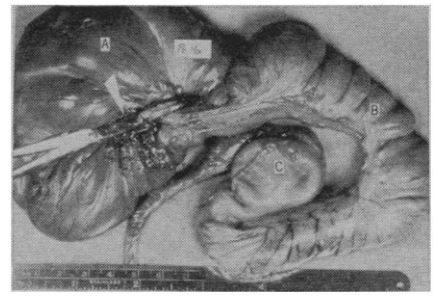

Fig. 1 (left). Stomach of *Presbytis cristatus*. Cardiac orifice indicated by an arrow. *A*, fundus; *B*, tubular portion; *C*, pyloric portion.

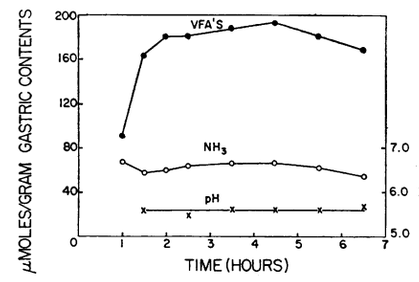

Fig. 2 (right). Changes in fermentation products in the gastric contents of *Presbytis cristatus*.

Table 1. Volatile fatty acids (VFA) in gastric contents of langur monkeys. Volatile fatty acids were determined by gas chromatography with a hydrogen flame detector.

Sample			Total VFA's (μmole/g wet matter)	Molecular proportions				
Hours after feeding	Dry matter (%)	*p*H		Acetic	Propionic	Butyric	*iso*-Valeric	Valeric
			Presbytis cristatus					
1	17.8	5.6	93	56	24	13	1	7
2	9.9	5.2	125	51	25	16	2	5
5	16.6	6.1	133	52	26	10	5	6
10	27.2	5.0	130	47	26	18	2	7
			Presbytis entellus					
2	14.2	6.3	103	50	28	14	4	5
3	16.7	5.8	233	46	22	23	0	9
3	12.9	6.5	89	48	30	14	1	7
3	17.2	5.4	124	47	33	14	1	6

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Olefins of High Molecular Weight in Two Microscopic Algae

Abstract. *The hydrocarbon composition of two algae, a golden-brown* (Botryococcus braunii) *and a blue-green* (Anacystis montana), *has been investigated by gas chromatography–mass spectrometry. Both show distributions of aliphatic hydrocarbons of odd carbon numbers in the medium and high ranges of molecular weight, with maxima at n-C_{17} and n-C_{29} for* B. braunii *and n-C_{17} and n-C_{29} for* A. montana. *With the exception of the* n*-heptadecane of* A. montana *all the hydrocarbons are monoenes, dienes, or trienes. Since certain continental sediments and oils show similar distributions of alkanes with respect to carbon number, these organisms may be the precursors of the hydrocarbons in these formations.*

There is very little data on the biogenesis of hydrocarbons by microorganisms (*1*). Most of the few microscopic algae and related organisms analyzed thus far have shown only small amounts of aliphatic hydrocarbons of relatively low molecular weight, with a maximum at about C_{17} (*2–5*). This is in contrast to the fact that higher plants synthesize substantial amounts of alkanes of high molecular weight, in the C_{23} to C_{33} range (*6, 7*), which have been usually considered the source of the paraffin wax in certain continental shales and petroleum crudes (*8*).

In a continuation of our studies on the distribution and genesis of hydrocarbons in nature (*1–3, 6, 9*), we have now found two algae, a golden-brown (*Botryococcus braunii*) and a blue-green (*Anacystis montana*) which, in addition to the common C_{17} aliphatic hydrocarbons, biosynthesize relatively large amounts of hydrocarbons of higher molecular weight.

We have selected *B. braunii* because it has been implicated in the formation of oil in tertiary sediments (*10*) and *A. montana* because it is considered a typical representative of one of the earliest forms of terrestrial life (*11*). Thus the new observations presented here may have significance not only on the formation of precursors of petroleum paraffins, but also on the interpretation of the alkane distributions reported for microfossil-bearing Precambrian rocks (*12, 13*).

In essence the experimental method followed consisted in growing the algae in the laboratory, extracting and fractionating their lipids, and analyzing the aliphatic hydrocarbon fraction by combined gas chromatography–mass spectrometry. The experimental details and analytical results are summarized below.

Botryococcus braunii and *Anacystis montana* were grown autotrophically in the light at 28°C. Bacteria-free cultures were employed and each culture was grown in three liters of D medium (*14*) and aerated continuously with filtered air. All cells were harvested by centrifugation, washed with a saline solution, and dried over P_2O_5 under vacuum.

The methods of extraction and fractionation, used to obtain the aliphatic hydrocarbon content of the organisms, have been reported previously (*3, 6, 9, 12*). Gas chromatographic analyses were performed on an F & M 810 gas chromatograph equipped with a flame ionization detector. An electronic digital integrator (Infotronics CRS 11/AB/H/41) provided an accurate quantitative analysis of the samples at the same time that the gas chromatographic pattern was obtained. Gas chromatographic–mass spectrometric analyses of the hydrocarbon fraction were carried out on an LKB 9000 gas chromatograph–mass spectrometer (*15*).

After these procedures the aliphatic hydrocarbons of *Botryococcus braunii* (Fig. 1) were identified as alkenes, with one, two, or three double bonds, ranging from C_{17} to C_{33}. The C_{27}, C_{29}, and C_{31} diolefins were predominant, the major component being the C_{29} diolefin. *Anacystis montana* (Fig. 2) shows a similar distribution with some particular differences. The olefins are mainly monoenes ranging from C_{19} to C_{29}, the major peak being the C_{27} mono-olefin. In this case heptadecane represents the only paraffin present.

Proper controls were run and necessary precautions were taken to exclude any possible source of contamination. Moreover, the unique nature of the patterns by themselves tends to minimize the possible contribution of extraneous material. Table 1 shows the relative percent composition of hydrocarbons in the cells. In the case of *A. montana*, only 85 percent of the total hydrocarbon content is reported in the table. The remaining 15 percent is made up by the unlabeled hydrocarbon peaks that can be seen in Fig. 2 which existed in small amounts and were not identified.

Identifications were supported in all cases by mass spectrometric data, and although there is little doubt concerning the values obtained for the molecular masses of these compounds (Table 1), it could be argued that they may correspond to monocycloalkanes and monocycloalkenes instead of to straight

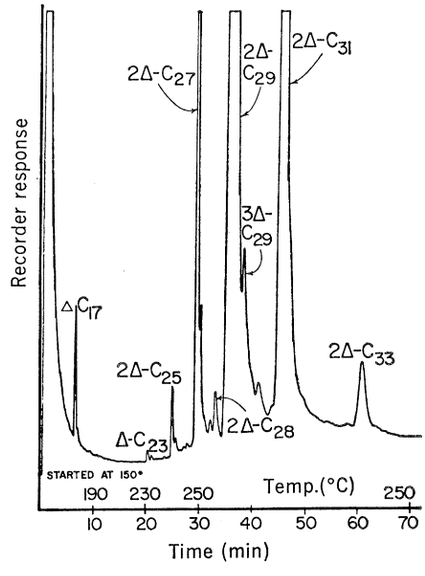

Fig. 1. Gas chromatographic separation of hydrocarbons of *Botryococcus braunii*, by use of an F & M 810 gas chromatograph equipped with a flame ionization detector. The glass column, 1.7 m by 0.3 cm inside diameter, was packed with OV-1 (methyl silicone fluid). The nitrogen pressure was 703 g/cm²; range was 10^2; and attenuation was 2.