Gondwana rocks are now seen to be preserved. These fractures were those most easily affected by stresses consequent upon vigorous sea-floor spreading relatively late in the history of the Indian Ocean when no further significant movement of India toward Asia could take place. This intracontinental orogenic uplift was facilitated (i) by the presence of a massive Phanerozoic succession lying on the Tibetan but not the Indian part and (ii) by the thick salt deposits at its base.

Association of Tibet with Australia explains the extraordinary distribution of the cladoceran *Daphniopsis* (*7*), recorded only in Kerguelen, Antarctica, Australia, Tibet, and Inner Mongolia. Search for a major plate boundary north of Tibet suggests the Tien Shan, a mountain system with a Phanerozoic history of great mobility and some peculiar features, rather than the Kun Lun–Astin Tagh which separate Tibet from the Tarim Basin. In the Kuruk Tagh in the eastern part of that basin, Norin (*8*) compared the tillite-bearing sequence, which would now be regarded as Precambrian-Cambrian, with that of the Adelaidean of the type sequence in South Australia. Today a more appropriate comparison would be with the Kimberley Basin and northern Western Australia, which on the suggested reassembly would be close.

The distribution of *Lystrosaurus* is in agreement with this hypothesis. Not only is it known in the Southern Hemisphere and India but it has been found in the Tien Shan and the Turfan Basin of Sinkiang (*9*). As related fossil reptilia occur in Shansi, it is probable that large areas of northern and northeastern China formed part of Gondwanaland (*10*). The suggested reassembly is shown in Fig. 2.

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10. P. M. Hurley, J. H. Lee, H. W. Fairbairn, W. H. Pinson, Jr. [MIT 19th Annual Progress Report (1971), pp. 5–13] suggest that China south of about latitude 40°N and Korea were both attached to India and lay west of Australia. While I agree with them that "it appears as if a Precambrian continental basement existed under much of what was formerly thought to be Tethys," it seems not possible for China south of about latitude 32°N and east of about longitude 102°E to be accommodated west of Australia, as these authors indicate diagrammatically. Allowance for Himalayan crustal contraction, together with the size of Tibet, fills the available space. If however northeastern China with much of Sinkiang was in Gondwanaland, southeastern China must have lain beyond it to become attached to Asia later. I agree with Hurley *et al.* that relationships of the floras of western North America and eastern Asia need checking by paleomagnetic work. A further problem is the occurrence of *Lystrosaurus* in Laos (E. H. Colbert, personal communication), which suggests a land communication with Gondwanaland; but paleomagnetic data from western Malaysia show that area to have been far distant from Australia in Mesozoic time (N. S. Haile and M. W. McElhinny, personal communication).
11. I thank colleagues in Australia and elsewhere, too numerous to name individually, for help and critical comment.

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Methane Formation in Living Trees: A Microbial Origin

Abstract. *Visibly healthy hardwood trees located on poorly drained soils contained high pressures of methane. Heartwood from these trees was water-soaked, neutral to alkaline in* pH, *fetid in odor, and infested with a diverse population of obligately anaerobic bacteria. The bacterium responsible for methane formation in trees was isolated and characterized as a member of the genus* Methanobacterium.

Methane has occasionally been either reported or inferred to be part of the composition of flammable gases that are sometimes trapped within the trunks of living trees (*1*). However, the factors responsible for the formation of methane in trees have remained a mystery. We report here a microbial origin for methane found in living trees.

Bushong (*1*) reported the first analysis of gases drawn from a tree and demonstrated the presence of flammable gas in a large cottonwood tree. During the course of microbial studies with forest trees we have observed high pressure gas release from newly made increment borer holes. The gas could usually be ignited producing a blue flame (Fig. 1). The wood from these trees was often so soaked with water that a stream of fetid liquid was ejected along with the gas to distances of 15 to 46 cm, thereby extinguishing the flame. Our field observations, in addition to reports by other investigators (*1*, *2*), indicate that high gas pressures do not occur in most trees and may be a condition not associated with normal growth. This study was initiated to determine the nature of high gas pressures in trees.

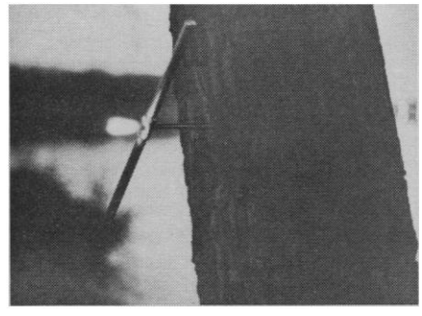

Fig. 1. Ignited methane gas emanating from a hollow increment borer bit drilled 20 cm into a cottonwood tree located on the shore of Lake Wingra, Wisconsin. Photograph is from a time exposure taken at night.

Visibly healthy trees with trunk diameters ranging from 50 to 90 cm (*3*) were selected at various locations in Wisconsin. Trees sampled included the following four species of hardwoods: American elm, *Ulmus americana* L.; black willow, *Salix nigra* Marsh.; white poplar, *Populus alba* L.; and eastern cottonwood, *P. deltoides* Bartr. An increment borer was used to sample tree trunks approximately 1 m above ground level. At intermittent distances inward boring was halted, a wood increment core was removed, and a rubber septum was immediately inserted into the borer orifice. Gas volumes twice the borer volume were first removed with a syringe, and then gas samples were collected for later analysis in a gas chromatograph equipped with a thermal conductivity detector. Methane was always a component of trunk gases that were under high pressure and was detected only in gas samples from cottonwood, elm, and willow trees that contained wetwood, an abnormal type of heartwood (*4*).

Wetwood cores removed from methane-positive trees were neutral to slightly alkaline (*p*H 6.8 to 7.8) and possessed a characteristic volatile fatty

Table 1. Composition, by volume, of gases in cored trees sampled in August 1973.

Specimen	Heartwood type	Percent of total gases				
		CO_2	CH_4	O_2	N_2	H_2
	*Alluvial sand and silt soil**					
Cottonwood A	Wetwood	14.1	61.7		23.2	1.0
	Lacustrine marl and peat soil†					
Cottonwood B	Wetwood	16.3	55.2		27.0	1.5
White poplar	Normal	18.5		6.4	75.1	

* Mississippi River, Wyalusing, Wisconsin. † Lake Wingra, Madison, Wisconsin.

acid odor which was almost indistinguishable from that of rumen fluid. At times, wetwood samples also possessed a pungent odor of hydrogen sulfide. Normal heartwood from trees lacking methane was acidic (*p*H 5.3 to 5.8) and was devoid of the volatile fatty acid and hydrogen sulfide odors. Methane was not found in all trees of the species studied, although trees containing methane were numerous and easily located on poorly drained soils, particularly in low-lying areas surrounding lakes and rivers.

The gas composition of two selected methane-positive trees growing in bottomlands of different soil types about 90 miles (1 mile = 1.6 km) apart is shown in Table 1. Methane was the major constituent in these trees, hydrogen was detected, and oxygen was absent. Since all cottonwoods sampled contained wetwood and methane, a white poplar growing near cottonwood B was selected for comparison of gases in trees with normal heartwood. The gas composition values for the white poplar reflect those of methane-negative trees. In these trees nitrogen was the major gaseous component, oxygen was present, and methane and hydrogen were absent. Our studies have shown that methane was the major component of trees containing high gas pressure, whereas nitrogen was the major gaseous component of normal trees.

The methanogenic activity present in heartwood removed from a tree containing methane is shown in Fig. 2. By means of an aseptic technique, an increment borer was used to obtain several different cores from the same area of cottonwood A listed in Table 1. Wetwood samples from each of the cores were immediately placed into anaerobic containers, sealed under nitrogen gas, and transported to the laboratory. Wetwood (2.0 to 2.3 g) from each core was added to separate anaerobic vessels containing 25 ml of phosphate-buffered (*p*H 7.2) inorganic salts media. These containers were sealed under an atmosphere of nitrogen or a mixture of hydrogen and carbon dioxide (H_2 and CO_2) and allowed to incubate at 25°C. Gas samples (0.4 cm^3) were withdrawn at intervals and analyzed for methane with a gas chromatograph equipped with a flame ionization detector. Methane was immediately detected when wetwood was incubated under an atmosphere of N_2 or of H_2 and CO_2. No methane was formed when wetwood was autoclaved prior to incubation under N_2 or H_2 and CO_2. In addition, methane formation was not detectable when wetwood was incubated in an atmosphere of H_2 and CO_2 with O_2. Wetwood incubated under H_2 and CO_2 produced methane during the first 48 hours of incubation at a much faster rate than that when incubated under N_2. This rapid rise in methane formation was probably a reflection of H_2 and CO_2 consumption by the indigenous methanogenic bacterial flora. Both H_2 and CO_2 individually are preferred substrates for growth of methane-producing bacteria (*5*). Methane formation in wetwood ceased when chloroform was added to a final concentration of 2 percent in an atmosphere of H_2 and CO_2. Chloroform is a potent inhibitor of methane-producing bacteria (*6*). These findings suggest that visibly healthy hardwood trees might be infested with a microbial population capable of producing methane.

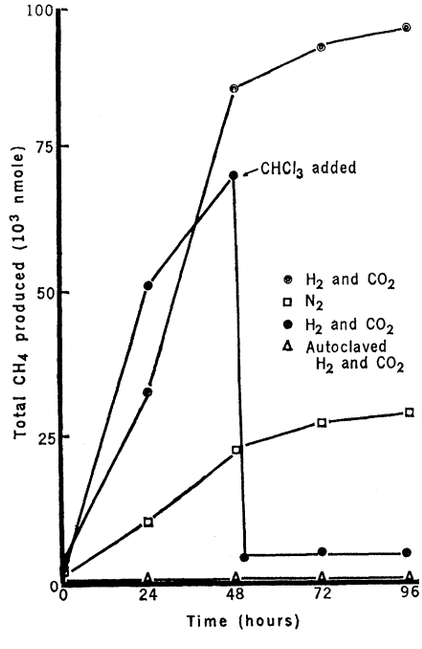

Fig. 2. Methane formation by wetwood samples removed from a cottonwood tree having high pressures of methane. Anaerobic incubation of wetwood in an atmosphere of H_2 and CO_2 (⊙) resulted in an increased rate of methane formation, when compared to wetwood incubated under N_2 (□). Autoclaving wetwood prior to incubation resulted in the loss of methanogenic activity (△). The addition of $CHCl_3$ to wetwood incubated with H_2 and CO_2 for 48 hours terminated methane production (●).

Microscopic observation of fetid liquid from methane-positive trees revealed a dense bacterial population of diverse morphological types including rods, cocci, spirals, and spores. By means of anaerobic culture techniques (*7*), fetid liquid from infested heartwood was serially diluted into a nonspecific complex medium, and a mineral salts medium was used for the enrichment of methanogenic bacteria. The diluted liquids were gased with an 80/20 mixture of H_2 and CO_2 and allowed to incubate for 2 weeks at 30°C. Growth in end dilution tubes of complex media demonstrated that wetwood from various cottonwood, elm, and willow trees contained between 10^7 to 10^9 bacteria per milliliter of fetid liquid. Growth and methane production in end dilution tubes of both media revealed that methane-positive trees contain between 10^3 to 10^5 methane-producing bacteria per milliliter of fetid liquid.

A methane-producing bacterium was isolated from the heartwood of cottonwood A (Table 1). This strict anaerobe was a gram-positive, nonmotile, curved rod that proliferated and produced under an atmosphere of H_2 and CO_2 methane in an inorganic salts medium. On the basis of these characteristics, this organism can be classified as a member of the genus *Methanobacterium* (*8*). It appears likely that this bacterium was responsible for methane formation in infested trees. This methanogenic organism was observed in all enrichment cultures obtained from either fetid liquid or wetwood cores from cottonwood, elm, and willow trees containing methane. Other types of methanogenic bacteria were not isolated from these samples.

These data reveal that bacteria are the causative agents for methane formation in living trees. The growth of

anaerobic bacteria in heartwood accounts for the high gas pressures observed in trees. The factors responsible for proliferation of methanogenic bacteria in trees are questions of considerable biological interest. We have detected methane only in trees that contain wetwood with a distinct volatile fatty acid odor, neutral to alkaline *p*H, and under strictly anaerobic conditions.

The high number of methanogenic bacteria and other anaerobes found in wetwood indicates vigorous microbial fermentation. The presence of these anaerobic bacteria in heartwood may constitute an infection of trees. It is difficult to ascertain whether the wood tissue itself is being decomposed or whether other nutrients serve as the substrates for this methane fermentation. The formation of heartwood in a tree is thought to involve, in part, translocation of excretory products from living xylem tissue toward the center of the stem (*9*). However, wetwood or bacterially infected heartwood from some trees is noticeably weakened (*10*), and this may be an indicator of microbial decomposition of the wood tissue. Methane-producing bacteria are unable to degrade wood, but convert end products from the anaerobic decomposition of organic matter, namely, H_2 and CO_2, into methane. Various kinds of bacteria reside in infested trees in addition to the methanogenic species isolated. Previous investigators (*11*) have described facultative and obligate anaerobic bacteria isolated from wetwood which decompose organic matter. The establishment of an anaerobic bacterial population in trees capable of producing methane probably results from root injury, especially in water-saturated soils, which provides a path of entry for indigenous soil microorganisms.

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12. We thank the University of Wisconsin Arboretum Committee and H. A. Peterson, Wisconsin Department of Natural Resources, for the use of field facilities and D. L. Henning for technical assistance. This work was supported by grant GB 41925 from the National Science Foundation.

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Lateral Phase Separation of Lipids in Plasma Membranes: Effect of Temperature on the Mobility of Membrane Antigens

Abstract. *Cooling populations of newly formed mouse human heterokaryons has effects on the intermixing of mouse and human surface antigens which indicate the occurrence of phase separations in membrane lipids. Antigen mixing, previously shown to be due to diffusion in the plane of the membrane, is retarded when cells are cooled from 37° to 21°C, but is then speeded by further cooling to 15°C. This result is in accord with observations on phase separations of lipids in artificial and bacterial membranes.*

Lipids of artificial and natural membranes have been shown by several physical methods to undergo changes from fluid to solid phase as the membranes are cooled (*1*). In artificial membranes these changes have generally been described as phase transitions, implying that an entire membrane changes from fluid (liquid-crystal) to solid (gel phase) over a narrow temperature range. However, phase changes especially in membranes of mixed lipid species have been discussed in terms of phase separations, rather than as phase transitions (*2, 3*). It has been suggested that several transition temperatures can be defined in mixed lipid membranes, in which classes of lipid molecules coaggregate and solidify when a membrane is cooled, leaving other lipids, with lower melting temperatures still fluid. Phase separations then result in a membrane containing areas of both solid and fluid phase lipids. One consequence of phase transition or phase separation may be an increase in the rate of movement of proteins or ions within or through a membrane as it is cooled. Such increased mobility due to phase separations of lipids has been shown for ion permeation and sugar transport in artificial and bacterial membranes (*4*). We now describe evidence for phase separations of membranes in mammalian cell plasma membranes. A detailed study of the effect of temperature on the intermixing of histocompatibility antigens in the surface membranes of virus-fused mouse and human cells (heterokaryons) shows that within a temperature range of 5° to 6°C cooling the cells speeds the rate of their antigens' diffusion within the plane of the membrane.

Earlier it was found that the surface antigens of heterokaryons formed from cultured mouse and human cells initially lay in separate areas of the membrane, but rapidly intermixed; antigens of both species come to lie randomly throughout the surface within 1 hour of heterokaryon formation (*5*). Antigen mixing, observed by reacting samples of the heterokaryon population with fluorescent antibodies, was not affected by metabolic or biosynthetic inhibitors but was slowed when the cells were incubated below 37°C after formation. The rapid rearrangement of surface antigens occurring at 37°C appears to be due to their diffusion in the plane of the plasma membrane, lateral diffusion. The temperature dependence of the process would then be due to increases in viscosity of membrane lipids with decreasing temperatures, in agreement with other data on protein diffusion in animal cell membranes (*6*). The relation between antigen mixing and temperature suggested a sharp phase transition in the membrane lipids around 15°C, since there was little or