5. C. J. Velz, in Proceedings of a Seminar on River-Water Quality Assessment, American Water Works Association, New Orleans, 20 to 25 June 1976. An excellent treatment of the nature of river quality problems and techniques for their assessment is given by C. J. Velz [*Applied Stream Sanitation* (Wiley, New York, 1970)].
6. D. A. Rickert, W. G. Hines, S. W. McKenzie, *Water Resour. Bull.* **11**, 1013 (1975); *U.S. Geol. Surv. Circ. 715-C* (1976).
7. River quality problems are not separable into disciplines. Any successful study of such problems requires a true interdisciplinary approach in which participants are committed to working as a problem-solving team.
8. Details of the assessment are described in the 13 chapters of *U.S. Geol. Surv. Circ. 715*. Some of the chapters are cited separately herein.
9. G. W. Gleeson, Advisory Committee on Environmental Science and Technology and the Water Resources Institute, Oregon State University, Corvallis (1972); W. G. Hines, S. W. McKenzie, D. A. Rickert, F. A. Rinella, *U.S. Geol. Surv. Circ. 715-I* (1978).
10. S. W. McKenzie, W. G. Hines, D. A. Rickert, F. A. Rinella, *U.S. Geol. Surv. Circ. 715-J*, in press.
11. D. A. Rickert, W. G. Hines, S. W. McKenzie, in *Urbanization and Water Quality Control*, W. Whipple, Jr., Ed. (American Water Resources Association, Minneapolis, 1975), pp. 70–84.
12. D. A. Rickert, R. Petersen, S. W. McKenzie, W. G. Hines, S. A. Wille, *U.S. Geol. Surv. Circ. 715-G* (1977).
13. D. A. Rickert, V. C. Kennedy, S. W. McKenzie, W. G. Hines, *U.S. Geol. Surv. Circ. 715-F* (1977).
14. W. M. Brown, W. G. Hines, D. A. Rickert, G. L. Beach, *U.S. Geol. Surv. Circ. 715-L*, in press.
15. W. G. Hines, D. A. Rickert, S. W. McKenzie, *U.S. Geol. Survey Circ. 715-D* (1976); *J. Water Pollut. Control Fed.* **49**, 2031 (1977).
16. W. G. Hines, D. A. Rickert, S. W. McKenzie, J. P. Bennett, *U.S. Geol. Surv. Circ. 715-B* (1975).
17. T. J. Tuffey, J. V. Hunter, V. A. Matulewich, *Water Resour. Bull.* **10**, 555 (1974).

Microtubules in Prokaryotes

Universally involved in mitosis and motility in eukaryotes, microtubules are seen in spirochetes.

Lynn Margulis, Leleng To, David Chase

Summary. Longitudinally aligned microtubules, about 220 Å in diameter, have been seen in the protoplasmic cylinders of the following spirochetes (symbiotic in the hindguts of dry-wood and subterranean termites): *Pillotina* sp., *Diplocalyx* sp., *Hollandina* sp. They are also present in a gliding bacterium from *Pterotermes occidentis*. These microtubules are probably composed of tubulin, as determined by staining with fluorescent antibodies to tubulin and comigration with authentic tubulin on acrylamide gels. *Treponema reiteri* lack tubulin by these same criteria. These observations support the hypothesis of the symbiotic origin of cilia and flagella from certain spirochetes.

Microtubules, 250 angstroms in diameter, composed of tubulin proteins are universal constituents of eukaryotic cells. They take part in flagella movement (*1*), and in intracellular transport such as that in nerve cells (*2*), protozoans (*3*), hydras (*4*), and fungi (*5*). Microtubules have a role in regenerative morphogenesis (*6*) and underlie many cell structures, especially those in protists and animals (*7*). They comprise the mitotic spindle (*8, 9*) and are intimately involved, in still incompletely known ways, in the segregation of the chromosomes to the poles in mitosis in nearly all eukaryotes (*8–11*). Tubulin proteins from very different sources show a great deal of homology (*12, 13*); most microtubules are sensitive to cold and most bind alkaloids such as colchicine, vinblastine, and lignans such as the podophyllotoxin derivatives (*12–14*). But some microtubules are insensitive to these agents (*15*).

In general, neither microtubules nor microtubule proteins are known to occur in prokaryotic organisms such as *Escherichia coli* or *Bacillus*. However, hollow tubular structures have been reported in cell walls in certain blue-green algae (*16*). Smaller tubular structures have also been reported in *Proteus mirabilis* and in *Treponema*, but not in *Borrelia* (*17, 18*). None of these prokaryotic tubules have been studied chemically. Microtubules have not been observed to be involved in prokaryotic cell division.

There are two classes of hypotheses for the origin of eukaryotic microtubules. The exogenous (*19*) hypothesis suggests the origin of tubules in eukaryotic cells by symbiotic acquisition of tubule-containing spirochetes. Originally selected because associated spirochetes conferred motility on their hosts, as time went on the spriochetes evolved into the ubiquitous (9 + 2) flagella or cilia (undulapodia) (*20*) of eukaryotes. If such ancestral spirochetes have not become extinct, it is expected that free-living descendant spirochetes, ancestors to the (9 + 2) flagellar organellar system, will be found and that these will contain microtubules. These hypothetical tubules are predicted, therefore, to be composed of tubulin proteins and to be homologous to those in eukaryotic cells. The endogenous hypotheses of Pickett-Heaps (*11*) and Taylor (*21*) of the origin of tubules suggests that tubulin differentiated in primitive photosynthetic organisms ancestral to modern red algae.

Electron Microscopy

The observations (*22, 23*) of intracytoplasmic microtubules, 250 Å in diameter, in large spirochetes—such as those in the hindguts of termites—did not come to our attention until 1972. Hollande and Gharagozlou placed the microtubule-containing spirochetes in the genus *Pillotina* and in a new family, the Pillotaceae. The difficulty of obtaining the termite host endemic to the island of Madeira limited further work until these same pillotinas were recognized in electron microscopic (EM) preparations of *Reticulitermes flavipes* (*24*). Since 1974 we have found the large distinctive pillotina spirochetes and their smaller hollandina relatives present in hindgut microbiota from 21 out of 21 species of dry-wood termites [family Kalotermitidae (Fig. 1)], from five out of five species of subterranean termites (family Rhinotermitidae, for example, *R. flavipes*, *R. flavicollis*, *R. hesperus*, and *Heterotermes aureus*), but absent in two out of

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two damp-wood termites (Hodotermitidae) and one unidentified species of nasutitermitid from Paquera, Puerto Rico (*25*). The abundance of these large spirochetes in the paunch (modified hindgut) (*26*) in dry-wood termites made biochemical studies possible although no attempts at in vitro growth of pillotinas have yet succeeded [motile spirochete maintenance of up to 5 days in culture under anaerobic conditions is described in (*25*)].

In this article, we present evidence for tubulin-containing microtubules in three types of related prokaryotic microbes. Our evidence is based on three different techniques: (i) observation of microtubules by transmission electron microscopy (TEM); (ii) isolation and separation of a soluble protein that comigrates electrophoretically on acrylamide gels with authentic tubulin from a hindgut fraction that contained only prokaryotes, primarily those spirochetes in which microtubules had been seen; and (iii) specific antitubulin immunofluorescent staining with fluorescent antibody to various authentic eukaryotic tubulins.

Micrographs of the basic structure of these extremely obscure symbionts are

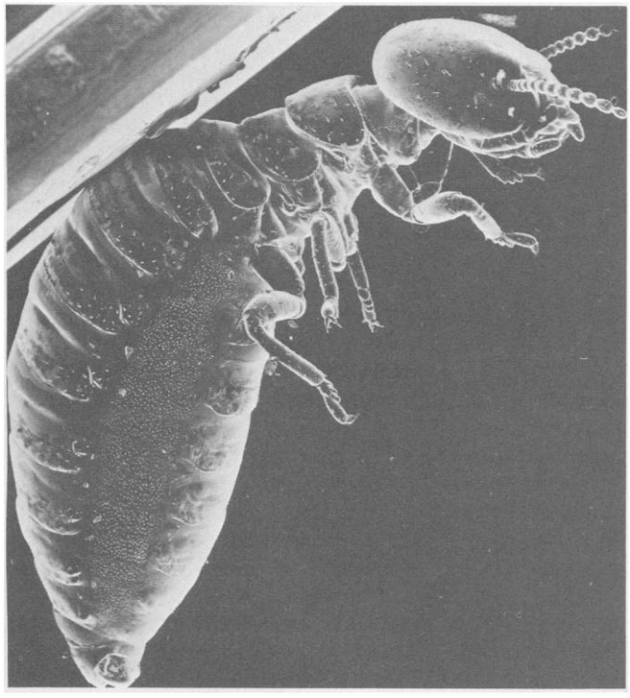
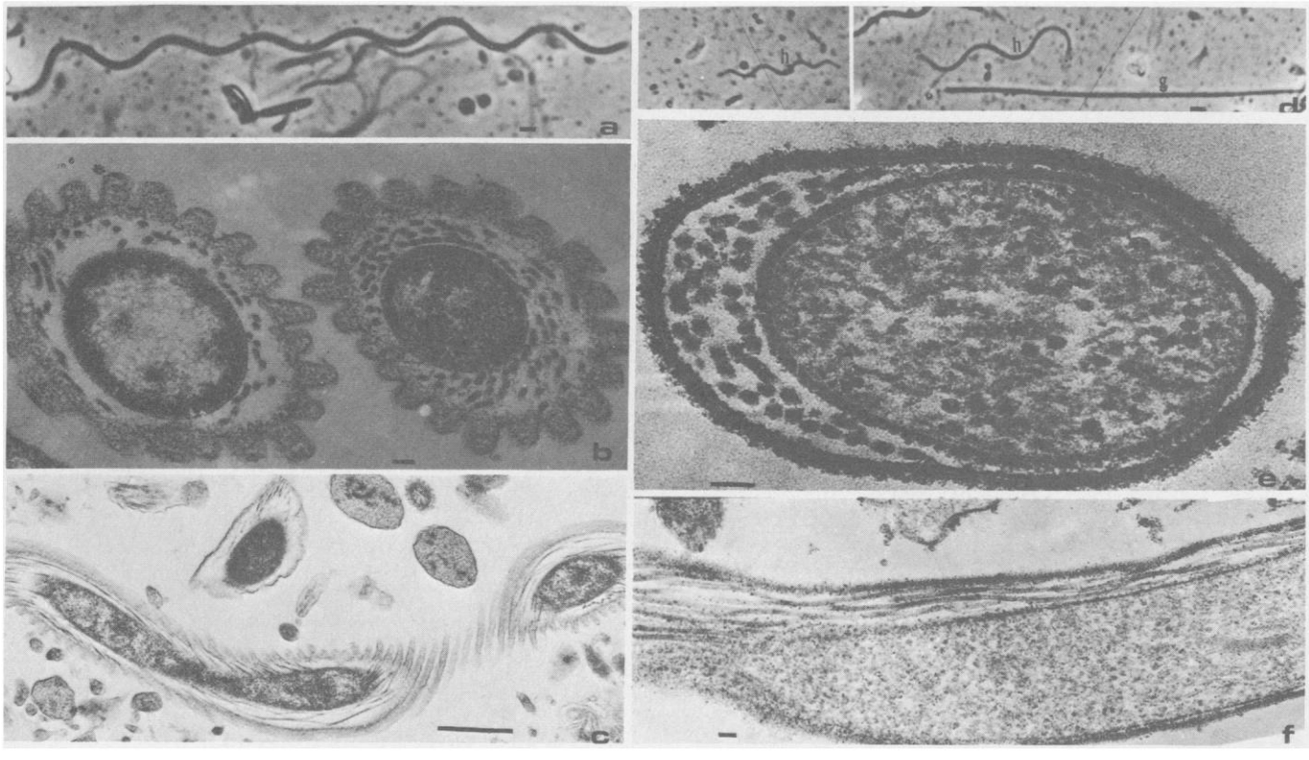


Fig. 1 (left). The Sonoran desert termite, *Pterotermes occidentis* (Walker), has been the best source for spirochetes and related bacteria in quantity that apparently contain microtubule protein. Tubulin protein previously has been thought to be limited to eukaryotes. The swollen abdomen of this pseudergate (wood-eating larva, or worker) is typical: within it lies the paunch or hypertrophied hindgut packed with many species of cellulolytic and nitrogen-fixing microorganisms which permit the termite colony to survive on an exclusive diet of wood, in this case, of the Palo verde (*Cercidium*) tree. Fig. 2 (below). Microorganisms symbiotic in termite hindguts in which microtubules have been found. (a) *Pillotina* sp. from *P. occidentis*. Phase contrast, live (bar, 1.0 μm). (b) Transverse section of *Pillotina* sp. from *Kalotermes schwarzi*. Transmission electron micrograph (bar, 0.1 μm). (c) Longitudinal section of *Pillotina* sp. from *Reticulitermes hesperus*. Transmission electron micrograph (bar, 1.0 μm). (d) *Hollandina pterotermitidis* (*h*), *Pillotina* sp., and unidentified gliding bacteria (*g*), from *Pterotermes occidentis* (bar, 10 μm). (e) Transverse section of *Hollandina* sp. Transmission electron micrograph (bar, 0.1 μm). (f) Longitudinal section of *Hollandina* sp. Transmission electron micrograph (bar, 0.1 μm).



presented in Fig. 2. All three—*Pillotina* sp. (Fig. 2, a to c), *Hollandina* sp. (Fig. 2, d to f), and the long skinny unidentified gliding rod [probably one of the organisms referred to as *Bacillus flexillus* in 1927 (*27*)] (Fig. 2d, labeled *g*)—are always extremely motile when healthy. Thus, the original observations of microtubules in pillotinas (*22, 23*) have been confirmed by us and extended to include a third spirochete and a gliding bacterium.

Termites were collected and identified as indicated in the tables; they were maintained as described (*25*). Electron microscopic techniques (*24*) were used, except that 5 percent tannic acid was added in some cases to the glutaraldehyde fixative to enhance the probability that the tubules would be seen, and impregnation times were lengthened to as much as 36 hours, depending on the size of the gut.

Occasional sections containing microtubules were seen in pillotinas from subterranean termites *R. flavipes* and *R. hesperus*. They were even more frequently seen in the medium-sized (0.75 to 0.90 micrometer in diameter) hindgut spirochetes described as *Hollandina calotermitidis*, from *Kalotermes schwarzi* (*25*). The microtubules are longitudinally aligned, and often look as though they follow the contours of the spirochetes' helical bodies, but they are not seen in all sections. Similar, if not identical, microtubules have also been seen in long skinny "gliding bacteria" unidentified but under study (Fig. 3c) and in hollandinas (Fig. 3d); both microbes come from the hindguts of the Sonoran desert dry-wood termite *Pterotermes occidentis* (Fig. 1). The outer diameters of the microtubules measured 240 ± 15 nanometers as determined on glossy prints (8 by 10 inches), with the aid of calipers and a dissecting microscope. The microtubules were always intracytoplasmic and were always larger than the atypically large axial filaments (spirochete endoflagella, 200 to 210 Å in diameter) of these microbes. In one case, tubules were seen in a gliding bacterium also infected with phage (Fig. 3e). No comparable tubules were seen in the many other termite hindgut prokaryotes studied (for example, spirilla; *Coleomitus* sp., the large filamentous endospore-forming bacterium, or the tiny treponeme-like spirochetes) nor have they been seen in *Treponema reiteri* (*18*).

Specific Immunofluorescence of Antibody to Tubulin

A specific antibody to microtubule protein has been developed (*8, 9, 28*). This antibody can be labeled with fluorescein and used in combination with fluorescence microscopy as a cytological stain; tubulin can be directly visualized, as can be seen in the micrographs of Fuller *et al.* (*9*). A Zeiss microscope was fitted with an AO xenon ultraviolet light

Table 1. Antibody tubulin; sources and results; SAA, same as above.

Antigen	Antibody originally tested against and result	F*	Microbe termites†	F	Comments
Source: G. M. Fuller and B. R. Brinkley					
Bovine brain 6*S* tubulin	Dividing and interphase human, kangaroo, mouse, monkey, rat, and hamster embryo cells (*9*)	+	*K. schwarzi*		Indirect technique‡
			Pillotinas	+	
			Hollandinas	+	
Rabbit serum					
			Tiny (treponeme-like) spirochetes	−	
			Flagellates	+	
			Spirilla	−	
			Coleomitus sp.	−	
			Skinny gliders	+	
			K. schwarzi		
			Fluorescein alone	−	
			IgG goat-fluorescein alone	−	
			Buffer preparations alone	−	
Source: K. Fujiwara					
Strongylocentrotus (sea urchin) unfertilized eggs vinblastine precipitated tubulin	Egg homogenate	+	*K. schwarzi*		Direct antitubulin labeled with fluorescein. Best preparations were with this antibody‡
	Tetrahymena cilia	+	SAA	SAA	
	Sperm (cranefly testes) flagella	+			
	Sea urchin sperm tail axonemes	+	*Pterotermes occidentis*		
	Rabbit muscle actin	−	Hollandinas	+	
	Myosin	−	Skinny gliders	+	
	Bovine serum albumin	−	Miscellaneous small bacteria and spirochetes	−	
	Chick, rat, mouse, fish frog brain tubulin	+	*Coleomitus* (two sp.)	−	
	HeLa cells, mitotic spindle	+			
			Treponema reiteri		
			Pelleted, pure culture	−	
Source: V. Kalnins					
Porcine brain tubulin	Embryonic chick and brain tubulin	+	*K. schwarzi*		Indirect technique, same as with Fuller antibody
	Porcine brain	+	Hindgut microbes, results identical to those with Fuller antibody listed above		
	Actin	−			

*Fluorescence. † *Kalotermes schwarzi* was collected by R. Syren and W. Ormerod in South Florida and identified by R. Syren, University of Miami. *Pterotermes occidentis* was collected by W. Nutting and L. Margulis in the Sonoran desert 20 miles south of Tucson, Arizona, and identified by W. Nutting, University of Arizona. ‡The best technique for indirect staining was as follows. Hindguts were removed with fine forceps on alcohol cleaned and flamed slides fixed with 5 percent formaldehyde in Trager's solution. The gut contents were drawn out into fixative with a fine-needle syringe. The preparation was washed with Trager's, placed in absolute acetone at − 8°C for 7 minutes, washed two to three times again with Trager's solution, and dried in air. It was incubated for 45 minutes at 35° ± 1°C with rabbit antibody to tubulin; the particles were then washed three times. When just a film of Trager's solution was left, goat antibody to normal rabbit IgG conjugated with fluorescein was added dropwise, and the preparation was incubated for 30 minutes in a humid chamber. It was washed thoroughly with Trager's and observed, and was then stored in a refrigerator with a desiccant. Direct techniques are the same except that only one 45-minute incubation with fluorescein-bound rabbit antibody to tubulin made against vinblastine precipitated sea urchin tubulin. Slides mounted for observation in 90 percent glycerin and 5 percent phosphate-buffered saline.

source and appropriate heat and interference filters to permit absorption of light at wavelengths from 490 to 495 nm. Guts were dissected, and the contents were removed to slides cleaned with alcohol. Formaldehyde (1 to 5 percent, or occasionally ethanol) was used as fixative; the hindgut preparations were stained for 45 minutes at 30°C with fluorescein-labeled antibody to tubulin directly or indirectly (Table 1). This experiment was repeated with slight variations five times, and the same observations were consistently made. In hindgut preparations of microbes from *Kalotermes schwarzi* or *Pterotermes occidentis*, the following organisms showed bright fluorescence: polymastigotes and hypermastigotes (particularly the rostral region although these fixatives were very poor for flagellates) and their flagella, pillotina and hollandina spirochetes, and the long, thin "gliding bacteria". In the same preparations the large *Coleomitus* sp., the smaller treponeme, spirilla, and other bacteria did not fluoresce; wood particles in the gut lumen and within the flagellates tended to fluoresce orange, whereas the spirochetes and flagella fluoresced green (Fig. 3, f and g). The consistent fluorescence of the thin gliding bacteria (0.45 by 15 μm) prompted our search for microtubules in them. These observations on the structure, behavior, and movement patterns of the gliders have led us to agree with Canale-Parola (*29*) that the "phylogenetic relationship between spirochetes and gliding bacteria may be closer than previously believed."

Significant and regular fluorescence was seen down the entire length of those spirochetes and gliding bacteria that gave positive signals. In fact, the fluorescent stain in the spirochetes was comparable in intensity to that of the flagella of neighboring polymastigotes and hypermastigotes. Preparations, even if refrigerated, aged in a day or so. The sharp image was replaced by a zone of fluorescence surrounding their contours or collecting toward the periphery of the spirochetes or flagella.

To verify the specificity of the fluorescence observations the following experiments were done with absorbed antiserum. Antiserum to tubulin (from K. Fujiwara, see Table 1) was absorbed with mouse brain tubulin for 18 hours at 4°C. It was also absorbed with a spirochete-enriched hindgut preparation free of flagellates, and washed three times with phosphate buffered saline. The indirect immunofluorescence, checked on slide preparations of hindgut microbes, treated with serum, yielded the following results: (i) antiserum to tubulin and fluorescein-conjugated immunoglobulin G (IgG)–stained preparations gave fluorescent spirochetes and flagellates as above and in Fig. 2, f and g; (ii) antitubulin absorbed with brain tubulin gave no fluorescence for either spirochetes or flagellates; (iii) antitubulin absorbed with the sonicated spirochete-enriched fraction gave positive but diminished fluorescence for both spirochetes and flagellates; (iv) normal rabbit serum poststained with fluorescein-conjugated IgG; and (v) fluorescein isothiocyanate alone gave no fluorescence of either spirochetes or flagellates. These results are consistent with our hypothesis that protein with antigenic sites that cross react with authentic tubulin is present in hollandina and pillotina spirochetes, as well as, of course, in the hypermastigote and polymastigote flagellates of the termite hindgut.

We feel that it is improbable that the fluorescing material is due to nonspecific adherence to the surface of the spirochetes and gliding bacteria, especially since aged preparations of flagella of hypermastigotes and polymastigotes behaved the same way, whereas filamentous spore-forming bacteria took up no stain whatsoever. It is more likely that the soluble complex of tubulin with the fluorescein-conjugated antibody to tubulin diffuses, over time, out of fixed material. In fact, the intensity of the spirochete fluorescence suggests that pillotinas, hollandinas, and gliding bacteria contain a large tubulin fraction that is not

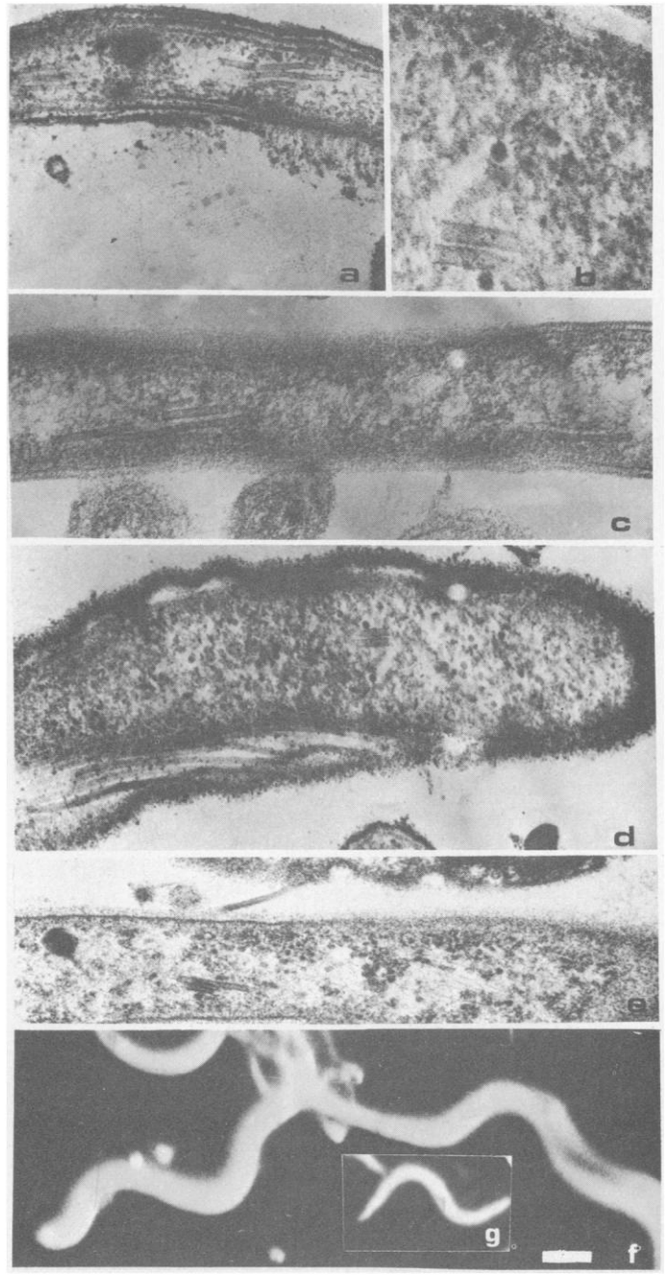


Fig. 3. Microtubules in the cytoplasm of the three types of prokaryotes shown in Fig. 2 and fluorescence micrographs of hindgut spirochetes stained with fluorescein - labeled antibody to tubulin. All microtubules are 240 ± 15 Å in diameter. (a) *Hollandina pterotermitidis*, longitudinal section. (b) *Hollandia pterotermitidis*, higher magnification, tannic acid added to fixative. Walls of tubules from 35 to 50 Å. (c) Unidentified gliding bacterium from *P. occidentis*. (d) *Hollandia pterotermitidis* showing microtubules and sheath bound flagella. (e) Unidentified gliding bacterium. Note both microtubules and bacteriophage with tail present in section. (f) Fluorescing *Pillotina* sp. from *Kalotermes schwarzi*. (g) Fluorescing *Hollandina* sp. from *K. schwarzi* (bar, 1.0 μm.).

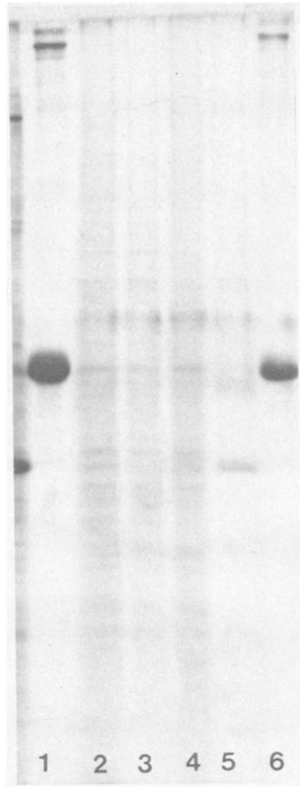


Fig. 4. Sample of gel electrophoresis from *Pterotermes occidentis*. 1, Tubulin standard, pig brain; 2, spirochete fraction separated by centrifugation, only prokaryotes; 3, polymastigote fraction unavoidably containing attached and associated spirochetes; 4, hypermastigote fraction unavoidably containing some polymastigotes, and attached and associated spirochetes; 5, termite hindgut wall fraction; 6, tubulin standard (1 and 6 contained 2 ml per well; 2 to 5 contained 30 ml per well).

necessarily always organized into classical tubules. The stained preparations were made and observed at 22°C. The ambient temperature of the termite hindgut is 28° to 36°C. Whether the organized tubules are sensitive to lower temperatures is not known.

Gel Electrophoresis

Although many controls were used (Table 1) to verify that the fluorescence was due to tubulin immunofluorescence, such techniques are intrinsically limited. Direct biochemical techniques comparing properties of the putative spirochete tubulin with those of well-known eukaryotic tubulin are potentially more definitive; thus, sodium dodecyl sulfate gel electrophoresis studies were undertaken.

Although they could not be grown in culture, large quantities of spirochetes could be separated from the other host termite microbiota by differential centrifugation (*24*). The hindguts were removed intact from the termites and collected in Trager's A solution (*30*). When enough pseudergates were available (60 to 70), they were used for the *R. hesperus* gel electrophoresis, whereas only eight to ten *Pterotermes* were required because of the much larger size of the their gut. Tissue homogenization was used to release the microbes all at once into a small volume of Trager's solution. The flagellates were easily sedimented by desk centrifugation at low speed. The resulting supernatants were collected and centrifuged at high speed for approximately 5 minutes. The pellets were centrifuged and washed several times in Trager's solution until microscopic examination showed that they contained large numbers of live spirochetes, both pillotinas and hollandinas, skinny gliding bacteria, and other smaller bacteria. Centrifugation was continued, if necessary, until screening for flagellates revealed none. Nearly all the visible flagellates were actively swimming even after they were collected in the pellet fraction, and all the flagellates appeared to be intact even if not motile. Therefore, we believe that the prokaryotic fractions could have only been minimally contaminated by tubulin of flagellate orgin.

The total cell protein was then solubilized in sodium dodecyl sulfate buffer, and boiled for 5 minutes; for electrophoresis, samples from various fractions were placed on slab gels made with 12.5 percent acrylamide running gels and 1 percent bis-acrylamide stacking gels (*31*). From 10 to 50 milliliters of total soluble protein was placed in each gel well. This experiment was repeated with minor variation three times. Electrophoresis of the proteins was conducted at least twice with the cleanest possible spirochete fraction from each species of termite host. In all cases, except when a pure culture of Reiter treponemes was used and when a slice of termite gut tissue was the sole protein source, a conspicuous band comigrating with authentic brain tubulin as standard was seen (Fig. 4). In one test, a sample of purified spirochete fraction was divided in half, and egg-white lysozyme at a final

Table 2. Summary of electrophoresis experiments.

Sample	Type of gel	Bands		Gel facilities, sources, comments
		Conspicuous (No.)	Identical with tubulin*	
Treponema reiteri pure culture	Tube, SDS–7.5 percent acrylamide	6 to 8	No	K. Fujiwara, 7-day cultures
Pterotermes occidentis	Slab, 12.5 percent acrylamide			E. Lazarides, 7 to 10 worker (pseudergates) termites per run; ran twice; same results
Hindgut microbes mixed bacterial spirochete fraction (no flagellates)		14 to 18	Yes	
Mixed bacterial spirochete flagellates—mainly hypermastigotes		8 to 12	Yes	
Mixed bacterial spirochete flagellates—mainly polymastigotes		12 to 14	Yes	
Termite gut wall tissue		30	Faint or no	
Reticulitermes hesperus	Slab 12.5 percent acrylamide			E. Lazarides, one actin-like band
Mainly pillotina and hollandina spirochetes		10 to 12	Yes†	
Mixed flagellates and spirochetes		6 to 8	Yes	Two actin-like bands; 70 to 80 worker termites per run; rabbit muscle actin run as internal standard

*That is, comigrating with standard brain tubulin. †Increased quantity of protein with lysozyme treatment.

concentration of 0.1 milligram per milliliter in Trager's solution was added to one member of the paired sample. To the other, Trager's solution alone was added. After a 15-minute incubation with lysozyme at room temperature, the proteins were solubilized in sodium dodecyl sulfate. After electrophoresis, it was obvious that lysozyme had increased the yield of all of the major protein bands, including the putative tubulin band. It seems unlikely that such a mild enzyme treatment with lysozyme, which is known to dissolve bacterial cell walls, could have released tubulin from contaminating flagellates, especially when no contaminating flagellates had been seen in the preparation before protein solubilization (Table 2).

Since *Treponema reiteri* could be axenically cultured (*32*) we attempted to find tubulin in these small spirochetes by the same methods: fluorescence of specific antibody to tubulin and gel electrophoresis. We were also able to test the effects of agents known to inhibit the polymerization of microtubule protein into tubules, such as podophyllotoxin, β-peltatin, and vinblastine (Table 3). The results of these experiments were consistent. On gel electrophoresis the treponemes have no protein band corresponding to tubulin, and they do not fluoresce when stained with fluorescein-conjugated antibody to tubulin. Their growth is not inhibited by any antimitotic drugs. These characteristics are consistent with the electron microscopic observations of Hovind-Hougen (*19*), who did not find 250 Å microtubules in *T. reiteri*.

There are many possible sources of error in our results. The mixed culture of bacteria may contain a nontubulin protein of molecular weight similar enough to that of tubulin to comigrate on gels; the fluorescense may be due to an unidentified artifact; and the microtubular structures may be convergent—similar to those of eukaryotes by chance alone. Furthermore, tubulin may be present in these spirochetes and gliding bacteria through phage transfer from flagellates in the crowded gut. Nothing short of detailed biochemistry on pure cultures of these large spirochetes will permit the definitive solution to this problem. However, if, as has been hypothesized, the cilia or flagellar system of eukaryotes was symbiotically acquired and later deployed in the origin of mitosis (*19*), the prediction is unambiguous. Tubulin protein of these spirochetes ought to be homologous to that of eukaryotic microtubules, and the nucleic acid responsible for the replication of microtubule organizing centers (*33*) should be homologous to nucleic acid of the relevant tubulin-containing spirochetes. Tubulin should not be found in cyanophyte tubules (*16*). Eventually, perhaps, colchicine-binding tubulin-containing spirochetes with a ninefold symmetry may even be discovered. At any rate, on the basis of our data (Table 3), the probability that tubulin-containing intracytoplasmic microtubules are present in the large symbiotic spirochetes and their relatives has risen significantly (*34*).

Conclusions

Microtubules, 240 ± 15 Å in diameter, have been found in the cytoplasm of large spirochetes (*Pillotina* sp. and *Hollandina* sp.) symbiotic in the hindguts of subterranean and dry-wood termites. Microtubules, aligned longitudinally down the long axes of these spirochetes, were also found in unidentified long, skinny, gliding bacteria. Large quantities of these prokaryotes were separated by centrifugation; their soluble proteins were released and studied by sodium dodecyl sulfate gel electrophoresis. Protein bands that comigrate with authentic brain tubulin were observed in preparations from these prokaryotes. The quantities of "tubulin" and other protein in solution were increased with gentle lysozyme treatment. Furthermore, specific antibodies to tubulin made from various antigenic sources (such as vinblastine-precipitated sea urchin egg tubulin and bovine brain tubulin) labeled with fluorescein was used as a stain on the hindgut microbiota. In such in situ cytological preparations, specific fluorescence was associated with hypermastigote and polymastigote flagellates, pillotinas, hollandinas, and the unidentified gliders (but none of the many other prokaryotes showed fluorescence). Cultivable treponemes (*Treponema reiteri*) do not contain microtubules, do not fluoresce in comparable cytological preparations, are not sensitive to antimitotic compounds such as vinblastine and podophyllotoxin, and show no tubulin-like band in sodium dodecyl sulfate gel electrophoretic preparations. Although alernative interpretations have not rigorously been excluded, we consider this presumptive evidence that there are bona fide tubulin microtubules in certain, but not all, spirochetes.

Note added in proof: We wish to make an important qualification concerning our claims of microtubule size in prokaryotes.

Hollande and Gharagozlou (*23*) reported microtubules 250 Å in diameter in *Diplocalyx* and *Pillotina*. We originally measured microtubules in fixed preparations in tannic acid. Subsequent work showed (i) that there was an error in magnification of microtubules in the slender rod and (ii) that tannic acid artificially inflates the diameter of microtubules so fixed. We then remeasured

Table 3. Summary of evidence for microtubules and microtubule protein in prokaryotes. The following antimitotic agents were tested and found to have no effect on growth of *T. reiteri* at concentrations between 10^{-7} to $10^{-3}M$: podophyllotoxin, β-peltatin, vinblastine, vincristine, and Colcemid. We have no comparable data for the other organisms. Symbols: +, observation positive; −, observation negative.

Termite and microbe organisms	TEM	Gel electrophoresis	Antibody to tubulin fluorescence
Kalotermes praecox (Madeira)*			
Pillotina calotermitidis	+ (*23*)		
Calotermes flavicollis (France)	+ (*22*)		
Diplocalyx calotermitidis			
Reticulitermes flavipes (Mississippi, Massachusetts)			
Pillotina sp.	+†		
Reticulitermes hesperus (San Diego)			
Skinny, gliding bacteria (unidentified)	+	+	
Pillotina sp.		+	
Hollandina sp.		+	
Pterotermes occidentis (southern Arizona)			
Pillotina sp.		+	+
Hollandina sp.	+	+	+
Gliding bacteria (unidentified)	+	+	+
Coleomitus sp.	−		−
Kalotermes schwarzi (Miami)‡			
Pillotina sp.	+		+
Gliding bacteria (unidentified)	+		+
Coleomitus sp.	−		−
Cultivable spirochetes			
Treponema reiteri	− (*19*)	−	−

*The same as *Postelectrotermes praecox*. †Only occasionally. ‡The same as *Incisitermes schwarzi*.

many prokaryotic and eukaryotic microtubules in the same preparations. We found that, in spirochetes, the microtubule size varied from 150 to 210 Å without tannic acid and from 150 to 250 Å with tannic acid. In both preparations eukaryotic microtubules were consistently larger. These data are taken to mean that both the size and size range of eukaryotic microtubules are larger than those of prokaryote microtubules. Most eukaryotic microtubules are 240 ± 20 Å in diameter, but in some protists a larger range has been observed. The published range for protist microtubules is from 150 to 300 Å (*35*). In order to make detailed morphological comparisons between prokaryotic and eukaryotic microtubules the presence, number, and dimensions of the microtubules must be determined.

References and Notes

1. S. Inoué and R. Stephens, Eds., *Molecules and Cell Movement* (Raven, New York, 1975); P. Satir, in *ibid.*, p. 143.
2. S. Ochs, *Ann. N.Y. Acad. Sci.* **253**, 470 (1975).
3. C. Bardele, *Sym. Soc. Exp. Biol.* **28**, 191 (1975); L. H. Tilney, in *Origin and Continuity of Cell Organelles*, J. Reinhert and H. Ursprung, Eds. (Springer-Verlag, New York, 1971), vol. 2, p. 222; M. O. Soyer, *C. R. Acad. Sci.*, in press.
4. G. Cooper and L. Margulis, *Cytobios*, in press.
5. W. Ormerod, S. Francis, L. Margulis, *Microbios* **17**, 189 (1976); M. Raudaskoski, *Arch. Mikrobiol.* **86**, 91 (1972).
6. S. Banerjee and L. Margulis, *Exp. Cell Res.* **78**, 314 (1973); J. K. Kelleher, *Mol. Pharmacol.* **13** 232 (1977); L. Margulis, *Int. Rev. Cytol.* **34**, 333 (1973).
7. R. A. Bloodgood and J. K. Kelleher, in *Biochemistry and Physiology of Protozoa*, S. Hutner and L. Provasoli, Eds. (Academic Press, New York, 1978); R. Goldman, T. Pollard, J. Rosenbaum, Eds., *Cell Motility* (Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. 1976), vols. A to C.
8. J. R. McIntosh, Z. Cande, J. Snyder, K. Vanderslice, *Ann. N.Y. Acad. Sci.* **253**, 407 (1975).
9. G. M. Fuller, B. R. Brinkley, J. M. Boughter, *Science* **187**, 948 (1975).
10. M. O. Soyer, *BioSystems* **7**, 306 (1975).
11. J. Pickett-Heaps, *ibid.* **6**, 37 (1974).
12. B. R. Brinkley, G. M. Fuller, D. P. Highfield, in *International Symposium on Microtubules and Microtubule Inhibitors*, M. DeBrabander and M. Borgers, Eds. (North-Holland, Amsterdam, 1975), p. 297.
13. R. F. Luduena and D. O. Woodward, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 3594 (1973).
14. G. G. Borisy and E. W. Taylor, *J. Cell Biol.* **34**, 535 (1967); J. K. Kelleher, *BioSystems* **9**, (1977); L. Wilson, K. Anderson, L. Grisham, D. Chin, in *Microtubules and Microtubule Inhibitors*, M. Borgers and M. DeBrabander, Eds. (North-Holland, Amsterdam, 1975), p. 103.
15. I. B. Heath, *Protoplasma* **85**, 147 (1975).
16. T. Bisalputra, B. R. Oakley, D. C. Walker, S. M. Shields, *ibid.* **86**, 19 (1975).
17. L. To and L. Margulis, *Int. Rev. Cytol.*, in press; W. Van Iterson, J. F. Hoeniger, E. N. VanVanten, *J. Cell Biol.* **32**, 1 (1967).
18. K. Hovind-Hougen, *Acta Pathol. Microbiol. Scand. Sec B* **255** (Suppl.), 1 (1976).
19. L. Margulis, *Origin of Eukaryotic Cells* (Yale Univ. Press, New Haven, Conn., 1970); in *International Symposium on Microtubules and Microtubule Inhibitors*, M. DeBrabander and M. Borgers, Eds. (North-Holland, Amsterdam, 1975), p. 3.
20. L. Kuznicki, T. L. Jahn, J. R. Fonesca, *J. Protozool.* **17**, 16 (1970).
21. F. J. R. Taylor, *Taxon* **25**, 377 (1976).
22. I. Gharagozlou [*C. R. Acad. Sci.* **266**, 494 (1968)] also discovered microtubules in a large spirochete, *Diplocalyx calotermitidis* (found in *Calotermes flavicollis* from southern France), an organism similar to the hollandinas.
23. A. Hollande and I. Gharagozlou, *C. R. Acad. Sci.* **265**, 1309 (1967).
24. R. A. Bloodgood, thesis, University of Colorado, Boulder (1974);_____, K. R. Miller, T. P. Fitzharris, J. R. McIntosh, *J. Morphol.* **143**, 77 (1974).
25. L. To, L. Margulis, A. T. W. Cheung, *Microbios*, in press.
26. J. Breznak and H. S. Pankratz, *J. Appl. Env. Microbiol.* **33**, 406 (1977).
27. O. Duboscq and P. Grassé, *Arch. Zool. Exp. Gen.* **66**, 451 (1927).
28. K. Weber, R. Pollack, T. Bibring, *Proc. Natl. Acad. Sci. U.S.A.* **72**, 459 (1975).
29. E. Canale-Parola, *Bacteriol. Rev.* **41**, 181 (1977).
30. W. Trager, *Biol. Bull.* (*Woods Hole, Mass.*) **66**, 182 (1934).
31. U. K. Laemmli, *Nature (London)* **227**, 680 (1970).
32. L. V. Holdeman and W. E. Moore, *Anaerobe Laboratory Manual* (Virginia Polytechnic Institute, Blacksburg, 1972).
33. C. F. Bardele, *J. Protozool.* **24**, 9 (1977); R. V. Dippell, *J. Cell Biol.* **69**, 622 (1976); S. R. Heidemann, G. Sander, M. W. Kirschner, *Cell* **10**, 337 (1977).
34. We recently have observed large free living spirochetes in the anaerobic zones of algal mats of Baja California, in an environment locally devoid of flagellates and ciliates.
35. M. Cachon and J. G. Cachon, *Ann. Biol.* **13**, 536 (1974).

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Macroeconomics of Disease Prevention in the United States

Prevention of major causes of mortality would alter life table assumptions and economic projections.

Gio B. Gori and Brian J. Richter

During the first half of this century the health and average life span of Americans improved considerably; epidemics of infectious diseases ceased to be a serious threat, and acute nutritional diseases were greatly reduced (*1, 2*). More recently, however, the aging of the population and certain life-style habits have brought about increasing rates of chronic illnesses (*3*). Healthy longevity has wide appeal, and over the last three decades the United States has invested an unprecedented portion of national income in the study and care of disease, but the health and longevity of Americans during that time have not shown commensurate improvement (*4*); a 1967 study suggested that neither a rise nor a decline in disease care expenditures would have a further impact on life expectancy (*5*). Of course, purely economic considerations ignore the fact that modern medicine aims beyond simple longevity scores and that it has made real advances, though difficult to measure economically, in improving the patient's quality of life and outlook for hope and in relieving societal and family anxieties about coping with the ill.

Evidence accumulated during the last 20 years indicates that the most important of modern diseases are caused by a variety of factors, most significantly by reckless personal and social habits such as improper diet, excessive drinking, smoking, drug abuse, lack of exercise, unsafe driving and working conditions, and inadvertent and deliberate environmental pollution (*3*). This suggests that at least partial prevention of important diseases may be possible.

Initially prevention can be expected to prolong life in the productive age groups, but with time the lives saved will accumulate in older brackets, aggravating the aging of the U.S. population projected by the current census and augmenting the consequent socioeconomic complications that some experts forecast (*6, 7*). Here we attempt to assess the economic

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