with its receptor site (or sites) on various proteins (*17*). Such a negative effect might be lessened or prevented by raising the intracellular concentration of cyclic AMP.

No matter which mechanism is operating, our results clearly indicate that *trans* fatty acids do provide sufficient fluidity to cell membranes to allow growth at 37°C. More important, however, their ability to support growth depends on the state of cellular metabolism—in which cyclic AMP is also involved. It seems desirable to reinterpret previous reports of *trans* fatty acid effects on cell physiology in terms of the metabolic state of the cells at the time of exposure to the acids. The finding that other fatty acids also exhibit regulatory interactions with cyclic AMP (*18*) opens the possibility that many exogenous fatty acids may form a class of inhibitory regulating agents that influence cell metabolism. Our results indicate that conditions that decrease cellular cyclic AMP may sensitize cells so that exogenous *trans* fatty acids add to the cell physiology a burden that is analogous to "total body burden" (*19*).

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Cellulose in the Cell Walls of the Bangiophyceae (Rhodophyta)

Abstract. *Mechanically isolated cell walls of the conchocelis phase of* Bangia fuscopurpurea *yield cellulose II (regenerated cellulose) upon treatment with Schweitzer's reagent. X-ray powder analysis and thin-layer chromatography of partial hydrolyzates confirm the presence of cellulose in this extract. Gas-liquid chromatographic analysis of wall hydrolyzates indicates that xylose, mannose, galactose, and glucose are major wall constituents. The presence of cellulose in the conchocelis provides evidence that this bangiophycean life cycle phase represents a transitional form or link between the two classes of red algae, Bangiophyceae and Florideophyceae. This suggests a close affinity of the two classes of the Rhodophyta and supports the hypothesis that bangiophycean algae were precursors of the Florideophyceae.*

Cell wall composition is one of four major characteristics used to distinguish algae at the class or divisional level (*1*). Although the algal division Rhodophyta has been systematically separated into two classes, the Bangiophyceae and the Florideophyceae, primarily on the basis of morphological and reproductive characteristics (*2*, *3*), the two classes also differ in the chemical composition of their cell walls. Cellulose is present in the Florideophyceae but has been thought to be absent in the Bangiophyceae (*4*).

The bangiophycean alga *Bangia fuscopurpurea* possesses two alternating life cycle phases, the bangia phase and the conchocelis phase. Although the generic phase is typically bangiophycean, the conchocelis phase possesses some morphological and ultrastructural features of the Florideophyceae (*5*). Chemical and physical analysis of mechanically isolated cell walls of the conchocelis phase reveals the presence of cellulose. The cellulosic content of the wall is low (about 3 percent, dry weight) but similar in quantity to that reported in the Florideophyceae (*4*).

We obtained a clean cell wall preparation from the laboratory-cultured conchocelis phase of *B. fuscopurpurea* (*6*) by using a modification of the techniques

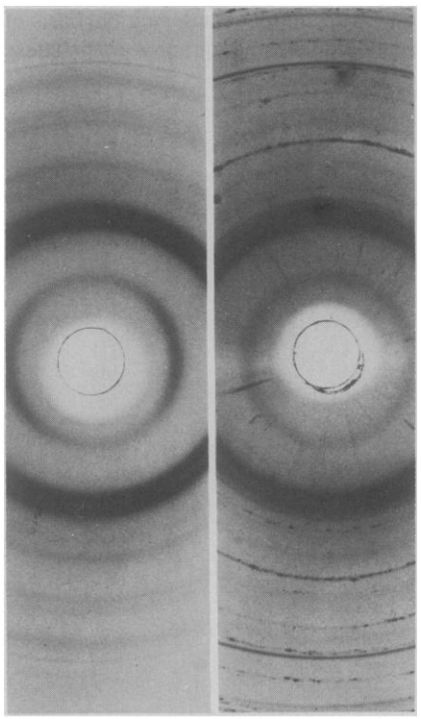
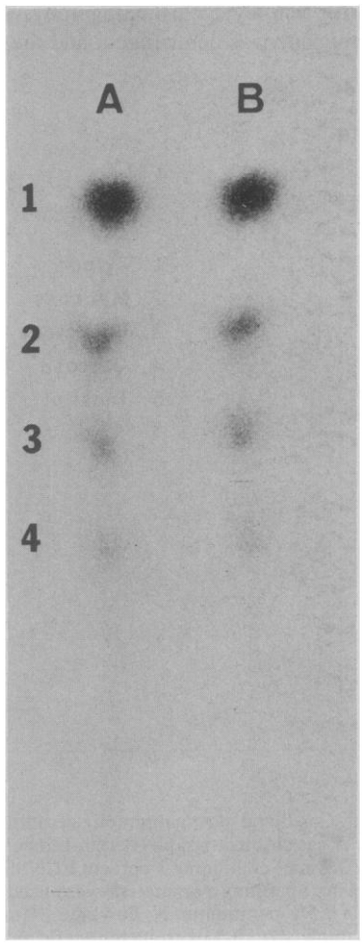


Fig. 1 (left). X-ray powder diagrams of regenerated cellulose from an Avicel standard (left) and from cell walls of the conchocelis phase (right). Fig. 2 (right). Thin-layer chromatogram comparing partial hydrolyzates of the cellulose fraction of the walls of the conchocelis phase (A) with an Avicel standard (B). Numbers denote sugars as follows: 1, glucose; 2, cellobiose; 3, cellotriose; and 4, cellotetraose.



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of Dodson and Aronson (*7*). Walls were fragmented with a Braun cell homogenizer cooled with liquid CO_2 and separated from cytoplasmic components by repeated centrifugation and washing in 0.1*M* tris-HCl buffer. The washing was repeated until the protein content of the isolated walls could not be further reduced. When viewed microscopically and stained with KI_3, cell walls did not show evidence of cytoplasmic contamination. Isolated walls were lyophilized, weighed, and treated with Schweitzer's reagent. Polysaccharide regenerated by acidification of the extract was treated with concentrated NH_4OH to remove other alkali-soluble polymers extracted with the cellulose and then dried and weighed. The regenerated cellulose (cellulose II) was packed into a thin-walled, glass capillary and placed in a CuK_α x-ray beam (λ = 1.5418 Å) and analyzed with a 114.6-mm Philips Debey-Scherrer type powder camera. Exposure times ranged from 7 to 8 hours at 40 kV and 19 mA (*8*). Interplanar spacings (*d*) were calculated from Bragg's equation. The lattice spacings agree, within accepted errors (*9*), with those reported for cellulose II (*4*) (Table 1 and Fig. 1).

The cellulose fraction from *B. fusco-purpurea* was partially hydrolyzed for 1 hour at 22°C in fuming HCl in preparation for thin-layer chromatography. The hydrolyzate was centrifuged, and the remaining turbidity was removed by filtration of the supernatant. Acid was removed by repeated drying at 40°C under N_2 with ethanol assists. The hydrolyzate was redissolved in 10 percent isopropanol and spotted on Eastman 13255 (cellulose) or 13181 (silica gel) Chromagram sheets. The sheets were irrigated with a mixture of *n*-butanol, ethanol, and water (13:8:4), and sugars were detected with alkaline $AgNO_3$ (*10*). Spots corresponding to glucose, cellobiose, cellotriose, and cellotetraose were detected (Fig. 2). The oligosaccharides were confirmed as a polymer homologous series (*11*).

Table 1. Lattice plane spacings (in angstroms) of cellulose II (regenerated cellulose) from conchocelis phase walls compared with that from an Avicel standard (commercially available microcrystalline cellulose) and reported values.

Conchocelis phase	Avicel	Reported values (*4*)
7.37	7.37	7.35
4.46	4.46	4.42
4.08	4.08	4.03
3.12	3.12	3.14
2.59	2.59	2.58
	2.22	2.21

Isolated cell walls were also hydrolyzed in 3*N* HCl at 100°C for 4 hours, and monosaccharide products were characterized as alditol acetates on the basis of gas-liquid chromatography (*12*). These analyses revealed that xylose, mannose, galactose, and glucose were components of the cell wall of the conchocelis phase (Fig. 3). Although xylose, glucose, and galactose polymers have been reported in both bangiophycean and florideophycean algae (*13*), the presence of mannose in cell walls appears to be restricted to the Bangiophyceae and to the cell wall mucilages of the simpler (or more primitive) representatives of the Florideophyceae, specifically members of the Nemaliales (*14*). Thus, the cell wall composition of the conchocelis phase appears to be somewhat intermediate between the wall composition reported for the two classes of red algae.

This study thus provides the first evidence of cellulose in the cell walls of a bangiophycean alga. The presence of cellulose in the conchocelis phase and its reported absence in the bangia phase (*15*) demonstrate that life cycle alternates of certain red algae may have cell walls of differing composition, suggest that the conchocelis phase represents a structural and biochemical link between the two classes of red algae, and support the hypothesis (*2, 16*) that bangiophycean algae were precursors of florideophycean forms.

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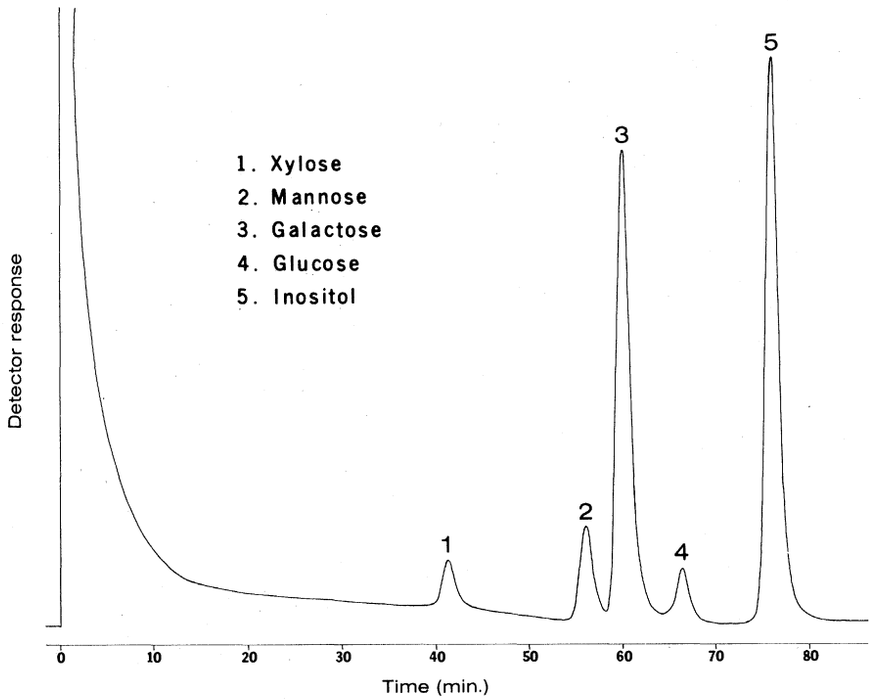

Fig. 3. Gas-liquid chromatography of alditol acetate derivatives of sugars in a cell wall hydrolyzate. A gas chromatograph (Perkin-Elmer 990) equipped with dual stainless steel columns (2.13 m by 3.2 mm) containing 3 percent ECNSS-M coated on 100 to 120 mesh Gas Chrom Q and the following operating parameters were used: temperature programmed from 155° to 185°C at a rate of 0.5°C per minute; N_2 flow rate, 30 ml/min; flame ionization detector, range 1, attenuation 32; sample size, 0.5 μl; solvent, acetic anhydride.

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Access of Urinary Nonvolatiles to the Mammalian Vomeronasal Organ

Abstract. *Guinea pigs were allowed to investigate urine that contained rhodamine, a nonvolatile fluorescent dye. Guinea pigs given free access to dyed urine exhibited fluorescence in their vomeronasal and septal organs but not on their olfactory epithelium. Fluorescence was not seen when unadulterated urine was presented. Thus compounds of low volatility, which do not reach the olfactory epithelium, may stimulate the vomeronasal system and provide information that is normally not provided by gustation or olfaction.*

Peripheral chemoreception has traditionally been thought to consist of two systems, gustation and olfaction. In air-breathing organisms, these two systems have often been distinguished by the method of stimulus access; that is, by "direct contact between the stimulus source and the receptor sheet (gustation) or migration of the molecules over distance from the stimulus source to the receptor sheet (olfaction)" (*1*). Until recently (*2*), one of the receptor organs in the mammalian nose, the vomeronasal organ (or Jacobson's organ), has been considered a redundant olfactory organ. However, the vomernasal organ may be part of a unique chemosensory system (*3*). The olfactory and vomeronasal systems exhibit a substantial degree of anatomical independence in both the nasal cavity (*3*, *4*) and the central nervous system (*2*, *5*). Although there has been much speculation about the possible functions of the vomeronasal sensory epithelium (*3*), the compounds that normally stimulate this olfactory-like epithelium (*6*) remain unknown (*7*, *8*).

Recent studies indicate that substances that communicate social and sexual information often appear to be of low volatility (*9*, *10*). Consequently, the sensory structure responsible for the detection of these substances is called into question. In this report, we present evidence that the vomeronasal receptor sheet may be stimulated by liquid-borne compounds of low volatility—specifically, substances in conspecific urine that are transported to the organ in a liquid medium. Thus the vomeronasal system is an anatomically distinct system that possesses characteristics of both gustation and olfaction.

When presented with conspecific urine, guinea pigs approach the stimulus and spend considerable time bobbing their heads and investigating, sniffing, and licking it. The amount of time devoted to these activities is influenced by the sex of the urine donor and of the recipient (*11*). We hypothesized that urine is transported to the vomeronasal organ for sensory processing during these investigatory behaviors. To test this hypothesis, we used urine mixed with rhodamine (B or 6G) hydrochloride, a nonvolatile fluorescent dye (*12*). Eighteen healthy guinea pigs were offered conspecific urine with or without the dye (*13*). Thirteen additional guinea pigs were subjected to other experimental manipulations (see Table 1). After a brief exposure to the urine (*14*), each guinea pig was killed with an overdose of pentobarbital. The bilateral vomeronasal organs and, in some cases, septal organs ($N = 4$) (*15*), the olfactory organs ($N = 7$), the external nares ($N = 7$), and the nasopalatine ducts ($N = 8$) were removed, sectioned (*16*), and examined by epifluorescence microscopy. We used two filter sets that are normally employed to visualize fluorescein isothiocyanate (FITC) and rhodamine fluorescence (*17*). No attempt was made to measure the degree of fluorescence.

When viewed with the rhodamine filters, fluorescence was seen in the vomeronasal organ of every guinea pig that had been exposed to rhodamine-dyed urine while awake (Fig. 1, left). Rhodamine fluorescence was absent from the vomeronasal organs of guinea pigs that had been exposed to stimuli that lacked the dye (Fig. 1, right). Also, no rhodamine was seen on the olfactory epithelium of any guinea pig, regardless of exposure condition (*18*). In the cases sampled, rhodamine fluorescence was observed on the septal organ of each guinea pig offered dyed urine. To control for the possibility of passive diffusion of rhodamine into the vomeronasal organ during dissection or sectioning, rhoda-

Table 1. Presence or absence of rhodamine fluorescence in the vomeronasal organ after various experimental treatments.

Treatment	Sample	Rhodamine fluorescence
Removal from home cage	2 males	No
Contact with female urine	3 males	No
Contact with female urine mixed with rhodamine (*20*)	7 males	Yes, bilaterally
Contact with male urine mixed with rhodamine (*20*)	8 females	Yes, bilaterally*
Contact with female urine mixed with rhodamine after unilateral nasal closure†	4 males	No, ipsilateral to closure; yes, contralateral to closure
Contact with female urine mixed with rhodamine while wearing Plexiglas nasal tube (*19*)	2 males	No, ipsilateral to tube; yes, contralateral to tube
Contact with drinking water mixed with rhodamine	2 males	Yes in one male (bilaterally); no in the other
Water mixed with rhodamine flushed through mouth of anesthetized animal	1 female, 1 male	No
Male urine mixed with rhodamine flushed through mouth of anesthetized animal	1 female	No

*One female did not contact the urine. Rhodamine was not seen in the mouth or on the rhinarium, nor was rhodamine fluorescence seen in the vomeronasal organ. †The right naris was closed for two males; the left for the other two.

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