

sperm death was approximately linear at each concentration of gossypol used (Fig. 1d), and the rates of death increased with increasing gossypol concentration. For example, death rate is 1%/min at 10 μM gossypol, and 40%/min at 100 μM gossypol (Fig. 1e). Control sperm in the absence of gossypol remained alive and motile for more than 2 h.

It is not clear at the present what the molecular mechanism is of gossypol action on sperm. However, our light microscopic study is consistent with the hypothesis that gossypol directly perturbs the mitochondria, creating an imbalance in the mitochondrial transmembrane potential, and inducing osmotic swelling.

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Effect of Gossypol on the Ultrastructure of *Spisula* Sperm

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Gossypol is a phenolic dialdehyde derived from the cotton plant (*Gossypium herbaceum*). It decreases sperm motility and is being studied as a potential male contraceptive (1).

This report describes the action of gossypol acetate extracted from *Gossypium herbaceum* grown in Brazil on structural aspects of *Spisula* sperm, as analyzed by scanning-electron-microscopy (SEM), transmission-electron-microscopy (TEM), and freeze-fracture.

Control sperm and sperm exposed to 50 μM gossypol for 10 to 30 min were fixed in 2.5% glutaraldehyde in filtered seawater and processed for SEM, TEM, and freeze-fracture according to conventional methodology (2).

SEM reveals that after 10 min exposure to 50 μM gossypol, *Spisula* sperm undergo a pronounced swelling of the midpiece accompanied by an irregular appearance of the cell membrane surrounding both the midpiece and sperm head (Fig. 1B). Control sperm have a smooth cell surface and a mitochondrial midpiece of normal size (Fig. 1A).

When ultrathin sections of similarly treated sperm cells (10 min exposure to 50 μM gossypol) are studied, the midpiece can be observed to be swollen (Fig. 1D) compared to control sperm (Fig. 1C). The treated sperm have small cytoplasmic granules among the mitochondria, which appear normal. These granules

are absent in control sperm. With longer exposure to gossypol (50 μM) for up to 30 min, the accumulation of granules increases and swelling of the midpiece continues.

Freeze-fracture replicas of sperm exposed to 50 μM gossypol for 10 min show swelling of the midpiece and reveal that the internal layer of the cell membrane has an altered distribution of particles, compared to control sperm. In the cell membrane of non-treated sperm, particles in the internal layer are distributed randomly (Fig. 1E). In the membrane of treated cells, particles are completely absent in some areas (Fig. 1F).

We conclude that exposure to gossypol alters the structure of the cell membrane of *Spisula* sperm, initially at the midpiece and subsequently around the sperm head. This morphological effect is closely related to changes in sperm motility, membrane permeability, and cell viability.

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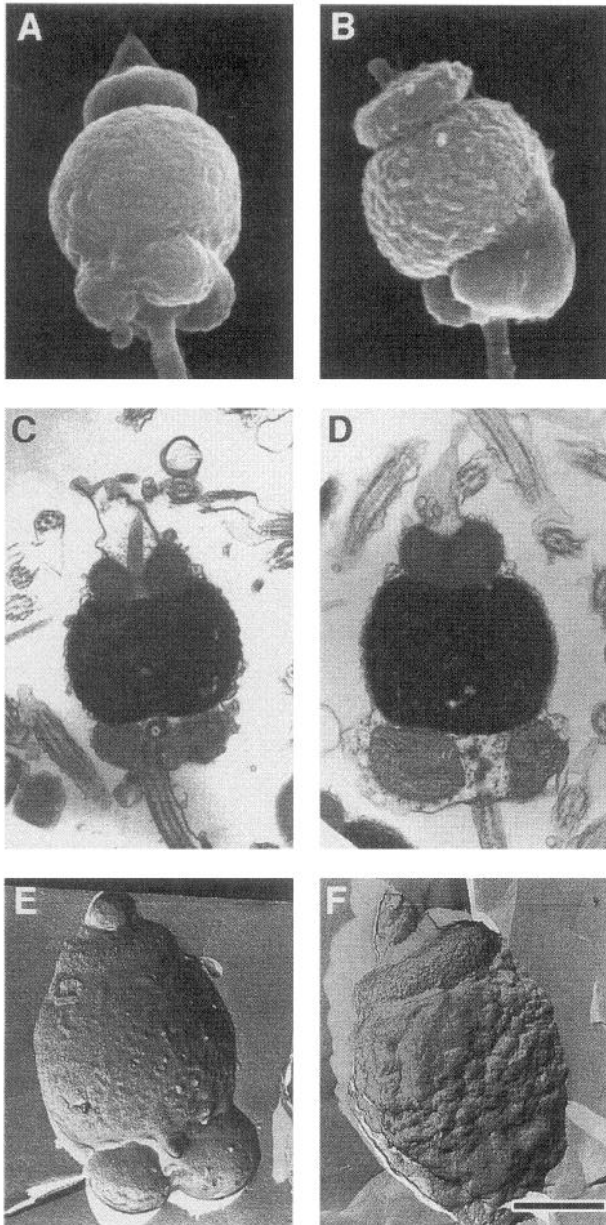


Figure 1. Electron micrographs of *Spisula* sperm; midpiece and head. Control sperm (A, C, E) and sperm exposed to 50 μ M gossypol for 10 min (B, D, F). SEM shows the change in the head surface and swelling of the midpiece (B) as compared to the control (A). TEM reveals swelling of the midpiece with the appearance of many cytoplasmic particles in the gossypol treated sperm (D); control sperm appears normal (C). Freeze-fracture replicas also reveal changes in the cell membrane with altered distribution of intramembrane particles in the gossypol treated sperm (F) as compared to the control sperm (E). For identification of cellular components, see schematic in S. Inoue et al. (3). Scale bar = 1 μ m.

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Orientation of Motile Unicellular Algae to Oxygen: Oxytaxis in *Euglena*

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Oxygen sensing by a microorganism was first demonstrated by Engelmann (1) in his experiment that determined the photosynthetic action spectrum. In motile bacteria, a positive chemotactic response to oxygen was subsequently observed as a net migration to environments that are better aerated (2). In fungi, growth responses toward oxygen are thought to be responsible

for self avoidance in hyphae (3). In higher plants, oxygen has only recently been shown to play a direct role in determining root orientation (oxytropism) (4). To understand these responses and the associated pathways of signal transduction in higher plants, it may be easier to first study the response in an algal system.