ide ($10^{-4}M$) to the mucosal bathing solution decreased the I_{sc}, reflecting decreased Na^+ absorption, but did not alter ψ or f_r of crypt cells. This finding suggests that the crypt cells are not involved in amiloride-sensitive electrogenic Na^+ absorption.

Our results indicate that cells of the colonic crypts and not the surface epithelium are responsible for cyclic AMP–mediated electrogenic Cl^- secretion. These results, plus those of previous studies (*5*), also indicate that surface epithelial cells and not crypt cells are responsible for electrogenic Na^+ absorption. Thus, the structural heterogeneity of the colon is paralleled by a functional heterogeneity: the surface epithelium absorbs and the crypts secrete.

MICHAEL J. WELSH*
PHILIP L. SMITH†
MICHAEL FROMM‡
RAYMOND A. FRIZZELL§
Department of Physiology, University of Texas Medical School, Houston 77025

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* Present address: Laboratory of Epithelial Transport and Kidney Physiology, Department of Internal Medicine, University of Iowa College of Medicine, Iowa City 52242.
† Present address: Department of Physiology, University of Kansas Medical Center, Kansas City 66103.
‡ Present address: Institute Klinische Physiologie, Freie Universitaet Berlin, Klinikum Steglitz, Hendenburgdamm 30, D-1000 Berlin 45, Federal Republic of Germany.
§ Present address: Department of Physiology and Biophysics, University of Alabama, Birmingham 35294.

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Nature's Ballistic Missile

Abstract. *The parasitic fungus* Haptoglossa mirabilis *infects its rotifer host by means of a gun-shaped attack cell. The anterior end of the cell is elongated to form a barrel; the wall at the mouth is invaginated deep into the cell to form a bore. A walled chamber at the base of the bore houses a complex, missile-like attack apparatus. The projectile is fired from the gun cell at high speed to accomplish initial penetration of the host.*

The endoparasitic fungus *Haptoglossa mirabilis* (Eumycota) infects its Adineta (Rotifera) host by means of a gun-like injection cell. When a moving rotifer strikes this cell, a missile is shot through the host's cuticle, a hypodermic-like structure is inserted into the body, and a walled sporidium is pumped in to initiate infection (*1*). The sporidium grows into a large, cylindrical thallus, which at maturity produces several evacuation tubes through which biflagellate zoospores escape to the exterior. After a swarming period, each zoospore produces a spherical cyst that germinates almost immediately to produce a "gun" cell. The mature cell with attached cyst (Fig. 1) is shaped like a miniature cannon and is anchored to the substrate by an adhesive pad in such a way that the "barrel" is raised at an angle to the substrate to facilitate host encounters. Only a fraction of a second elapses between contact with the host and release of the infective sporidium (*2*).

Barron (*1*) suggested that the mechanism of attack in *Haptoglossa* might be similar to that described for the unrelated, plant-parasitic *Polymyxa betae* Keskin (*3*) and *Plasmodiophora brassicae* Wor. (*4*) of the Plasmodiophorales (Myxomycota). In the Plasmodiophorales an electron-dense, bullet-like structure ("stachel") forms within a tubular cavity ("rohr") inside the zoospore cyst. The rohr possesses a short, narrow tail ("schlauch"). At the moment of attack, the rohr is evaginated to form a bulbous structure (adhesorium), which attaches to the host. The stachel passes down the rohr and penetrates the host wall. The schlauch evaginates through the hole in the wall and an amoeboid infection unit is injected into a host cell. In *P. brassicae* it takes about 1 minute to produce the adhesorium, penetrate the wall, and infect the host.

Fig. 1. Light micrograph of a cluster of five gun cells of *Haptoglossa mirabilis*. The four peripheral cells are in a mature but unfired state. The central cell has fired but failed to penetrate a rotifer; the sporidium (*s*) is still attached to the empty gun cell by the hypodermic-like tube (×3300).

Even though *H. mirabilis* and the Plasmodiophorales may not be closely related, their mode of attack is very similar. However, the gun cell of *H. mirabilis* is more sophisticated in structure and design than the encysted zoospore of the Plasmodiophorales. We report here preliminary ultrastructural observations of the gun cell, with special reference to the unique structure of the missile itself.

Methods for recovering and culturing *Adineta* rotifers and *H. mirabilis* have been outlined elsewhere (*5*). The rotifers were used as bait to recover the fungus from greenhouse soil and to maintain the fungus in nonaxenic culture. A petri dish containing 2 percent water agar to which gun cells had attached themselves was rinsed gently in running tap water to remove surface debris. The washed plate was then flooded with fixative solution containing 3 percent glutaraldehyde and 1.5 percent acrolein in $0.07M$ phosphate buffer (*p*H 6.8). The fluid was drawn off slowly with a Pasteur pipette and the dish containing the parasite was overlaid with lukewarm water agar. Small blocks of agar (1 by 1 mm) with the gun cells sandwiched between them were cut out and placed in fresh fixative solution for 4 to 5 hours. The material was postfixed in 1 percent OsO_4 made up in the same buffer, dehydrated through a graded acetone series, and embedded in Spurr's plastic. Sections were cut with diamond knives, stained with 4 percent uranyl acetate in absolute methanol and Reynold's lead citrate, and viewed with a Phillips 200A or a JEOL JEM 100 CX electron microscope operating at 60 kV.

Figure 1 is a light micrograph of a cluster of five gun cells of *H. mirabilis*. The four peripheral cells are in a mature but unfired state. In these the protoplasm is concentrated in the tapering forward section (barrel) and the swollen

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basal section is highly vacuolated. A long tubular inclusion runs from the tip of the cell toward the vacuole. The central cell has fired but failed to penetrate a rotifer; the ellipsoidal sporidium is still attached to the empty gun cell by a firing tube.

Figure 2 is an electron micrograph of a longitudinal section through the forward part of the gun cell. Part of the zoospore cyst and the trailing edge of the adhesive pad is visible in the lower right corner. The cell tip is open, forming a large pore that is at the mouth of a short, broad bore. The bore is about 1.5 μm long and has a diameter of about 0.2 μm. It ends in an enlarged chamber, which narrows abruptly to form a flexuous tail. The anterior part of the tail (*bt* in Fig. 3A) is tube-like and is filled with electron-dense material that appears to be attached to the base of the missile. The outside wall of the cell continues over the lip of the muzzle and extends inward to line the bore, chamber, and at least part of the basal tube. In the unfired cell the missile is sealed into the chamber by wall material situated across the base of the bore.

The dominant feature of the gun cell is the harpoon-shaped missile housed in the chamber. In *Polymyxa* (*3*) the projectile is a simple bullet-shaped structure, and in *Plasmodiophora* (*4*) it is bullet-shaped with a ring of electron-dense material at the forward end. In *H. mirabilis* the structure (about 2 μm long) appears to be more complex, consisting of a shaft and an electron-dense head that is buttressed by three concentric, cone-shaped struts anchored proximally to the chamber wall (Fig. 3, A to C). Filaments form lateral connections between the supporting struts and between the outer strut and the chamber wall (Fig. 3C). The shaft (Fig. 3A) tapers gradually toward the head and has an inner electron-dense core surrounded by a heterogeneous casing (Fig. 3D). In Fig. 3A the shaft does not appear to be directly continuous with the head, but rather is connected by a short buttress element. Although it seems most probable that the head, buttress, and shaft all form part of the missile that is fired from the bore, it remains possible that the head and buttress simply serve as a wadding. The missile is oriented toward the apical pore, aligning the projectile for forward discharge.

Haptoglossa mirabilis is currently placed in the Oomycetes of the Eumycota. *Plasmodiophora* and *Polymyxa* are in the Plasmodiophoromycetes, but the disposition of this class is controversial. Some authors (*6*), emphasizing the zoosporic phase, place this class close to the Oomycetes in the Mastigomycotina of the Eumycota. Others (*7*), emphasizing the naked thallus, consider the Plasmodiophoromycetes as phylogenetically distinct from the Oomycetes and place them with the slime molds in the Myxomycota. Both *Haptoglossa* and the parasitic Plasmodiophoromycetes penetrate the host with a sharp projectile. The evaginated rohr and schlauch of the Plasmodiophoromycetes are clearly homologous with the bore, chamber, and basal channel that form the injection tube of *H. mirabilis*. A full appreciation of the relations between the *Haptoglossa* group of animal parasites and the plant-parasitic Plasmodiophoromycetes requires more detailed ultrastructural comparisons of the vegetative and reproductive stages.

Barron (*2*) referred to the attack cell of *H. mirabilis* as an injection cell and suggested, on the basis of light microscopic evidence, that a hypodermic-like structure penetrates the host wall and that the initial wound might be caused by a projectile. The present ultrastructural study confirms the presence of a gun-like bore and a complex harpoon-like attack apparatus in the parasite. However, since the act of infection is virtually instantaneous, the term gun cell seems more appropriate than injection cell to describe this remarkable biological structure. These preliminary observations establish that the attack apparatus of *H. mirabilis* is one of the most unique subcellular fungal structures yet described.

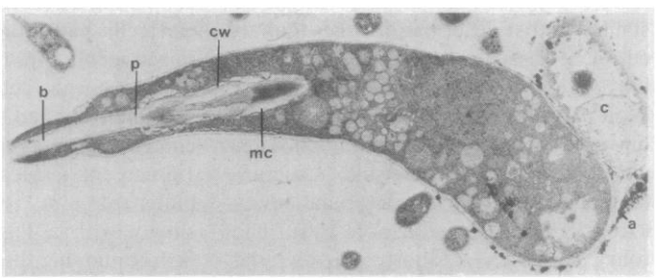

Fig. 2. Longitudinal section through a mature, unfired gun cell of *H. mirabilis* showing the plug (*p*) sealing the missile into the missile chamber (*mc*) beneath the bore (*b*). A continuation of the fungal cell wall (*cw*) lines the bore and missile chamber. Part of the empty zoospore cyst (*c*) and the adhesive pad (*a*) of the gun cell can be seen in the lower right corner (×18,560).

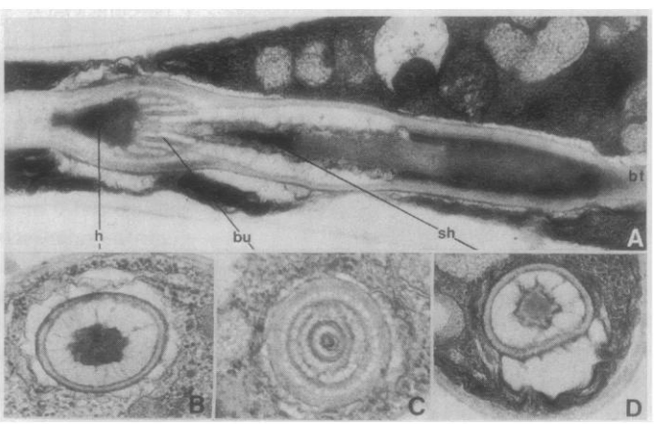

Fig. 3. (A) High-magnification longitudinal view through the anterior end of the attack apparatus showing the head (*h*), conical buttresses (*bu*), shaft (*sh*), and upper part of the flexuous basal tube (*bt*) (×56,000). (B) Cross section through missile chamber and head (×66,000). (C) Cross section through chamber and buttresses (×66,000). (D) Cross section through chamber and shaft (×66,000).

E. JANE ROBB
Department of Botany and Genetics, University of Guelph, Guelph, Ontario, Canada N1G 2W1
G. L. BARRON
Department of Environmental Biology, University of Guelph

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