Scanning Electron Microscopy of Human Head Louse (Anoplura: Pediculidae) Egg and its Clinical Ramifications

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ABSTRACT Pediculosis affects more elementary school students than all other communicable childhood diseases combined, excluding the common cold. The current study using scanning electron microscopy visualized operculums through which developing embryos received oxygen, as well as fracture lines within the adherent sheath by which the egg is attached to the hair. Using microscopic techniques, including freeze-fracturing, the mode of attachment of the sheath to the hair follicle was observed, in addition to the existence of several inner membranes enclosing the embryo within the egg structure. The chemical nature of the sheath is also analyzed using electron dispersive x-ray analysis.

KEY WORDS *Pediculus humanus capitis*, nit sheath, scanning electron microscopy

PEDICULOSIS IS A prevalent and highly communicable condition infesting over 6 million elementary school-aged children per year in the United States (Anonymous 1998). Excluding the common cold, more young students are affected by head lice, *Pediculus humanus capitis* De Geer, than all other communicable childhood diseases combined. Whether one considers head lice a serious medical problem or not, it is paramount to have a basic knowledge of such a common medical condition. Treatment of pediculosis consists of killing the crawling stages, egg removal, and elimination of fomite transmission (Burgess 1995, Burgess 1998). Inasmuch as insecticides have not been shown to be 100% ovicidal, existence of any eggs signifies that the infestation may still be present. Most schools adhere to a "no-nit" policy before children can reenter school after being infested, thereby forcing prolonged absenteeism. Thus, eggs are significant in terms of finding better ovicidal capabilities with our available treatment modalities, and in their removal from the hair shafts. The current study focuses on the morphology of the head louse egg using scanning electron microscopy, with emphasis placed on the clinical implications of our findings as related to ovicidal potential and egg cast removal.

Materials and Methods

Head lice eggs were obtained from private patients of Craig G. Burkhart. The eggs were considered homogeneous and no attempt was made to identify the egg from any particular patient. The specimens were fixed with 2% glutaraldehyde in 0.1 M cacodylate buffer before being dehydrated in ethanol, freeze fractured in liquid nitrogen, and critical point dried with CO_2. The specimens were either sputter coated with gold palladium on a Polaron E 500 sputter-coating apparatus or coated with evaporated carbon using a Denton vacuum evaporator (Moorestown, NJ). The specimens were then observed using a Cambridge 180 scanning electron microscope (Cambridge, MA) equipped with a Tracor Northern TN 5500 energy dispersive x-ray (EDX) spectrometer and analyzer (Madison, WI).

Results

The oval egg was shown to be cemented to the hair shaft by a cylindrical sheath that surrounds both egg and shaft (Fig. 1). A domed operculum is located on the free distal end of the egg, and it is the only portion of the egg not encased by the sheath. The operculum by which the developing embryo receives its oxygen was found to consist of 7–11 aeropyles occupying half of the surface of the operculum. There is a distinct ridge to the egg at this interface (Fig. 2).

At higher magnification, the sheath is noted to have fracture lines and some breakage at the ends of its follicular attachments (Fig. 3). On removing the hair and observing the inside of the sheath, the surface was shown to have minimally elevated ridges with no follicular remnants (Fig. 4). Using the freeze-fracture technique, several membranes were observed encircling the egg (Fig. 4).

Electron dispersive x-ray analysis revealed sulfur to be in the egg component, whereas both sulfur and silicon were detected in the egg sheath. No other

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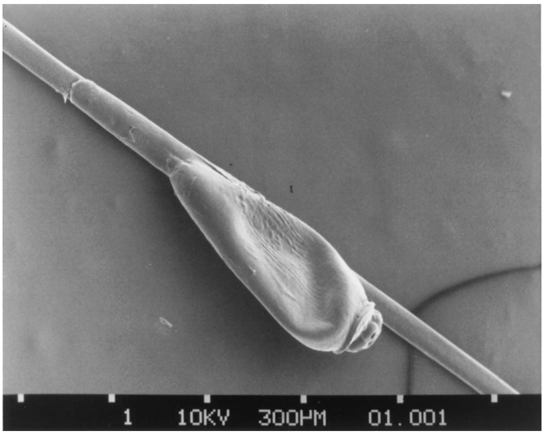

Fig. 1. Egg of *P. humanus capitis* with sheath casing surrounding both egg and hair shaft. Domed operculum is located on the free distal end (85×).

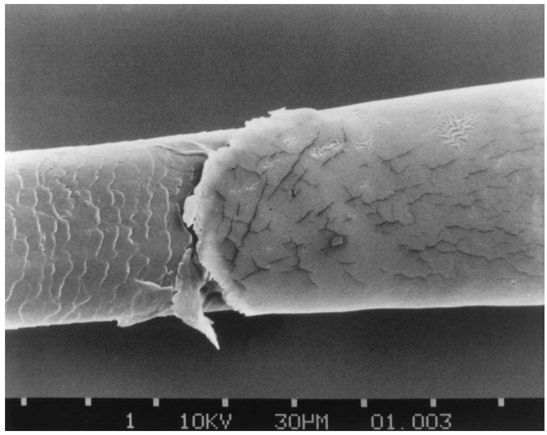

Fig. 3. The rim of the sheath of *P. humanus capitis* overlaying the hair follicle has fracture lines as well as breakage at the ends of follicular attachments (400×).

elements could be ascertained from the background radiation.

Discussion

The cylindrical sheath enclosing the egg and hair shaft is formed by vaginal secretions from the female louse (Burgess 1995). Although the length of the cylindrical band varies along the hair shaft (Burkhart et al. 1998), the sheath covers the entire egg except for portions of the operculum. Current scanning electron studies of the egg of the head louse differ from those of the crab louse, *Pthirus pubis* (L.), in which 14–20 aeropyles occupy two-thirds of the operculum surface and a reticular matrix is seen on the operculum surface between aeropyles (Ubelaker et al. 1973). Despite the morphological differences between the eggs of these 2 species, the opercular region of both was shown to be the most likely portion of the egg to crack when nymphs emerge from eggs (Harwood and James 1979).

As seen on the micrograph, the sheath is not very resilient, because it reveals fracture lines and small portions that break off at the edges from frictional forces such as combing. By flash pyrolysis–gas chromatography/mass spectrometry, the chemical composition of the sheath proves to be almost entirely proteinaceous, with a protein profile similar to that of the hair shaft itself (Burkhart et al. 1999). Therefore, breaks and fissures within its matrix would be consistent with its chemical composition. Of note, sulfur and silicon were demonstrated by EDX to be present in the sheath structure.

Scanning electron microscopy revealed minimally elevated ridges inside the egg sheath revealed but no hair cuticle cells, suggesting that a tight grip holds the sheath to the shaft, rather than a sticky glue attachment that would have detached cuticle cells from the hair. This tight band attachment is consistent with the

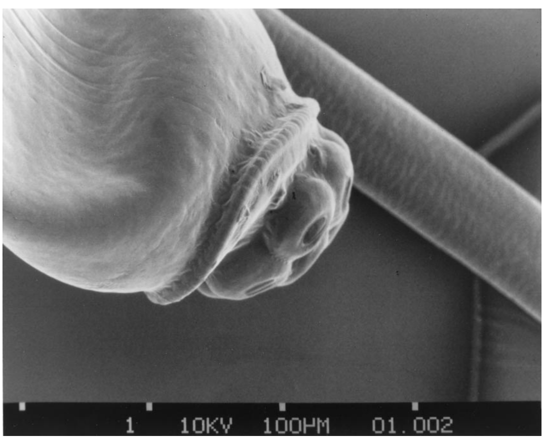

Fig. 2. A distinct ridge of the sheath of *P. humanus capitis* is noted at the interface of the egg and operculum. Several convex protrusions with central aeropyles are noted (350×).

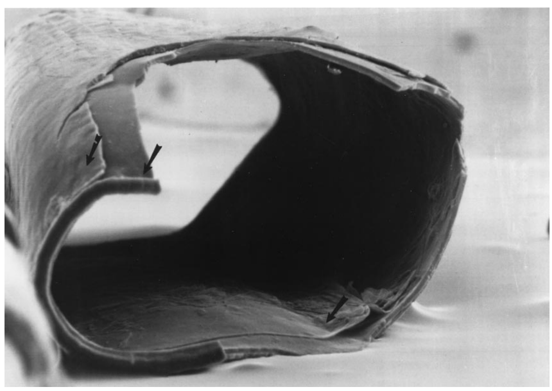

Fig. 4. The inside of the sheath of *P. humanus capitis* displays minimally elevated ridges with no follicular remnants or signs of tearing. Three distinct inner membranes (see arrows) are detected that encapsulate the egg (400×).

egg and hair retaining their structural integrity after detachment (Burkhart et al. 1998).

The egg sheath can be more easily removed by agents, like bleach, that damage the outer layers of the hair shaft, or by agents, like heated vinegar, that cause some egg dissolution or chemical weakening of the sheath (Burkhart et al. 1998). More effective solutions for egg removal will be problematic because the proteinaceous delineation of the nit sheath mirrors the hair follicle to which it is attached.

The freeze fracture technique revealed the existence of at least 3 membranes enclosing the louse embryo. Thus, an insecticide has to penetrate the aeropyles and several lipid membranes to reach the embryo. Such tissue consists of mostly lipid in its prenatal development (Fernstrom and Edmond 1998). Our findings suggest that a lipophilic base, not an aqueous or alcoholic base, would alleviate the transport of any insecticide to its destination (the neural tissue of the egg) for ovicidal activity.

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