SHORT COMMUNICATION

Histochemical Analysis of the Nit of *Pediculus humanus capitis* (Anoplura: Pediculidae)

C. N. BURKHART,[1] J. ARBOGAST,[2] P. SMYTHE,[3] AND C. G. BURKHART[4]

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ABSTRACT Histochemical stainings of head louse nits were performed and observations indicated that the nit sheath was nonchitinous. One discernable membrane composed of hyaluronic acid was established that encircled the larvae within the egg. The structural framework of the egg included a definite lattice around each aeropyle of the operculum, through which oxygen exchange occurred.

KEY WORDS head lice, nit sheath, hyaluronic acid

THE ENDLESS SCOURGE of head lice is caused by the ectoparasite *Pediculosis humanus capitis* De Geer. The female louse lives for 30 d during which time she lays ≈300 eggs (Burgess 1995). Each egg is firmly cemented to individual hairs ≈1 cm from the scalp surface with a glue-like substance. Inasmuch as no current therapy guarantees 100% ovicidal activity, the existence of nits signifies the possibility that infestation is still present. Anatomical analysis of the louse nit may assist in assessing the safeguards that nature has developed to protect the developing louse.

Materials and Methods

Hairs with attached nits were obtained from patients presenting with head lice. No attempt was made to designate specific hair from any particular person, because all nits were considered homogeneous for our investigation. All the samples were processed at Toledo Hospital Department of Pathology.

Results

Using light microscopy, the louse nit was cemented to the hair shaft with an adherent cylindrical sheath. The sheath was of variable length on the hair shaft and encapsuled the entire egg save for the domed operculum, which allows the maturing larvae to have ambient oxygen to breathe. With routine hemotoxylin and eosin, the sheath stained pink, whereas the egg was a mass of blue and pink sections. Of note, each aeropyle of the operculum was framed by the egg wall structure (Fig. 1). The periodic acid-Schiff (PAS) demonstrates the presence of glycogen and neutral polysaccharides by staining tissues red. The egg stained red by PAS and was amylase-resistant, signifying the existence of neutral polysaccharides. The sheath was pink and was negative by PAS staining.

The alcian blue reaction demonstrates the presence of acid polysaccharides by staining them blue. The egg revealed a stippled blue staining of the structure at pH 2.5, but the sheath was negative. At ph 0.5, the alcian blue did not react with either the egg or the nit sheath. There was noted however a definite inner membrane circumventing the entire egg with both alcian blue stains. The colloidal iron stain demonstrates hyaluronic acid with a bright blue staining, and a bright blue staining was found encircling the larva within the egg structure (Fig. 2). Neither the egg nor sheath revealed any staining on Verhoeff van Gieson elastic stain.

Discussion

By histochemical staining, the egg contains neutral, and nonsulfated acid polysaccharides. Complex carbohydrates, a major constituent of chitin, stain with alcian blue and PAS and were present in the egg in our testing. Chitin is one of the most abundant macromolecules in the world, and is the major constituent of exoskeletons of crustacea and insects. It is water insoluble, and viscoelastic with high tensile strength. In nature it is bound tightly with protein, minerals, and lipids.

The chemical structure of the sheath is becoming less of an enigma. Barat and Scaria (1962) reported that the louse sheath is chitinous, while Carter (1992) denied the existence of chitin in this glue-like substance. Histochemical staining by PAS and alcian blue was negative. Inasmuch as complex carbohydrates, like chitin, should stain with both reagents, our study supports Carter's assessment that the sheath is nonchitinous. More definitive work with flash pyrolysis-gas chromotography/mass spectrometry has revealed

[1] Department of Biology, University of Toledo, Toledo, OH 43606.
[2] Head of Chemistry, Lourdes College, Sylvania, OH 43560.
[3] Department of Pathology, Toledo Hospital, Toledo, OH 43606.
[4] Department of Medicine, Medical College of Ohio, Toledo, OH 43614.

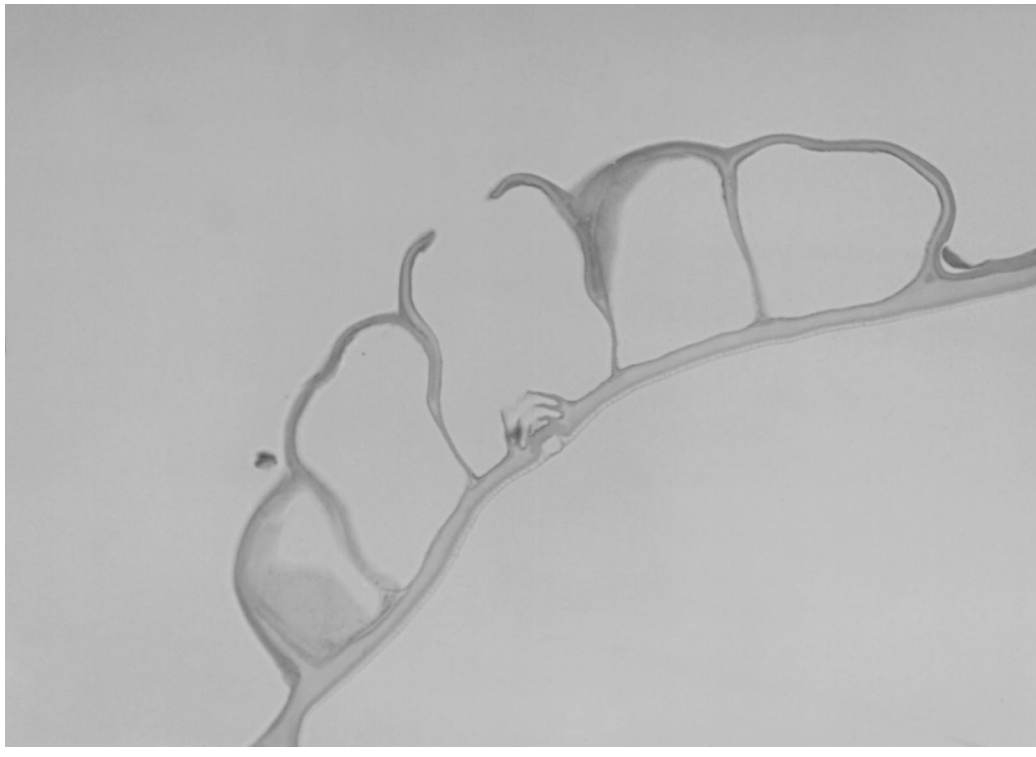

Fig. 1. By hemotoxylin and eosin, a definite structural framework of the egg encircling each aeropyle of the operculum is noted (magnification 400×).

Fig. 2. Colloidal iron stain is specifically taken up by the inner membranous layer of the egg (100×).

definitive proof that the nit sheath is indeed proteinaceous, and not chitin (Burkhart et al. 1999).

At least 1, and possibly a few, discernable membranes were noted encircling the larvae within the egg as noted by several histochemical stains. The positive colloidal iron stain and presence with alcian blue suggests hyaluronic acid as the component between membranes. Hyaluronic acid is known to have protective properties for joints, ocular stuctures, and umbilical tissues. In similar circumstances in nature, hyaluronic acid, which is water soluble, is walled by lipophilic membranes. This would seemingly be the case with head lice as well.

The operculum of the head louse is known to consist of 7–11 aeropyles and has a distinct ridge at the egg interface. Whereas, the operculum of the pubic louse consists of a reticular matrix between aeropyles seen on scanning electron microscopy (Khudobin 1995), no such structure has been seen with the head louse. Through all of our histochemical stains, a definite structural framework of the egg encircling each aeropyle of the operculum is seen. Thus, the operculum, and each individual aeropyle, is a functional part of the structural integrity of the nit capsule.

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