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Feature Articles

Electron Microscopic Examination of Various Types of Ancient Specimens

Jeanne M. Riddle, PhD*

Paleopathology (the study of disease in ancient remains) is an important and interesting area of investigation. Through these studies, we can: 1) learn the time frame in which specific diseases emerge within identified cultures, 2) determine if the tissue response to the pathologic alteration remains constant over long periods of time, and 3) assess whether or not modern populations share the same diseases with their ancient counterparts.

On the morphologic level, tissue samples obtained from ancient humans were first examined histologically with the light microscope. More recent literature contains a limited number of ultrastructural and topographic studies in which samples removed from or associated with mummified ancient bodies were investigated with either the transmission electron microscope or the scanning electron microscope (1-4).

Because it is necessary to fix tissues and cells immediately in order to preserve the full range of their ultrastructural features, fine structural details of ancient specimens are frequently compromised at the increased resolution and magnification levels possible with the transmission electron microscope. However, sufficient morphologic characteristics remain in mummified tissue specimens to identify positively classes of blood cells, specific types of fibers such as collagen, and characteristic developmental stages of parasites. The addition of the scanning electron microscope to the armamentarium of the paleopathologist has increased the types of ancient samples that can be studied. Using this instrument, we can gain for the first time a view of the topography of these materials in apparent three-dimension.

Over the past 12 years, I have had the opportunity to study a variety of samples collected from Egyptian mummies autopsied under the auspices of the Paleopathology Association, an international organization comprised of basic scientists, physicians, anthropologists and archeologists, founded by the late T. Aidan Cockburn, MD, Detroit, Michigan. Additional insect remains were obtained from Peruvian mummy bundles during their dissection by James M. Vreeland, Jr, archeologist, University of Texas, Austin, Texas. Selected examples from these samples will be presented in this publication.

Background Information on the Various Mummies

Egyptian mummies, obtained from the Pennsylvania University Museum, are identified by an acronym PUM and a Roman numeral which indicates the order in which the mummies were received and autopsied (II, III and IV).

PUM II was carbon dated at 170 BC \pm 70 years. This mummy was an adult man, 35 to 40 years old with a height of 5 feet 4 inches. He was thought to be an important person, possibly a priest. The method used for his embalming was classic, that is, his organs were preserved, the body was wrapped in linen, and the mummy was placed into an elaborately decorated sarcophagus (5).

PUM III was carbon dated at 835 BC \pm 70 years. This mummy, an adult woman approximately 35 years old, was 5 feet 1 inch tall with a calculated weight of 95 pounds. In contrast to PUM II, this mummy was eviscerated per ano, wrapped in pieces of medium quality linen, and was not contained within a sarcophagus. Tissue preservation of this body was generally of poor quality (6).

PUM IV was dated at the first or second century AD (Roman period). This mummy was a male child, 8 to 10 years in age and 3 feet 5 inches tall. This mummy had also been eviscerated per ano. Its wrappings consisted of linen strips, and the body was dressed in a number of full or shorter length shroudlike garments. The body tissues of this mummy were also severely degenerated (6).

Insect specimens were also collected from three Peruvian mummy bundles, the major form of burial for central and south coastal Peruvian cultures. Each bundle contained a mummy in a flexed body position. The mummies were wrapped in successive layers of plain-weave and/or patterned textiles. Layers of the textiles were often alternated with other materials such as seed

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*Research Electron Microscopy Laboratory, Henry Ford Hospital

Address reprint requests to Dr Riddle, Research Electron Microscopy Laboratory, Henry Ford Hospital, 2799 W Grand Blvd, Detroit, MI 48202.

cotton, leaves, grass or hides. Each of these mummy bundles was associated with three distinct cultural complexes: Paracas (86 AD), Epigonal (1231 AD), and Huancho (1240 AD). The bodies contained within the bundles were those of a 40- to 60-year-old woman, an 18- to 20-year-old woman, and a 12-year-old child, respectively (4).

Ancient Specimens Surveyed

Outer wrappings

Wrappings collected from the Egyptian mummy PUM II consisted of a fabric constructed from relatively fine threads (Fig 1). When individual threads of this cloth were surveyed with the scanning electron microscope, we found that they were composed of slender, straight fibers that showed jointlike markings (Fig 2). These fibers showed a topographic morphology that was identical with reference flax fibers. By studying this ancient textile with the scanning electron microscope, we were able both to view its pattern of weaving and to confirm that it was linen, the traditional cloth in which Egyptian mummies were wrapped.



Fig 1
Scanning electron micrograph of the linen wrappings removed from PUM II x 38.

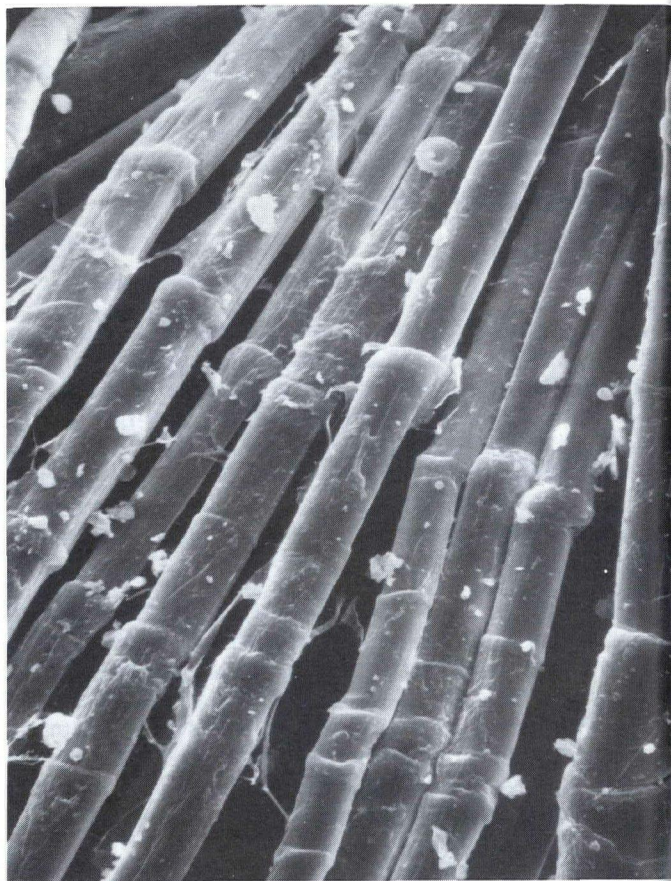


Fig 2
Individual flax fibers which make up the threads of the linen wrappings of PUM II x 800.

During the unwrapping process on PUM II, we found a small ball that was composed of whitish fibers incrustated with possibly dried unguents. On gross observation, this appeared to be a ball of cotton wrapped between two small pieces of linen. Examination of the individual whitish fibers by scanning electron microscope showed that they were straight fibers that had a repeating, inherent twist (Fig 3), a morphology that corresponded exactly to the three dimensional appearance of reference cotton fibers. Electron microscope confirmation that this ball of material was indeed cotton was an important piece of information, since cotton had not previously been found in the western world before the time of Christ. The earliest identification of cotton until its discovery among the linen wrapping of PUM II was from a Roman grave of about 200 AD.

Human blood cells

During the autopsy of PUM III, a small plaquelike mass of brown, granular material that had adhered to the inner aspect of the occipital bone was discovered and removed for further study. This material was originally

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Fig 3

Individual fibers of cotton showing their inherent twist removed from the cotton ball found on PUM II x 650.

thought to be brain tissue because of its anatomic location, but after it was rehydrated, sectioned and examined by light microscopy was found to contain numerous peripheral blood elements.

While it is quite common to observe well-preserved erythrocytes in the tissues of mummified bodies, leukocytes are rarely found. This mass of material, which was either an antemortem hematoma or a postmortem blood clot, gave us the opportunity to observe both the topographic features and the quality of ultrastructural preservation of human blood cells over 2,500 years old. Some of the ancient erythrocytes retained their biconcave shape (Fig 4) and still contained an electron-dense material, presumably hemoglobin (Fig 5). The whole erythrocytes were first viewed with the scanning electron microscope; then the same cells were studied in ultra-thin sections with the transmission electron microscope. It was possible to identify various classes of leukocytes, ie, lymphocytes, neutrophils, and eosinophils. The limiting plasma membrane of these cells was generally intact. Their nuclei were distinct and showed

characteristic distributions of heterochromatin and euchromatin. The cytoplasm of leukocytes recognized as lymphocytes showed swollen mitochondria and ribosomes (Fig 6). The cytoplasm of neutrophils showed numerous typical membrane-bound neutrophil granules (Fig 7).

Hair

Although the soft tissues of PUM IV were degenerated, the hair on the head of this mummy remained intact. When we examined some of these hairs by scanning electron microscopy, we found that the microarchitecture was preserved. The prominent scalelike plates (epicuticles) were seen to have retained their transverse orientation and regular spacing (Fig 8).

Insects

Adult insects and their larval forms are sometimes found between the wrappings of mummies and mummy bundles or even associated with the fleshy parts of the bodies.

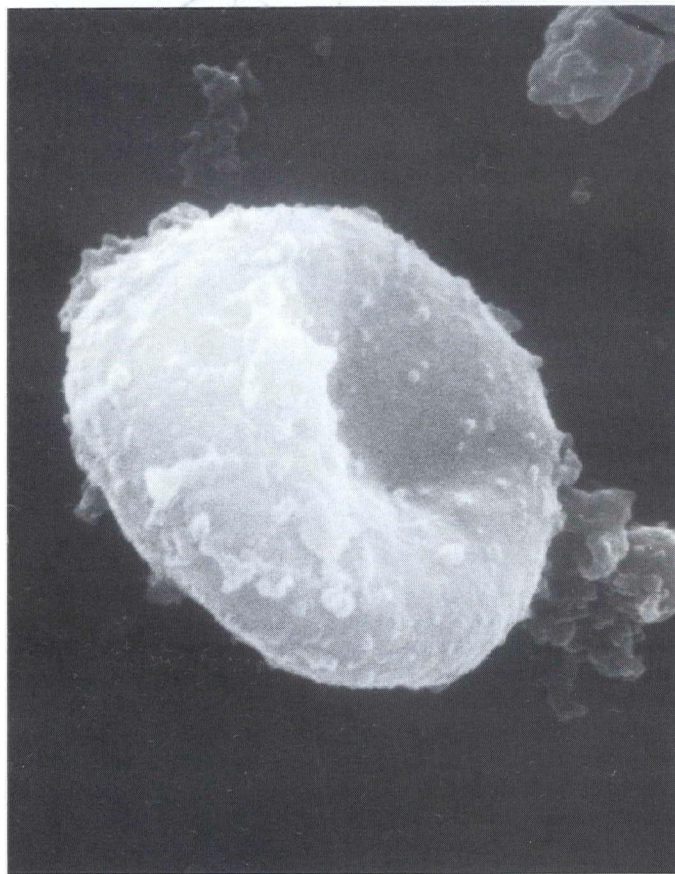


Fig 4

Scanning electron micrograph of an ancient erythrocyte—note central concavity x 14,500.

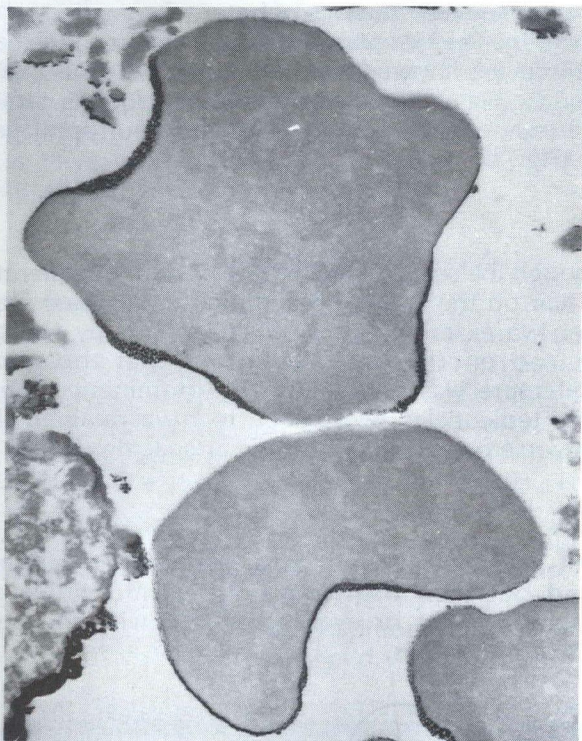


Fig 5

Sectioned ancient erythrocytes from PUM III show internal electron-dense hemoglobin. Black material on surface is layer of deposited gold-palladium added to perform SEM studies x 15,000.



Fig 7

Ancient neutrophils contained a distinct nucleus and well-preserved neutrophilic granules within their cytoplasm x 15,000.



Fig 6

Ancient lymphocytes showed a distinct nucleus, swollen mitochondria and ribosomes within their cytoplasm x 23,000.



Fig 8

Topographic details of a single hair removed from the head of PUM IV x 630.

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Using the scanning electron microscope, different areas on these immature and mature insects such as their mouthparts, appendages, and integument can be observed in detail (Fig 9). Compilation of this information in most instances provides the necessary clues for positive identification and classification of these ancient insects. Those associated with the fleshy parts of PUM IV were identified as members of the Anobiidae family, known as the drugstore beetle, *Stegobium paniceum* (Linnaeus).

A variety of insect forms were found associated with the Peruvian mummy bundles that we investigated. Two distinct species of flies and five distinct genera of beetles (Fig 10) were identified by using scanning electron micrographs. In addition, a previously unknown feature of the integument of the Calliphorid puparium was displayed using this three-dimensional survey approach (Fig 11).

Discussion

The application of electron microscopy to the study of samples collected from various mummies and their associated wrappings has produced new information. Examination of blood cells found in an Egyptian mummy

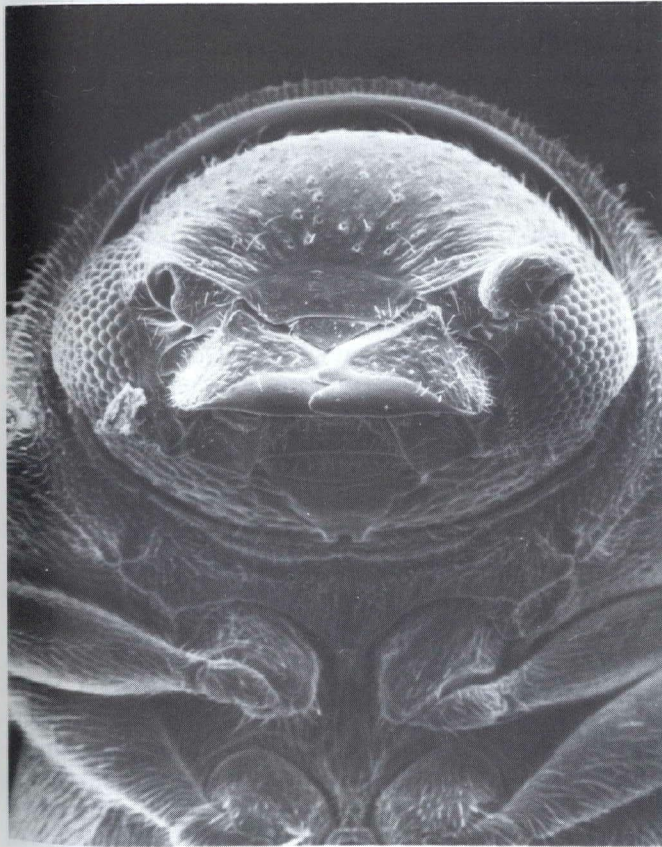


Fig 9
A so-called "drugstore beetle" removed from the soft tissues of PUM IV x 125.



Fig 10
Adult beetle, Anobiidae *Stichoptychus* on near, found within the Paracas mummy bundle x 40 (Photo credit, Paleopathology Newsletter 1982;39:5-9).

and studied by a continuum of microscopy (light, transmission and scanning electron microscopy) showed that erythrocytes and leukocytes over 2000 years old had the same ultrastructural features as their modern counterparts. Even though the time span of 28 centuries represents a brief period on the total evolutionary scale, our study showing the overall constancy of the construction of peripheral blood elements represents an important and previously unknown finding.

The ability to analyze fabrics using the scanning electron microscope also provides a more detailed way of confirming the method of their construction (pattern of weaving) as well as the basic fiber used than does the more conventional examination with the light, dissecting and polarizing microscope.

Finally, identifying insects found either in the flesh or contained between the various wrappings gives us insight into the effectiveness of different preservation processes (embalming techniques) and burial practices in general.

We should continue to use modern technology and newly developed instruments to study ancient specimens in order to further our understanding of earlier

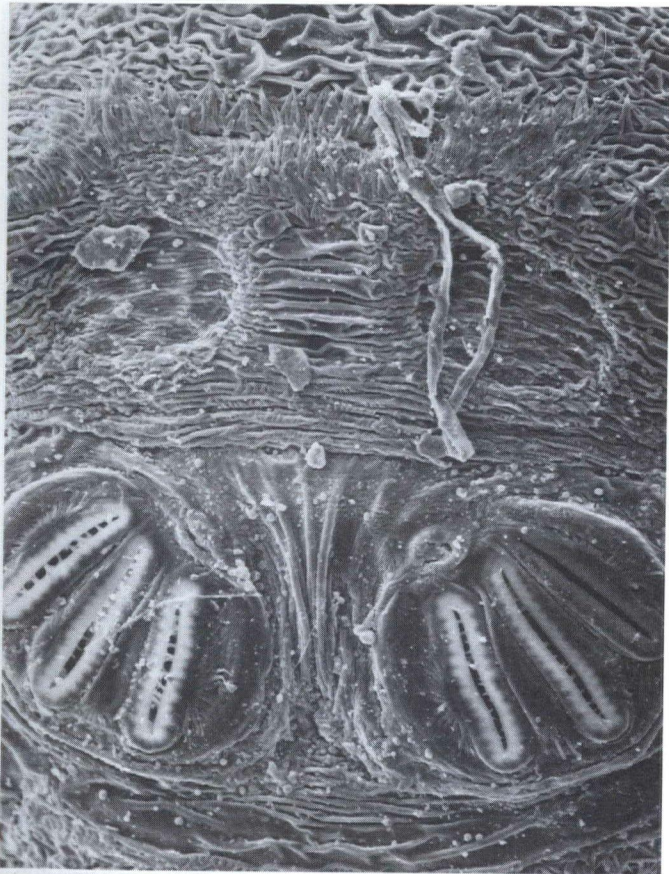


Fig 11

A previously undiscovered feature of the integument of a puparium of the fly Calliphoridae *Cochliomyia macellaria* (Fabricus) recovered from the Paracas mummy bundle x 132 (Photo credit, Paleopathology Newsletter 1982;39:5-9).

cultures. However, we should be aware that these samples, which offer a unique opportunity to explore our past, must be used judiciously as a nonrenewable resource.

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