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THE ELECTRON MICROSCOPE IN PALAEOPATHOLOGY

INTRODUCTION

THE earliest palaeopathological studies were made in the eighteenth century and were concerned with the results of trauma and disease in fossil animals. In 1825 Granville made a careful macroscopic study of a Ptolemaic mummy and diagnosed ovarian dropsy: this was almost certainly an instance of cystadenocarcinoma of the ovary. Twenty-seven years later Johan Czermak, a distinguished Viennese laryngologist, made the first microscopic studies of ancient mummified tissues. He employed simple but effective methods, teasing out the tissues in a solution of caustic soda. He was also a pioneer, being the first person to use a micrometer in the study of such preparations. The light microscope was therefore employed more than one hundred years ago in palaeohistological investigation.

A long fallow period followed, broken only by some obscure investigations made by Fouquet in 1889 (Moodie 1921) until in 1904 Wilder made sections of Peruvian mummy and dried Utah Amerindian bodies following rehydration of the tissues in a solution of caustic potash. Not long afterwards Shattock (1909) made frozen sections of portions of the calcified aorta of the Pharaoh Merneptah given to him by Grafton Elliot Smith. At about this time Ruffer commenced his classical palaeopathological studies in Cairo (Sandison 1967b). He issued a series of papers from the year 1910 until his tragic death at sea in 1917. Those published in 1909, 1910, 1911 are of histological interest. They may readily be consulted in the collected works edited by Roy Moodie (1921). Ruffer employed a rehydrating fluid which contained alcohol and sodium carbonate and which is still used today (Sandison 1963b).

Further light microscope studies came from Wilson (1927), Williams (1927, 1929), Aichel (1927), Simandl (1928), Shaw (1938), Busse-Grawitz (1942), Gürtler and Langegger (1942), Graf (1949), Schlabow *et al.* (1958), Rowling (1961) and Sandison (1955, 1957, 1959, 1962, 1963a, 1963b, 1967a, 1967b, 1968). The majority of these studies were made on Egyptian mummies but others included Egyptian Canopic material, and tissues from Peruvian mummies, a Guanche body, Amerindian bodies, German and Scandinavian *Moorleichen*, and Scandinavian and British skeletal material. For an assessment of these investigations reference may be made to Sandison (1963b).

THE ELECTRON MICROSCOPE

The light microscope suffers from an inherent limitation, i.e. that although empty *magnification* may be obtained by manipulating the optical system there is a limit to

the *resolution* of fine detail. This depends on the wavelength of visible light and was first demonstrated by Ernst Abbe in 1878. On 13 May 1927 Stintzing took out a patent application for overcoming the resolving limit of the light microscope by using cathode rays and in 1928 Ruska and Knoll began to investigate systematically the potentials of electron microscopy.

By 1933 Ruska had constructed an instrument capable of magnifying x 12,000 times with a resolution capacity of 5 mμ. The first commercial electron microscope was ordered by Imperial College in 1936 from Metropolitan Vickers Electrical Company. However, although powerful instruments were available they could not be used in histological studies because the available techniques of specimen fixation, embedding and microtomy were too crude to produce material which allowed direct visualization of macromolecular structure. Not until the end of the sixth decade of the twentieth century were these preparatory techniques sufficiently developed and capable of being reproduced in the study of biological materials.

ELECTRON MICROSCOPE INVESTIGATIONS IN PALAEONTOLOGY

It was inevitable that with the perfection of electron microscope techniques these would be applied to palaeontological material, and such studies have been used to supplement biochemical and X-ray and electron diffraction investigations of ancient and fossil bones (Ascenzi, 1955, 1963, Little 1960, Little *et al.* 1962, Wyckoff *et al.* 1963, Isaacs *et al.* 1963 and Race *et al.* 1966). Ascenzi succeeded in demonstrating the persistence of 640 Å periodicity in Neanderthal bone collagen and Wyckoff *et al.* a fairly similar periodicity in a Pleistocene *Equus*. Little *et al.* found collagen-like fibrillar fragments present in bone of Miocene age (25 million years old) and other workers have made similar observations on Miocene and Pleistocene fossil bones. It is interesting to note that electron microscopy was also used in the demonstration of disparity of the Piltdown cranium and jaw (Ascenzi 1963). All of these studies were directed to matrix material and cellular remains were not present.

ELECTRON MICROSCOPE STUDIES OF DRIED HUMAN SOFT TISSUES

Leeson, however, in 1959 made electron microscope studies of the skin of an Amerindian dried body of uncertain date from British Columbia. Leeson used the conventional rehydration techniques of Sandison (1955), fixed in osmium tetroxide, embedded in methacrylate and cut on a Porter-Blum ultramicrotome. He demonstrated cell membranes, nuclear membranes and chromatin. These findings were not surprising since light microscope studies of Egyptian mummy skin made by Ruffer (1921) and Sandison (1967a) had shown nuclei and cell membranes.

The first published accounts of electron microscope studies of ancient material of undoubted antiquity came from Lewin (1967, 1968). He investigated skin and muscle from a mummified Ancient Egyptian hand dating approximately to 600 B.C. After rehydration the tissues were fixed in osmium tetroxide, embedded in Epon-Araldite mixture and stained with lead hydroxide. Lewin published photographs which he claimed to show nuclear and cytoplasmic membranes, possible nuclear pores and possible mitochondrial organelles and tonofilaments.

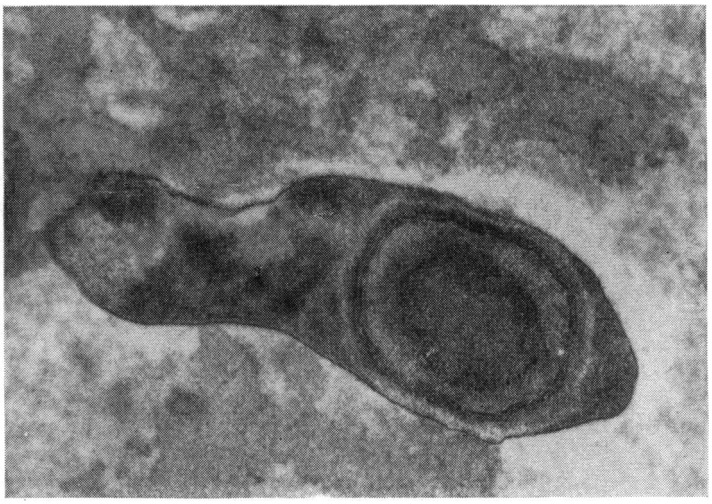

Figure 1.

Forearm muscle. A cell-like structure is lying within a heterogeneous background material (presumed degenerate skeletal muscle sarcoplasm). Note the nuclear and cytoplasmic compartments and the intracytoplasmic membranes. (X 38,500).

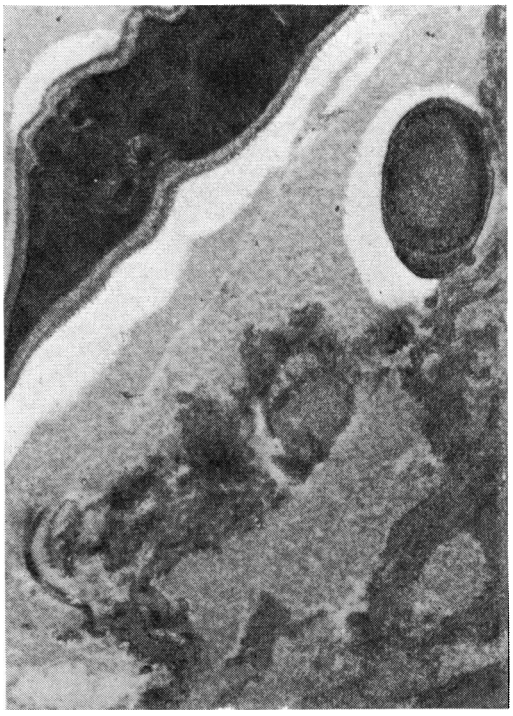

Figure 2.

Forearm muscle. A cell-like structure along with a cleft-like zone filled with pleomorphic masses of very dense homogeneous material is illustrated. (X 18,000).

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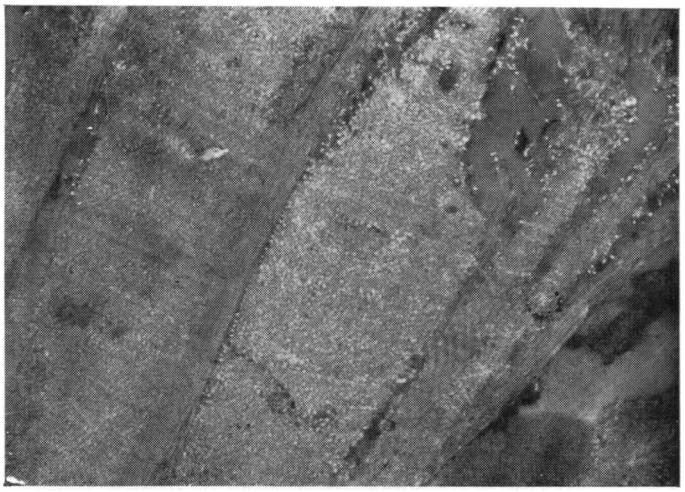

Figure 3.

Sclera. Substantial bundles of collagen fibrils alternate with non-organized materials of varying density. (X 18,000).

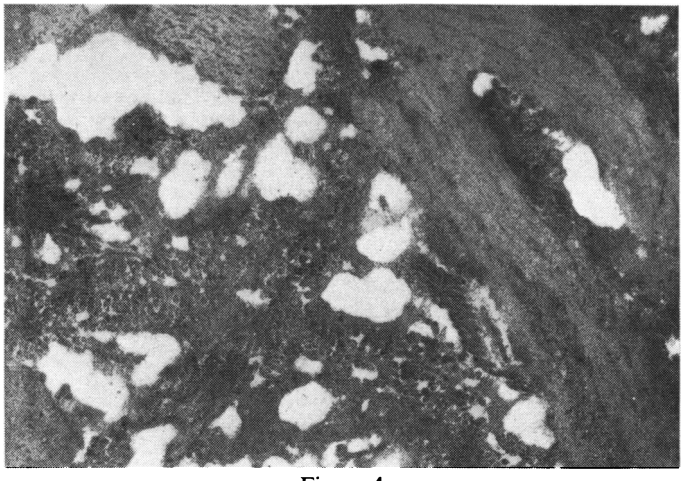

Figure 4.

Skin. Dermis only is present and consists of bundles of collagen fibrils, presumed fat spaces and unidentifiable material. (X 21,000).

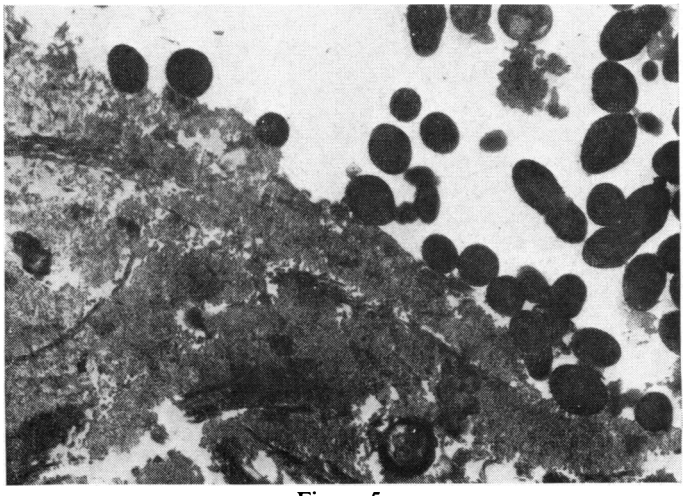

Figure 5.

Skin. Dermis is covered by large numbers of micro-organisms, probably cocci. (X 10,500).

MATERIAL AND METHODS USED IN THE PRESENT STUDIES

We have studied skeletal tissues from a well-preserved young female mummy of the 26th Dynasty, scleral material from another elderly male mummy of New Kingdom date, and dermal tissue from a young female Peruvian mummy of probable pre-Columbian date.

Following tissue rehydration using the method of Sandison (1955) 1 mm. cubic blocks were fixed for one hour in phosphate-buffered 2% osmium tetroxide at pH 7.4. They were embedded in Araldite and sectioned on an LKB ultrotome. All sections were stained with uranyl acetate and lead citrate and examined in a Siemen's Elmiskop 1A microscope. Original magnifications between x 2–11,000 were later photographically enlarged.

RESULTS

1. The tissues from the forearm flexor skeletal muscles of the young female mummy showed frequent cell-like structures within an unorganized heterogeneous background (fig. 1). These structures were spindle-shaped, measuring on average 2.5 x 0.9 μ and were limited from the surrounding tissues by a prominent membrane. This membrane could not be resolved into a triple-layered unit membrane. Within the structural membrane apparent 'nuclear' and 'cytoplasmic' components were present. The ovoid 'nuclear' zone averaged 0.7 μ in diameter, had a density comparable to normal chromatin and was faintly outlined by a double membrane profile. The 'cytoplasmic' compartment consisted largely of unorganized zones of variable density but all cell-like structures contained, in addition, membraneous structures concentrically arranged and often in tramline fashion. Other organized structures present consisted of tubular profiles (fig. 2)—1.5 μ in diameter—bordered by a definite zone of uniformly amorphous material and completely filled with pleomorphic masses of very dense but uniform structureless material.

2. Scleral tissue from the elderly male mummy was shown to be acellular and largely composed of interweaving bundles of collagen fibrils (fig. 3). Vague fibril periodicity was noted but could not satisfactorily be measured, although the fibril diameter was normal at 40 mμ. Non-fibrillar materials of variable density occupied the zones between collagen bundles and resembled elastica and neutral lipid.

3. Dermal tissue (fig. 4) from a young female Peruvian mummy consisted of irregularly dispersed bundles of collagen fibrils. The denuded surface from which the epidermis had been lost was peppered with large numbers of micro-organisms (fig. 5) measuring 0.6–1.5μ with spherical ovoid and dividing forms.

DISCUSSION

We believe that these electron microscope palaeopathological studies are the first to be made outside the North American continent and the first to report on the examination of a tissue other than skin or muscle. We hope that further investigations will be made and that such investigations might be useful.

Valuable observations have been made in palaeohistopathology with the light microscope employing conventional and histochemical staining methods on paraffin sections and on frozen sections (Sandison 1963b). Such techniques will continue to

be of service. Palaeopathology must however avail itself of the opportunities offered by other investigational methods and of these electron microscopy is important.

Some points which arise from the present studies give weight to similar observations made on light microscopy of dried human tissues. It is obvious that surface contamination of skin by bacterial and fungal organisms is a more serious impediment to electron than to light microscope studies. Some of these organisms have gained access to the tissues in ancient times during the embalming or drying process but others of contemporary origin may flourish on such specimens after unwrapping.

Light microscope studies have shown less prominent but similar involvement of the deeper tissues by putrefactive organisms. These may readily be disregarded by the eye on light microscopy but are a serious hazard in electron microscope studies. It is also more obvious on electron microscope examination that marked shrinkage has occurred in all structures due to the prolonged process of embalming, dehydration and fixation. Due allowance must be made for this in the interpretation of electron micrographs.

The cell-like structures seen in our studies of skeletal muscles might conceivably be of protozoon type. Protozoa are most unlikely to invade tissues in post-mortem putrefaction and if these structures were indeed protozoal it would seem to indicate an *in vivo* infestation.

The tubular profiles noted in our studies of skeletal tissue may possibly be interpreted as capillary structures containing haemoglobin.

SUMMARY

Palaeopathology, if it is to advance, must make use of all available advanced techniques. Electron microscopy is coming into use and may add useful information on preservation of ultrastructure as well as evidence of disease.

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