Electron Microscopy of the Manchester Mummies

by

A. Curry, C. Anfield and E. Tapp

Summary

Transmission electron microscopy (TEM), analytical electron microscopy (AEM) and scanning electron microscopy (SEM) have been used in the examination of various Egyptian mummy tissues. A liver was found to be well preserved with cell membranes and nuclei discernable. Centrioles with typical 9-fold symmetry have been identified in this tissue. Other tissues were found to be poorly preserved. Remains identified as parasites have been identified in the liver and an intestine. Bacteria, bacterial spores and hyphae-like structures were commonly observed in the tissues.

Crystals found surrounded by fibrous tissue in lung contained Si, Fe and Ti. It was concluded that this man suffered from sand pneumoconiosis during life. Heavy metal pollutants were not found in the various tissues examined by AEM.

The hair of two brothers was examined by SEM in an attempt to elucidate their parentage.

Introduction

Electron microscopy comprises of a number of distinct methods, some of which have been applied to the study of Egyptian remains. The transmission electron microscope (TEM) enables scientists to visualize structure finer than that previously accessible. The fineness of detail discernable is referred to as the resolving power and is a function of the wavelength used to illuminate the specimen. The shorter the wavelength the greater the resolving power. Electrons exhibit wave properties and their wavelength is a fraction of that of visible light giving a thousandfold improvement in resolving power.

Analytical electron microscopy is one of the latest developments in electron microscopy This technique utilizes the electron beam as a probe which analyses the elements contained within the area undergoing examination by the collection and analysis of the x-rays produced by interaction of the electrons and the specimen.

The scanning electron microscope (SEM) is used to examine the surfaces of solid objects. A fine beam of electrons is made to scan the specimen and an image is built up sequentially on a cathode ray tube, analogous to the formation of a television picture giving a topographical image with a good depth of field.

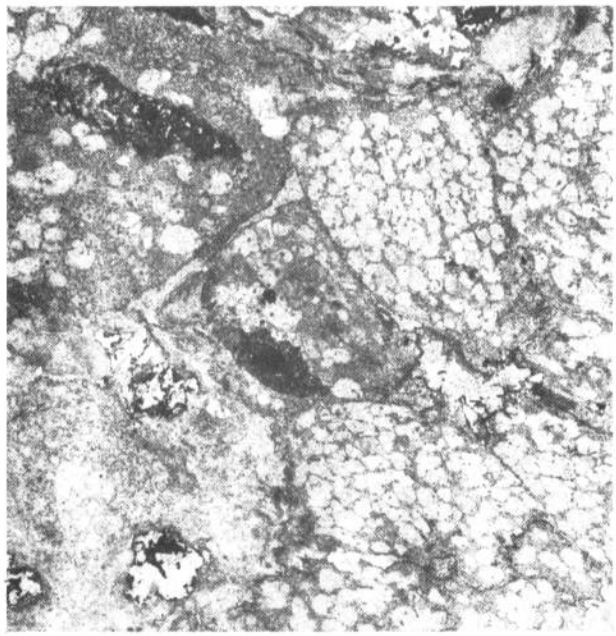
These three electron-optical techniques were used in the following study to investigate various tissues from mummified bodies in the Manchester Museum collection. Further details of these techniques can be found in Meek (1976).

Transmission Electron Microscopy (TEM)

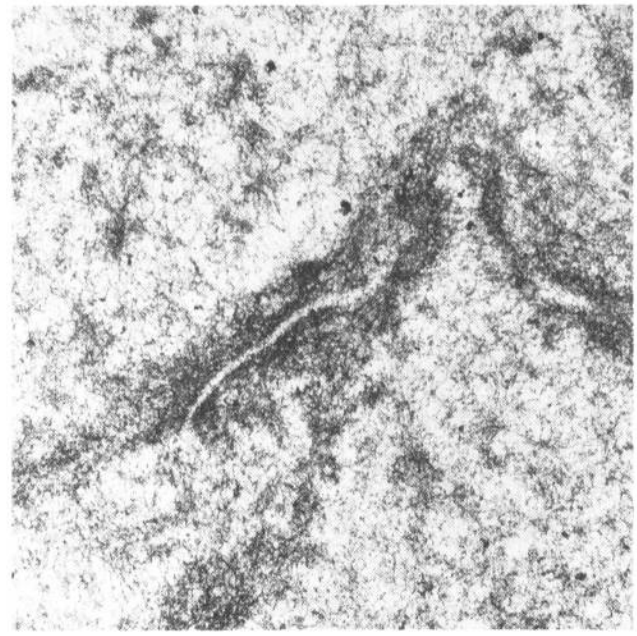
The transmission electron microscope has not been extensively employed in the field of palaeopathology even though instruments have been available for well over two decades. Early investigations all concentrated on the study of fossilized and ancient bone, not least of which was that of Ascenzi (1963) who used the electron microscope in the demonstration of the disparity of the Piltdown cranium and jaw. Leeson (1959) published the first study of dried human soft tissues, rehydrated according to Sandison (1955). He described cell membranes, nuclear membranes and chromatin in the skin of an Amerindian dried body from Columbia. These findings were not remarkable, for similar cellular components had been seen at light microscope level by Ruffer as early as 1921. Lewin (1967 and 1968) was the first to investigate with TEM the ultrastructure of ancient Egyptian mummified material. He processed skin and muscle tissue from an Egyptian head, dated at approximately 600 B.C., and published electron micrographs showing nuclear and cytoplasmic membranes, nuclear pores and tonofilaments.

Skeletal muscle and scleral material from two Egyptian mummies, and dermal tissue from a young Peruvian mummy of probable pre-Columbian date were studied by Macadam and Sandison (1969). The results were disappointing in that only vague cellular structure was observed in the skeletal muscle and the dermal tissue had been extensively infiltrated by micro-organisms. Yeatman (1971) described nucleoli and rough endoplasmic reticulum in cartilage tissue from a naturally dessicated Aleutian mummy some two to three hundred years old. He suggested that the cartilaginous matrix supported the cellular components preventing them from undergoing the profound shrinkage which normally occurs in drying soft tissue. Hufnagel (1973) studied several different tissues by TEM, including abdominal wall, aorta and trachea, from an Egyptian mummy 2,700 years old. At the ultrastructural level she described an abundance of fibrous material resembling collagen, trilaminar membranes and myelin-like configurations. Round, dense bodies, comparable in size to nuclei and mitochondria were also observed.

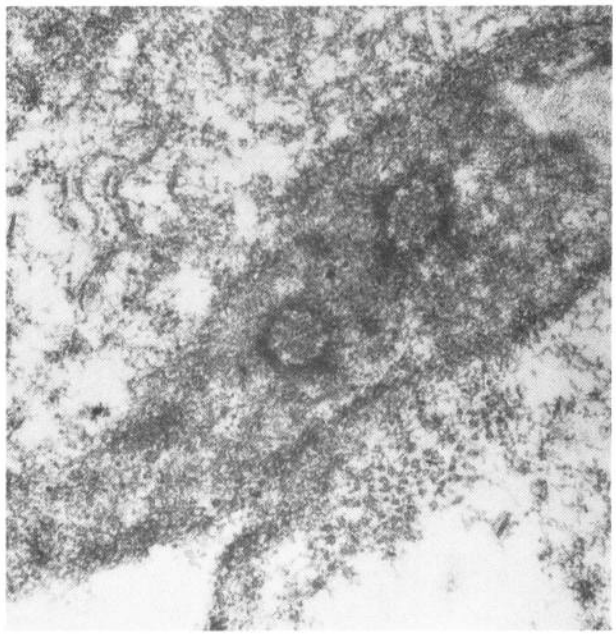
With these promising results in mind, tissues were taken from mummy '1770' which was unwrapped in 1975 at the Manchester Medical School. Unfortunately the tissues were unsuitable for TEM due to the poor state of preservation of the body. Other tissues, however, were available from the extensive collection of Egyptian



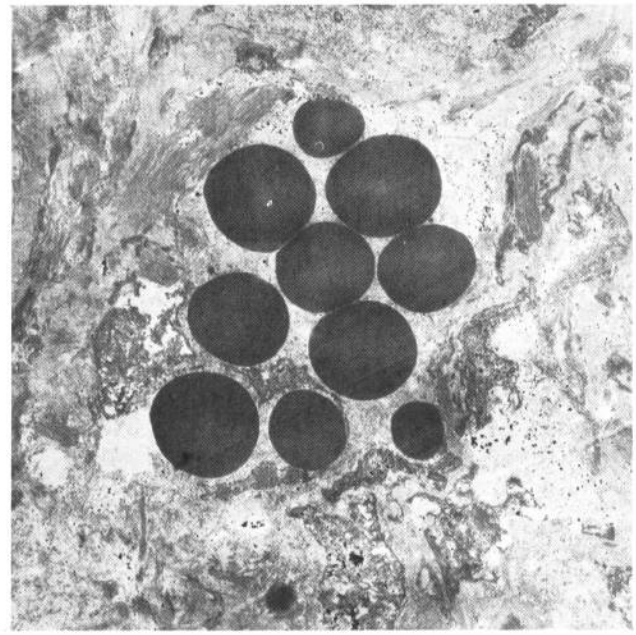
(1) A section of liver showing cell outlines, nuclei and electron lucent areas, interpreted as positions of mitochondria during life. ×2,700.



(2) Remains of two closely apposed cell membranes with an electron dense desmosome from liver. ×50,000.



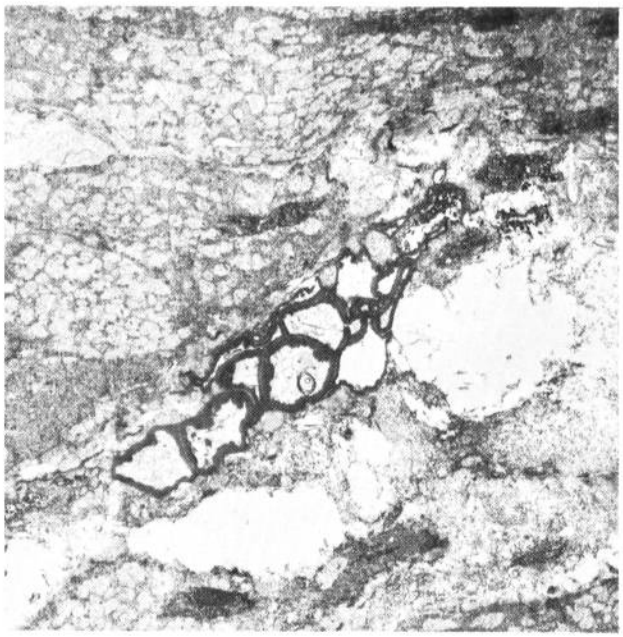
(3) A pair of centrioles from liver. ×38,000.



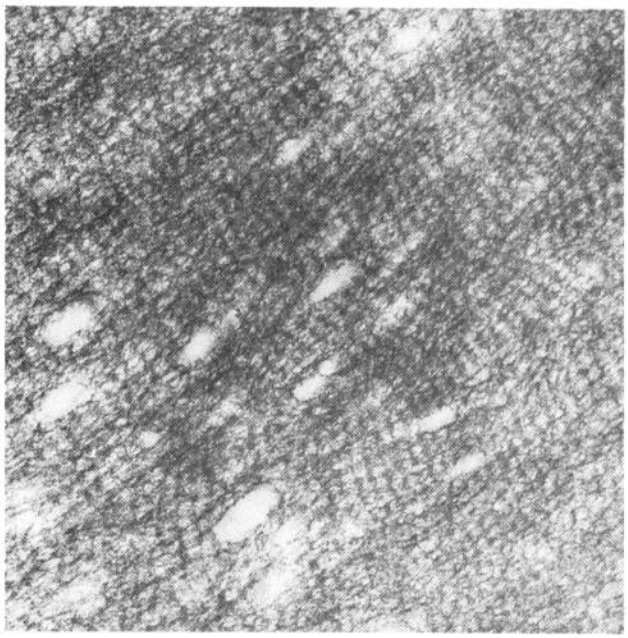
(4) Liver section showing intercellular space, interpreted as a capillary containing blood cells. ×3,700.

mummies and associated canopic jar material in the Manchester Museum.

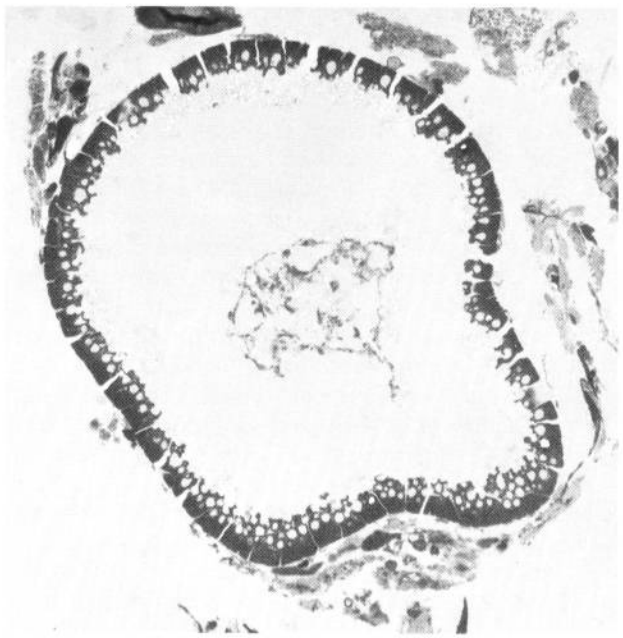
Materials and Methods Material for TEM came from three sources: the canopic jars from a tomb of two brothers, Nekht-ankh and Khnum-Nakht of the 12th Dynasty; the mummy of Asru (a female of the Late Period); and an isolated head labelled 'no 7740'. Pieces of tissue approximately $5mm^3$ were taken from areas adjacent to those removed for histological examination, and placed in a 4 per cent glutaraldehyde solution made up in 0.1M Sorenson's phosphate buffer pH7.4. Twenty-four hours later the rehydrated pieces of tissue were the consistency of firm agar and could be cut into $1mm^3$ blocks. The blocks were placed in fresh glutaraldehyde solution for four hours, followed by phosphate buffer for



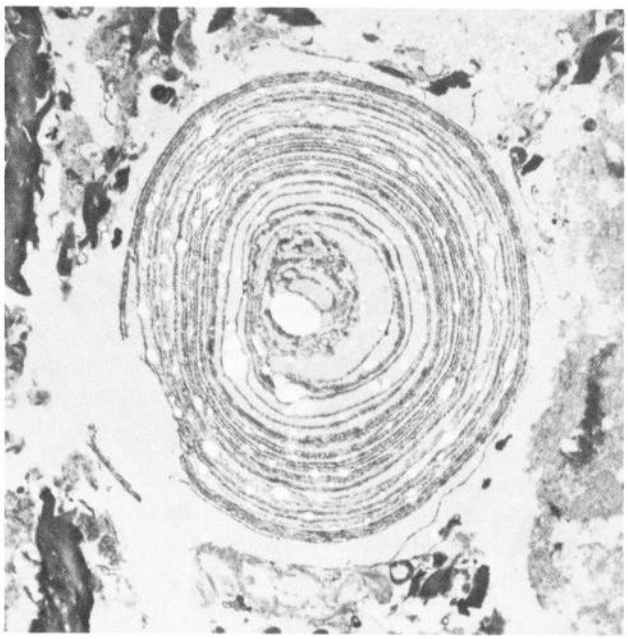
(5) Section of liver containing a group of cells with thickened walls, possibly the remains of a liver fluke. ×2,000.



(6) Longitudinally arranged fibrils with a cross-banding pattern from skeletal muscle. ×55,000.



(7) Section through intestinal wall showing remains of a parasitic worm. ×3,000.



(8) Section through intestinal wall showing a structure composed of concentric layers, interpreted as a parasite cyst. ×5,000.

storage pending the light microscope reports.

Selected tissues were post-fixed with 1 per cent osmium tetroxide in 0.1M Sorenson's phosphate buffer pH7.4, dehydrated in a graded series of alcohols and embedded in an Epon/Araldite mixture. Ultrathin sections were cut on an LKB Ultratome III ultramicrotome using glass or diamond knives, collected on to uncoated copper grids, stained with uranyl acetate and lead citrate and examined in an A.E.I. EM801 transmission electron microscope.

Results The ultrastructure of liver tissue from one of the two brothers was well preserved (**1**). Easily recognizable cell membranes were present with desmosomes at intervals along their length (**2**). Desmosomes are areas of intimate cell contact where cell to cell communication

is thought to occur. The cytoplasm showed relatively electron lucent areas of mitochondrial size probably indicating the sites of these organelles prior to their disintegration (**1**). A cell nucleus of medium electron density with crenated margin was present in most of the cells examined (**1**). Centrioles were identified in two cells, their typical tubular configuration with walls composed of nine segments being remarkably well preserved (**3**). Inter-cellular bile canaliculi were present but the expected microvilli were absent (**1**). Structures resembling red blood cells occurred in an intercellular space that was possibly a small capillary (**4**). Characteristic bundles of collagen fibres, exhibiting a 64 n.m. periodicity, were in close proximity to this blood vessel and in most other intercellular locations of the tissue examined. After examination of sections of a modern liver fluke and much consultation with parasitologists it was decided that a flattened group of cells with thickened walls (**5**) were possibly the remains of part of the liver fluke, *Fasciola hepatica*.

Skeletal muscle (**6**) from the canopic jars of the two brothers exhibited longitudinally arranged fibrils with a cross-banding pattern but did not display the organized regular cross-banding sequences of freshly prepared skeletal muscle tissue. Electron lucent vacuoles were apparent between some of the fibres, possibly indicating the location of mitochondria.

The intestinal tissue remains of Asru were poorly preserved, there being no remnant of cellular organization, only an amorphous ground substance interrupted by collagen fibres. However, Dr Tapp's histological examination had already revealed structures which were obviously not of human origin. Transmission electron microscope examination of this tissue showed two types of structure present, the first was of cylindrical appearance with a thick fenestrated wall (**7**) and the second was spherical with a wall composed of concentric layers (**8**). These were interpreted as being worm and cyst remains respectively. Photomicrographs of these remains were sent to Mr P. Gooch at the Commonwealth Institute of Helminthology, who suggested that they may have been the remains of *Strongyloides*, a parasitic worm which invades the intestinal wall, but this identification is by no means conclusive.

Lung tissue, again from the two brothers, was also shown to be largely acellular. The surviving composition was predominantly of bundles of collagen fibres and elastic tissue (**9**). The collagen, in many areas, consisted of fibrous capsules surrounding numbers of electron dense, crystalline particles (**9**). These particles were later investigated by analytical electron microscopy.

The brain/dura from the isolated head was disappointing at the ultrastructural level. No cellular organization remained, only amorphous material, large multilaminar bodies, resembling myelin figures, and remains of large numbers of micro-organisms were present (**10**).

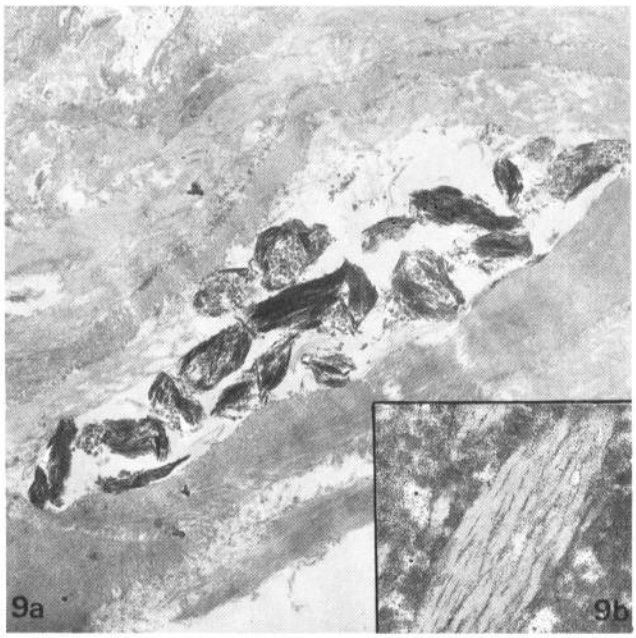
Two types of micro-organism have been identified. Filamentous structures resembling fungal hyphae (**11**) were present in the brain/dura, lung and intestinal tissues. Almost spherical structures 0.7μm in diameter, indicative of bacterial cocci, were found in most of the tissue samples examined. A wall up to 30 nm thick was easily identifiable surrounding many of these bacteria and on occasion a division septum was apparent. Within the brain/dura, lung and intestines many bacterial spores were readily observed (**12**). These have a complex envelope composed of many concentric layers.

Discussion Previous transmission electron microscope studies on dried, soft tissues from mummified bodies have not been very successful in demonstrating a wealth of detail at ultrastructural level. The presence of connective tissue components, however, have been consistently documented (Lewin 1967, 1968; Macadam and Sandison 1969; Yeatman 1971; Hufnagel 1973). It seems likely that the rigid, structural organization of collagen and elastic tissues prevent their total decomposition. A similar situation was demonstrated by the skeletal muscle examined, the fibrillar pattern being reasonably well preserved.

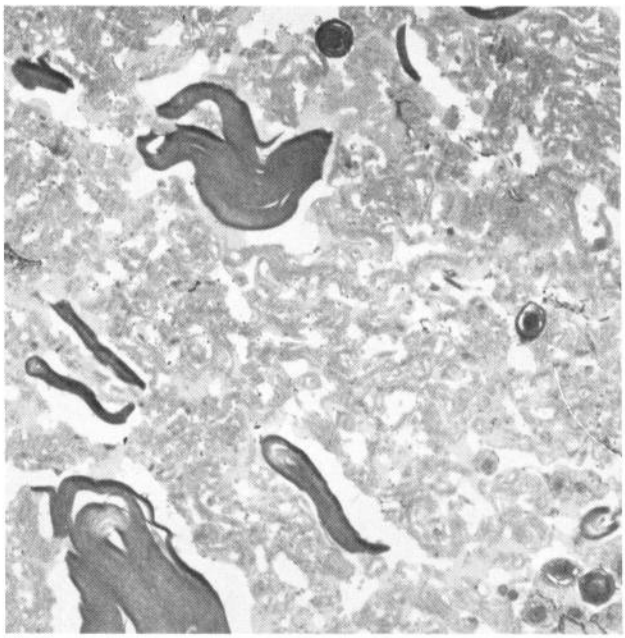
Soft body tissues putrefy quite rapidly after death and consequently it was not surprising that little cellular detail was discernable in the intestine, lung and brain/dura examined. The structural preservation of the liver tissue was therefore quite remarkable. The presence of cell and nuclear membranes are well documented (Leeson 1959) but the finding of desmosomes and centrioles is very interesting. The liver must have been removed from the body very soon after death and dried quickly to preserve the sub-cellular structure to any extent.

Worm infestations in the Ancient World and Egypt have been well documented (Brothwell and Sandison 1967) and appear to have been of common occurrence. Most modern findings of parasitic worm infections in ancient peoples have come from analysis of faecal remains (Gooch 1975). The eggs or cysts of most parasitic worms are extremely resistant to decay and are passed out of the intestine in large numbers. Remains of adult parasitic worms are difficult to identify as these would simply putrefy on the death of the host. However, if the worms become calcified during the life of the host, or if the body organs containing these worms were dried soon after death, then the worms would remain relatively intact.

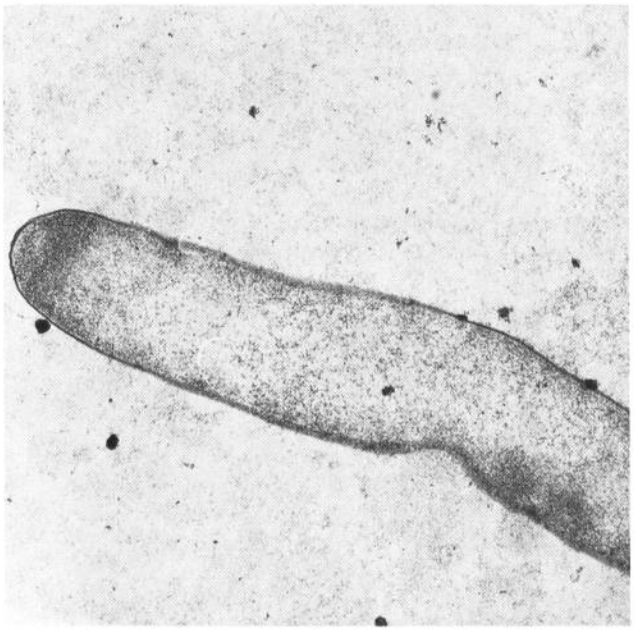
The calcified remains of the parasite Guinea worm, *Dracunculus*, were discovered by radiology, as discussed by Dr Isherwood. The identification of the two worms discovered by TEM is tentative. *Fasciola* has been identified previously in archaeological deposits (see annotated bibliography of Gooch 1975) from their characteristic eggs. If the remains found in the liver of Nekht-ankh are *Fasciola*, then this was probably an early invasive stage. The second worm, *Strongyloides*, differs from most other nematodes found in the ileum, by living inside the intestinal wall. From here the female worm releases parthenogenetic eggs which pass out of the intestines and hatch. Larvae reinfect humans by burrowing through the skin. After migrating around the body they finally settle in the wall of the ileum. The occurrence of nematode remains within the epithelium of the ileum of Asru suggests that she suffered from an infection of *Strongyloides*, which can cause anaemia in



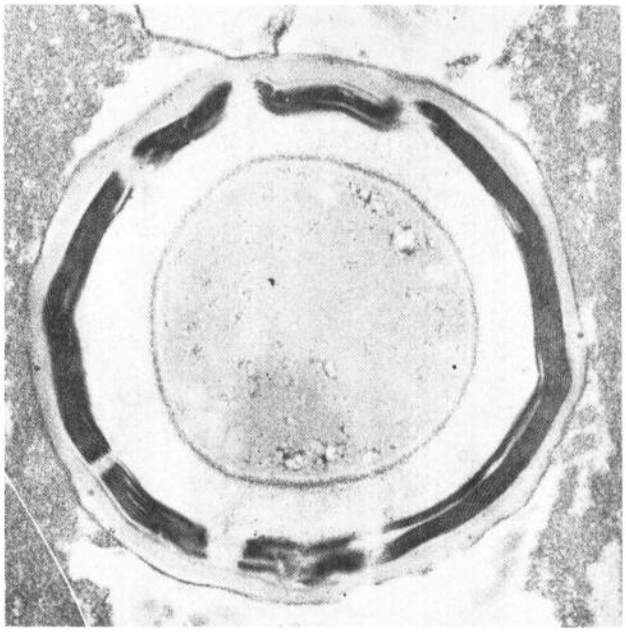
(**9**) Section through part of a lung containing sand particles. Note that fibrous tissue completely surrounds these particles. ×12,500. (*Inset*) Remains of bundles of elastic tissue from lung. ×17,000.



(**10**) A section of mummified brain showing large myelin bodies and bacterial spores. ×3,700.



(**11**) Filamentous structure resembling a fungal hypha. ×25,000.



(**12**) A bacterial spore. Note concentric layers. ×80,000.

life by destroying the intestinal mucosa (Borradaile et el. 1967).

The age of the micro-organisms present in the mummies is problematical. The spores and hyphae-like structures found within the tissues are probably of ancient origin, for they would sporulate as the tissues dried out. The abundance of bacterial spores can give an indication of the state of the tissues when they were mummified. For comparison pieces of fresh brain tissue were allowed to putrefy over a period of twelve days. Some of the pieces were kept in a humid atmosphere while others were kept dry. The former contained numerous bacterial spores whereas the latter contained very few. The brain/dura from the isolated mummified head contained many bacterial spores indicating that this tissue has been allowed to putrefy. If the brain had been removed soon after death and the body packed with 'natron' the fragments of brain tissue remaining along with the dura

would have dried out relatively rapidly and would not have been extensively infiltrated by bacterial spores.

Analytical Electron Microscopy (AEM)

The application of analytical electron microscopy to the study of Egyptology was first reported in 1975 (Tapp, Curry and Anfield 1975). AEM was used to analyse crystalline material found within lung tissue. Since this publication we have used AEM to examine certain mummies for the presence of heavy metals.

Present-day disorders caused by lead, mercury or other metal poisoning are particularly unpleasant. In most cases such poisoning is the result of the inadequate disposal of industrial waste and hence receives much publicity (Hunter 1971). Thus, modern man is exposed to small quantities of certain heavy metals because he lives in an industrial society. We therefore regard such problems as being of fairly recent origin, but did ancient civilizations suffer from cases of metal poisoning and what quantities of such metals occur in Ancient Egyptians? Examination of Egyptian mummy material for chemical poisoning has been previously undertaken by an American group using the neutron activation test and atomic absorption (Cockburn, Borraco, Reyman and Peck 1975) and they have found significantly lower concentrations of lead in bone compared to modern man, although the concentration of mercury was about the same.

Materials and Methods Material was rehydrated (see Materials and Methods, TEM), but was not post-fixed in osmium tetroxide. The small pieces were dehydrated in a graded series of alcohols and embedded in an Epon/Araldite mixture. Sections were cut on an LKB Ultratome III ultramicrotome using a diamond knife, and coated in carbon in a vacuum coating unit. The sections were examined, unstained, in either an A.E.I. Corinth 275 electron microscope fitted with a Kevex Si (Li) x-ray detector or an A.E.I. Cora analytical electron microscope.

An A.E.I. EM801 was used for the electron micro-diffraction studies.

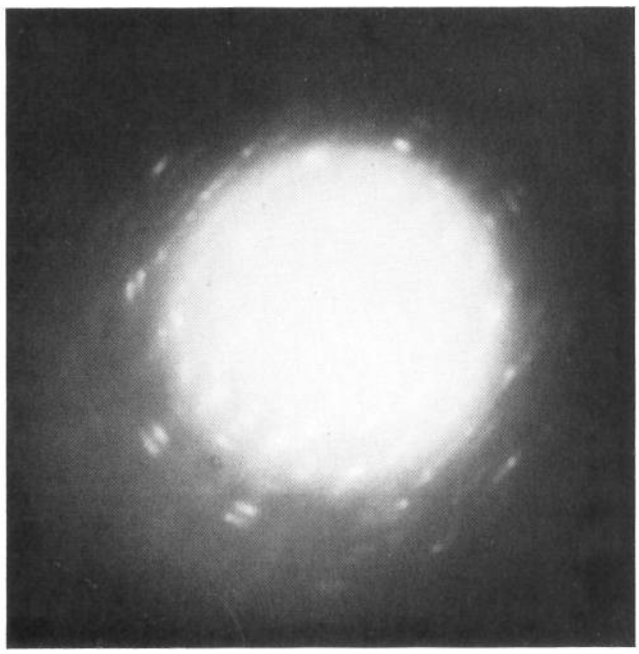
Results (a) *The examination of diseased lung material* The discovery of diseased lung in Nekht-ankh and its full histological examination has been described in Dr Tapp's earlier chapter. In essence, birefringent particles were seen around blood vessels and in fibrotic areas of the lung indicating disease during life. These particles proved to be of a crystalline nature as they produced an electron diffraction pattern when examined in a transmission electron microscope operating in the electron diffraction mode (**13**).

The elemental nature of these crystals was examined on three occasions and the surrounding tissues and resin were taken as control areas. A typical result of the crystal analysis is illustrated (**14**) and the results tabulated (**15**).

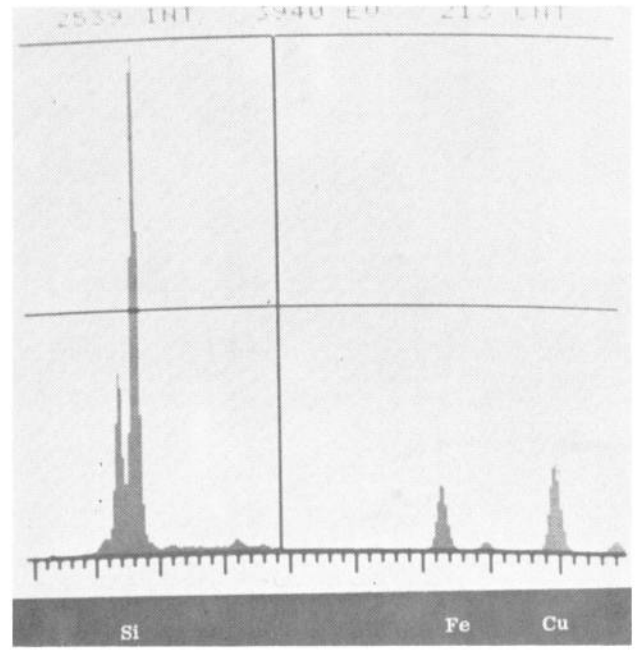
These results indicate that there is a high proportion of silicon, iron and titanium in the crystals with significantly lower levels in the tissue and resin. This would seem to indicate that the crystals are detrital quartz (SiO_2) grains.

(b) *Examination of mummy material for heavy metals* Lung and liver from Nekht-ankh, intestine from Asru and brain/dura from an isolated head were examined in A.E.I. Cora for the presence of heavy metals. No trace was found of any heavy metal in any of these tissues.

Discussion Pulmonary silicosis is common in miners, quarry workers and potters and may produce consider-



(**13**) Electron diffraction pattern of crystals found in lung tissue indicating that crystal symmetry is present.



(**14**) A typical elemental analysis result of the crystals found in lung tissue. Cu peak is from copper specimen grid.

able morbidity and mortality (Cockburn et al. 1975). According to Berry et al. (1976) deposits in lungs of patients with pneumoconiosis were demonstrated to be of crystalline nature by electron diffraction. By contrast, 'normal' patient lung deposits were amorphous in nature; Nekht-ankh undoubtedly had deposits in his lungs and these were of a crystalline nature. The analysis of the deposits suggests that they were sand and thus Nekht-ankh undoubtedly suffered from sand pneumoconiosis. This condition is known in modern Bedouins (Bar-Ziv and Goldberg 1974). Nekht-ankh is unlikely to have been a stone worker and it therefore suggests that his condition was a result of inhalation of sand, silt and/or clay-sized particles during dust-storms. This condition of sand pneumoconiosis in Nekht-ankh is not unique as it is known that Pum II also suffered from this condition (Cockburn et al. 1975).

A.E.I. Cora can detect 10^{-17}–10^{-18} grams of an element if it is present in a sample. Thus, the absence of lead, mercury or other heavy metals in the tissues of the mummies examined is interesting. Perhaps the preparation of the samples for examination eliminated these elements. However, this explanation seems unlikely in view of the low solubility of compounds containing lead or mercury. It seems that heavy metal pollution or conditions caused by exposure to these toxic elements were not common in ancient Egypt. Similar results were obtained by Cockburn et al. (1975) who found that the lead content of two Egyptian mummies examined was significantly smaller than that of modern man. The mercury level in Pum I and Pum II was, however, found to be the same as that found in modern man. This last result is surprising and is in contrast to the results obtained from the Manchester mummies. It is obvious that this aspect of examination of ancient bodies deserves more attention, and certainly needs to be tackled using several analytical methods. In conclusion it would seem that modern man is exposed to considerable lead pollution compared to Egyptian man.

Scanning Electron Microscopy (SEM)

Scanning electron microscopy was principally used to examine the insect remains found in the mummies, but was also used to examine the surface structure of some mummy hair in the hope of elucidating the race of each of the two brothers, Nekht-ankh and Khnum-Nakht.

The sarcophagi of the two brothers depict one to be negroid and the other of the 'semitic' type. This puzzling situation obviously leads to some interesting speculation as to the parentage of these men. However, was there any race difference between these two brothers? There are pigmentatory and structural differences between the hair of these two races and it was hoped to elucidate any differences by examination of the hair remains from these men whose mummified bodies had been unwrapped earlier this century by Margaret Murray who placed the remains in large storage jars.

FIG. 15 *Major elements found in the crystals of Nekht-ankh's lungs*

Elements: Si, S (small amount), Cl, K, Ca, Fe.
Surrounding tissue (control): Si (small amount), S (small amount), Cl.
Surrounding tissue-free resin (control): Si (small amount), S (small amount), Cl.

Quantitative analysis with reference to the elements Si, Fe, Ti

	crystals	crystals	crystals	crystals	resin (control)
Si peak (P)	3899	3926	14505	11576	1096
background (B)	990	1332	3816	2853	666
P – B	2909	2594	10689	8723	430
$\frac{P-B}{B}$	2.94	2.94	2.80	3.08	0.65
Fe peak	1031	1044	5147	2903	535
B	279	324	1494	1350	405
$\frac{P-B}{B}$	2.69	2.22	2.45	1.15	0.32
Ti peak	546	—	2524	1877	—
B	414	—	1782	1476	—
$\frac{P-B}{B}$	0.32	—	0.42	0.27	—

Quantitative analysis using A.E.I. Cora (no Ti found)

	crystals	tissue (control)	resin (control)
Si peak	1003	88	83
B	165	65	65
$\frac{P-B}{B}$	5.08	0.35	0.27
Fe peak	262	28	—
B	40	23	—
$\frac{P-B}{B}$	5.5	0.21	—

Materials and Methods The dried specimens of hair were attached to SEM stubs by conductive adhesive and coated in gold using either a sputter coater (Edwards) or a vacuum coating unit (A.E.I.). These were examined in either an A.E.I. Corinth 275 electron microscope fitted with an S.E.M. attachment (CESA) or a Cambridge S4–10 Stereoscan. Images were recorded on Ilford FP4 film.

Results All hair samples examined in this study were reddish in colour and examination under the scanning electron microscope showed samples to have an identical structure. The outer surface of the hair is covered by flattened imbricated scales.

Discussion The study of human hair is a much more complex subject than it first appears (Brothwell and Spearman 1963) as micro-organisms, bleaching in life, preparation of the body after death and exposure to atmosphere can alter the colour. Indeed the bodies found preserved in peat in Denmark (Glob 1969) had red hair, as have mammoths recovered from permafrost regions (Carrington 1958). In view of these facts the colour of hair would not appear to be useful. However, as the structure of the hair examined was all found to be identical, two conclusions are possible: The hair of one of the brothers was not preserved or both brothers were of identical race.

Assessment of the application of electron microscope techniques in palaeopathology

The interpretation of the ultrastructural appearance of freshly fixed tissues, under the transmission electron microscope is difficult, to say the least. Tissues, thousands of years old, which were either allowed to dry out naturally or subjected to mummification processes, present an almost overwhelming number of problems in interpretation of TEM results. There are bound to be many artefacts produced by shrinkage, putrefaction or embalming methods.

Subcellular components can be identified from dried soft tissues, as demonstrated by the liver tissue examined in this study. These organelles indicate the success or failure of embalming techniques. Microscopic diagnosis of disease is largely a matter of cellular arrangement and organization rather than the appearance of subcellular components. In this context TEM can only be used as an interesting extension of normal light microscope examinations.

The presence of foreign bodies or material is of diagnostic relevance. The identification of parasitic worm remains and the inference of associated disease is possible, though difficult. The number of micro-organisms present can also be an indication of the state of preservation of tissues prior to mummification.

The analytical electron microscope has been proven to be useful in this study. The structural preservation of the cellular components is not as important in this technique as in transmission electron microscopy and consequently the results are more meaningful. However, leaching of certain salts or addition of salts during embalming must add caution to the interpretation of results. Without the analytical electron microscope, the chemical compositon and therefore the identification of the crystalline deposits in the lung tissue from Nekht-ankh, would have been difficult.

The application of scanning electron microscopy to palaeopathology is limited. In this study it has been used to examine the surface structure of hair and the fragile insect remains.

In summary, it appears that transmission electron microscopy is of limited practical diagnostic use in palaeopathology unless disease is produced by the presence of foreign material. As an extension of routine light microscope investigations, it is interesting from an academic point of view. The analytical electron microscope results indicate a bright future for this relatively new technique in the field of palaeopathology.

Acknowledgements

We are grateful to A.E.I. Scientific Apparatus Ltd (Kratos), Mr P. Gooch and Mr Robin Grayson for their assistance in this project.

References

A. Ascenzi (1963), 'Microscopy and prehistoric bone', in *Science in Archaeology*, ed. D. Brothwell and E. Higgs, London.

J. Bar-Ziv and G. M. Goldberg (1974), *Archives of Environmental Health*, 29, 121.

J. P. Berry, P. Henoc, P. Galle and R. Pariente (1976), 'Pulmonary mineral dust', *American Journal of Pathology*, 83, 427.

L. A. Borradaile, F. A. Potts, L. E. S. Eastham and J. T. Saunders (1967), *The Invertebrata*, Cambridge.

D. Brothwell and A. T. Sandison (1967), *Diseases in Antiquity*, Thomas, Springfield, Illinois.

D. Brothwell and R. Spearman (1963), 'The hair of earlier peoples', in *Science in Archaeology*, ed. D. Brothwell and E. Higgs, London.

R. Carrington (1958), *Elephants, A short account of their natural history, evolution and influence on mankind*, The Scientific Book Club, London.

A. Cockburn, R. A. Barraco, T. A. Reyman and W. H. Peck (1975), 'Autopsy of an Egyptian mummy', *Science*, 187, 1155.

P. V. Glob (1969), *The Bog People*, Faber and Faber Ltd.

P. S. Gooch (1975), *Helminths in archaeological and prehistoric deposits*, Annotated bibliography No. 9, Commonwealth Institute of Helminthology, St Albans, Herts.

L. Hufnagel (1973), Communication in *Paleopathology Newsletter.*

D. Hunter (1971), *The diseases of occupations*, English Universities Press Ltd.

T. S. Leeson (1959), 'Electron microscopy of mummified material', *Stain Technol*, 34, 317.

P. Lewin (1967), 'Paleo-electron microscopy of mummified tissue', *Nature*, 213, 416.

P. Lewin (1968), 'The ultrastructure of mummified skin cells', *Can. Med. Ass. J.*, 98, 1011.

R. F. Macadam and A. T. Sandison (1969), 'The electron microscope in Palaeopathology', *Medical History*, 13, 81.

G. A. Meek (1976), *Practical electron microscopy for biologists*, Wiley-Interscience.
M. A. Ruffer (1921), *Studies in the paleopathology of Egypt*, ed. R. L. Moodie, Chicago.
A. T. Sandison (1955), 'The histological examination of mummified material', *Stain Technol.*, 30, 277.
E. Tapp, A. Curry and C. Anfield (1975), 'Sand pneumoconiosis in an Egyptian Mummy', *B.M.J.*, 3 May.
G. W. Yeatman (1971), 'Preservation of chondrocyte ultrastructure in an Aleutian Mummy', *Bull. N.Y. Acad. Med.*, 47, 104.