NON-FILARIAL ELEPHANTIASIS IN ETHIOPIA
ANALYTICAL STUDY OF INORGANIC MATERIAL IN LYMPH NODES

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Introduction

Elephantiasis is a condition of progressive diffuse thickening of the skin and subcutaneous tissues. This is associated with oedema and hypertrophy of the tissue. Elephantiasis develops most commonly around the feet and lower legs, the groin and scrotum, occasionally the arms, and, rarely, on the face. It is not of itself a fatal condition, but the disabling effects cause great hardship to the many sufferers. This condition is generally associated with filarial infections. However, not all cases of tropical elephantiasis can be directly attributed to parasitic infections.

Non-filarial elephantiasis is common in many parts of North-East and Central-East Africa, particularly along the Rift Valley region, and accounts of it are recorded from Uganda (LOWENTHAL, 1934; BURKITT, 1966); Kenya (CLARK, 1948); Nigeria (NGU and KONSTAM, 1962); and Ethiopia (COHEN, 1960; OOMEN, 1968) in which the important common factor is the assertion that both adult and larval filariae are absent from the areas investigated, while at the same time the authors were unable to specify the cause of the condition.

This emphasizes a major epidemiological problem, for in early cases it is usually impossible to distinguish filarial from non-filarial elephantiasis (NELSON et al., 1962) and the cause or causes of non-filarial elephantiasis are far from clear. Most opinions centre around pre-existing lymphatic deficiencies, and several classifications have been presented (e.g. the detailed classification given by OOMEN, 1969). No conclusive evidence exists for a hereditary factor.

In this paper, an account is given of analytical results obtained following a new approach to the study of this condition, based on transmission and scanning analytical electron microscopy, in conjunction with X-ray diffraction analysis, emission spectroscopy, microincineration and various histological techniques. As a result, hitherto undescribed accumulations of inorganic material, especially silicon and aluminium, have been located in lymph nodes draining affected areas. It is postulated that these elements, either directly, or in conjunction with other factors, are responsible for some types of swollen leg.

Preliminary observations on this condition indicated the presence of some silicate material, but in the absence of distinct crystals, giant cells, or epithelioid granulomas, they were considered to be of little significance. However, more recent studies on lymph node material, carried out in Ethiopia, have shown the presence of epithelioid granulomas and occasional giant cells (PRICE, 1972) and later studies on slides of lymph node material from patients with swollen legs sent to this laboratory from Ethiopia drew attention to cells containing very small particles of a crystalline nature, which proved birefringent and

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polarizable. Unfortunately, most were far too small to allow accurate petrological analysis, but they were so common as to suggest that they might be relevant to the condition, and that further more detailed investigations would be of value.

**Materials and methods**

Lymph node material was taken from 12 subjects; inguinal nodes draining affected limbs from 7, and inguinal nodes from clinically normal limbs from 5. The affected material came from 3 male and 2 female subjects with apparent non-filarial elephantiasis (NFE), and 2 male subjects known to have filariasis. In none of the patients did the swelling extend above the knee. The control material was taken during herniorraphy. In none of the subjects used was there any family history of the condition.

1. **Light microscopy**

Tissues were fixed in Carnoy fixative and 10% aqueous formalin, and processed according to standard methods for routine examination. Staining methods employed included haematoxylin and eosin, and periodic acid Schiff.

2. **Electron microscopy**

The basic pattern of these preparations is illustrated in Fig. 1. Small pieces of tissue, none larger than 1 mm.\(^2\) were fixed in buffered glutaraldehyde, washed in the buffer, then secondarily fixed in Palade buffered osmium tetroxide (Palade, 1952). After dehydration, the tissues were embedded in araldite. Thin sections were cut for examination using an AEI EM6B transmission electron microscope, after staining with uranyl acetate and Reynolds's lead citrate (Reynolds, 1963). For analysis, material processed as described above was sectioned at about 5 \(\mu\)m, and mounted on pure quartz slides for random spot analysis using a Microscan 5 micro-analyser, and on copper grids for similar analyses using a Stereoscan analytical electron microscope, both at the Cambridge Scientific Instrument Company, Applications Division. These sections were initially analysed unstained, being post-stained to allow identification of the cell types analysed. Information was plotted in graph form. The sections were photographed, the areas analysed located as accurately as possible, dissected out, and re-processed for the preparation of thin sections for transmission electron microscopy.

**Fig. 1.**

**ELECTRON MICROSCOPE**

**PREPARATIVE AND INVESTIGATIVE PROCEDURES**

Lymph node material

- glutaraldehyde
- osmium tetroxide
- araldite
- sections

- unstained
- stained
- copper grids
- quartz slides

STEREOSCAN

SCANNING E.M. and 
INORGANIC ANALYSIS

M ICROSCAN

AEI EM 6B

TRANSMISSION E.M.
The quartz slides were coated with carbon before and after the sections were applied, the first coat to assist later removal of the sections prior to re-processing and transmission electron microscope studies, and the second to assist electrical conductivity. The re-processed material was examined unstained to locate any electron-dense material in the areas analysed. 1 μ sections of all the material prepared for electron microscopy were stained with toluidine blue in borax for light microscope examination.

3. X-ray diffraction analysis was carried out on material from 3 of the nodes, prepared by the method of Gold (1967), and the diffraction patterns obtained recorded using a powder camera.

4. Microincineration procedure was carried out on sections from all the tissues, using alternate stained and incinerated serial sections, which were examined for birefringent and polarizable material.

5. Emission spectroscopy studies were carried out at the North-East London Regional Instrumentation Centre, using a Hilger Medium Quartz Spectrograph. Prepared samples were arced for 90 seconds to complete combustion, and the results compared with known standards.

Results

Using routinely prepared light microscope sections, crystalline material was located in macrophages (Fig. 2). With dark field reflected light, these were clearly seen, distributed at random throughout the nodes. Generally, their size did not allow a clear determination of their form, though in a few areas larger clusters could be seen, usually in the cortical regions of the lymph nodes. There was little birefringent material in the tissue obtained

Fig. 2. Particles (P) in macrophages within the medulla of an affected inguinal lymph node. Haematoxylin and eosin. × 250.
from normal subjects, and none was seen to polarize. Using polarized light, the crystals for which any form could be determined were very clear, and were seen to be fine acicular structures, usually associated with connective tissues (Figs. 3 and 4) or radiating groups (Figs. 5 and 6). Petrological features of crystals large enough to be studied included polarization colours of a very low order (whites and greys predominating), with a variable refractive index and a low birefringence. Extinction angles could not be determined due to the generally small size. None of the crystals appeared to be isotrophic.

Figs. 3 and 4. Light and corresponding dark-field photographs, showing acicular crystals in loose connective tissue in lymph node medulla. × 1,000.
Not all the apparently crystalline material observed by reflected light showed polarization phenomena, but this could be due to the fact that not all are crystals (e.g. some may be melanin granules, or cell debris), or that they are masked in some way, perhaps by protein deposited through cellular reactions against them.

Microincineration. All the samples were studied using this technique. Silicate particles were detected in 2 NFE patients, but not in the filarial material. All the normal material was negative.

X-ray diffraction techniques were carried out on 3 tissue block extracts, 2 NFE and one filarial. All were positive for silicon, one being almost as good as the standard. The results from the filarial material were not as distinct as those from the NFE material (Fig. 7).

Emission spectroscopy revealed the presence of silicon in all the samples analysed, but it was difficult to obtain constant results due to the unrepeatable nature of sparks. Other elements positively identified included boron, magnesium, calcium and phosphorus.

Electron microscopy. Particles were seen, though very difficult to find, in 3 of the nodes from NFE patients, but none in the filarial or normal material (Figs. 8 and 9). Using analytical techniques, silicon was found in all the material examined (patients and controls). The relative counts are shown in the Table, and a representative graph, prepared from information computed during several 200-channel analyses, shown in Fig. 10. It is clear that the counts for silicon in the patients, both NFE and filarial, average
25% higher than found in the normals, although there is a definite overlap in the ranges recorded.

![Fig. 7. Powder camera diffraction patterns. The silicon standard is on the extreme left. The other three patterns are NFE, known filarial, and NFE respectively. The two NFE patterns are very similar to the standard, that of the filarial material is not so distinct.](image)

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<th>Table. Relative silicon counts for the three groups.</th>
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<td>Patients (4)</td>
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<td>Totals</td>
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Some of the tissue analysed in the Microscan 5 was re-processed for examination using transmission electron microscopy, in order to identify if possible the form in which the silicon was present. There was no evidence of crystalline or particulate material in any sections examined after this process.
FIG. 8. Transmission electron micrograph of amorphous/acicular deposits in macrophage.
× 15,000.

Silicon is usually classified as a trace element in the higher animals, forming less than 0.05% of the total body weight in man. It occurs naturally in hair and nails, and has been detected in blood, urine, and pancreatic juice. In this study, attention is drawn to the presence of high quantities of silicate material in lymph nodes draining the affected limbs of elephantiasis patients. It has been found in normal subjects, but in relatively lower quantities than in either NFE or known filarial cases. The distribution of the element is very random, though certainly associated with cells of the macrophage series, but the amounts found can only be presented in a relative way at the present time, and the forms in which they occur are not easy to determine. Only X-ray diffraction and spectrophotometric methods positively identified silicon in all the tissues, while methods dependant upon direct optical phenomena (such as crystallographic or interference data) did not always confirm the presence of particulate material. This is interpreted as being due to the relatively small number and size of actual particles present, and the optical limitations of the methods employed, and also the high probability that not all the silicate present is in particulate form.

The origin of the crystals is uncertain, but there appear to be only two possible sources, one being the direct entry of particulate material into the body, and the other due to their formation from a saturated solution within the tissues following the gradual accumulation of soluble silicates. The presence of such silicate material within the body may preclude the idea that much particulate material enters the body directly, as one would expect to see at least some evidence of crystalline inclusions in the material from the normal subjects. Precipitation of crystalline material in an acid medium (caused by cell breakdown and the activity of released lysosomal enzymes) from a saturated solution...
once a critical 'threshold level' has been attained seems far more likely. Silicates dissolve far more readily in alkaline conditions, such as are common in many tropical soils, and it is considered that the greater part of the silicate content of these tissues entered the body in solution from the soil via abrasions on the feet and lower legs. It is quite possible that the results of chemical and physical weathering on the rocks within the area investigated could produce local accumulations of toxic silicates. The analytical results suggest a possible felspathic origin, and there are also indications that certain varieties of quartz may be present.

It is considered to be unlikely at this stage that the presence of silicates alone could give rise to the condition of swollen legs. There may be contributory factors, e.g. parasites, hereditary factors, and deficiencies in lymphatic channels. However it is felt that the overall effects that the silicates have on the cells, particularly of the reticulo-endothelial system, are dramatic enough to suggest that they play a significant part in establishing conditions favourable for the development of 'swollen leg,' even possibly providing the 'trigger mechanism' for the onset of filarial elephantiasis.

The effects caused by various silicates on biological tissues and systems have been studied by many workers. Further light and electron microscope observations made during the present study, and comparisons with the findings of other workers, will be the subject of a later paper, which is in the process of preparation.

Summary

A new approach has been made to the study of some cases of tropical elephantiasis, using transmission and scanning analytical electron microscopy, in conjunction with X-ray diffraction analysis, emission spectroscopy, microincineration, and various histological techniques. Hitherto undescribed accumulations of inorganic materials, particularly silicon and aluminium, have been located in lymph nodes draining affected areas. It is postulated that these elements, either directly or in conjunction with other factors, are responsible for the onset of some types of swollen leg.

REFERENCES