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# Fungal Osteoclasia: a Model of Dead Bone Resorption

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A morphological investigation has been carried out on the osteoclastic activity revealed by a fungus of the *Mucor* genus on buried bone. The hallmark of its activity in eroding bone is the finding of resorption pits and boring channels whose walls are sharp and well calcified up to the free edge, suggesting that bone resorption affects crystallites and the organic matrix simultaneously. Unlike normal osteoclastic cells, the fungal membrane in contact with the bone shows no brush border. As the electron microscope reveals no migration of material to the fungal membrane and its protoplasm, the view is expressed that the material which reaches the fungus has been previously solubilized. There is good reason for supposing that a substance capable of solubilizing the inorganic bone fraction spreads freely through bone tissue, decalcifying the matrix, where, but only where, hyphae show the effects of ageing. All the findings are thoroughly discussed and compared with those furnished by the boring channels in fossil bone.

*Key words:* Bone — Fossil bone — Bone resorption — Boring channels — Fungi.

L'action ostéoclasique exercée par un champignon appartenant au genre *Mucor* sur des os inhumés a été étudiée au point de vue morphologique. L'érosion osseuse donne lieu à des cavités et à des canalicules de percement, dont les parois apparaissent régulièrement calcifiées jusqu'a l'interface avec le champignon, ce qui veut dire que la réabsorption intéresse en même temps la matrice organique et les cristallites. Au contraire des ostéoclastes, la membrane du champignon ne montre jamais un bord en brosse au niveau des points où elle se met en contact avec le tissu osseux. De même l'examen au microscope électronique ne décele apparemment aucun passage de matériel dans l'épaisseur de la membrane ainsi que dans le protoplasme du champignon, ce qui porte à admettre qu'un processus de solubilisation a lieu au préalable. Seulement dans le cas où le champignon présente des phénomènes de vieillissement, des données font prévoir la libération d'une substance capable de décalcifier la matrice organique. Tous les résultats ci-dessu sont discutés de façon analytique et comparés à ceux fournis par les canalicules de percement des os fossiles.

Die osteoklastische Aktivität eines der Mucor-Gattung angehörenden Pilzes auf begrabene Knochen wurde morphologisch untersucht. Die Wirkung dieses Pilzes in der Knochenerosion führt zu Resorptionshöhlen und Bohrkanälen, deren Wände scharf und bis zur Oberfläche gut verkalkt sind; das bedeutet, daß die Knochenresorption gleichzeitig die Kristalliten und die organische Matrix in Mitleidenschaft zieht. Im Gegensatz zu den normalen Osteoklasten zeigt die Membran des Pilzes an den Berührungsstellen mit dem Knochengewebe keinen Bürstensaum. Ebenfalls läßt eine elektronenmikroskopische Untersuchung keinen Durchgang von Substanzen durch die Membran und das Protoplasma des Pilzes erkennen, so daß man annehmen muß, daß vorher ein Auflösungsprozeß stattgefunden hat. Die Vermutung liegt nahe, daß eine Substanz, welche den anorganischen Knochenanteil aufzulösen vermag, sich

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frei durch das Knochengewebe ausbreitet, wobei die Matrix demineralisiert wird; diese Erscheinung tritt jedoch nur ein, wenn der Pilz Alterserscheinungen aufweist. Alle oben aufgeführten Resultate werden analytisch erörtert und mit denjenigen verglichen, die durch Untersuchung der Bohrkanäle von fossilen Knochen erhalten werden.

## Introduction

As far back as 1864 Wedl described, in both recently deposited and fossil bones and teeth, canaliculi forming a labyrinth-like structure and measuring 4–7 μ in diameter. They were quite different from those present in normal tissue and contained fine brownish filaments. The author was of the opinion that these findings were caused by a fungus.

Canaliculi showing the same features were observed by Roux (1887) in fossil bones and fully confirmed by Schaffer (1889, 1890, 1894). Roux called them "Bohrkanäle", that is, bored channels and suggested the name *Mycelytes ossifragus* to indicate the fungus producing them.

In 1956 Morgenthaler and Baud observed "Bohrkanäle" in a series of bones ranging from the Magdalenian period to the 16th century and furnished evidence that the canaliculi contained an imperfect saprophytic fungus belonging to the genus *Dematiacea.*

A recent histologic and electron microscopic study of the *torus palatinus* in the Neandertal "Circeo I" skull revealed to Sergi, *et. al.* (1972) that some areas of the bone contained many canaliculi or microtubules interweaving irregularly and forming a complicated labyrinth-like structure. Although the canaliculi were empty, their features are all in line with the view that they were produced by mould colonies.

Starting from these premises we have carried out an investigation in order to give an explanation for the following points: (a) Are the "Bohrkanäle" really produced by mould colonies? (b) What is the nature of the mould responsible for the bone canaliculi? (c) Which morphological features found in bone are due to moulds?

## Material and Method

Fragments of human vertebrae, obtained from cadavers dissected in the autopsy room, were buried in flower-pots containing garden earth. The earth was kept wet and exposed at a room temperature of about 20°. A few days later a white mould masked the surface of the bone fragments.

On the 45th day after burial, when the bone had been thoroughly penetrated by a luxuriant mycelium, specimens were prepared from bone fragments for examination under both optical and electron microscopes. At the same time mould specimens were cultivated on potato infusion agar to allow isolation and identification of the mycete (or the mycetes).

For histologic examination, following EDTA (ethylene diamine tetraacetic acid) demineralization and embedding in paraffin, bone sections of approximately 8 μ were cut. They were stained with haematoxylin-eosin, or by the periodic acid-Schiff (P.A.S.) method or the van Gieson method.

The bone fragments to be examined under the electron microscope were fixed for 2 h in 4% formalin in phosphate buffer (pH 7.2) and refixed for one hour in cold (4°) 1% osmium tetroxide, buffered as above. The fragments were dehydrated in acetone and embedded in Araldite. Ultrathin and semi-thin sections were cut on a Porter-Blum microtome. The ultrathin sections were examined unstained, or after staining with uranyl acetate and lead citrate,

under an Elmiskop 1 A electron microscope. The semi-thin sections which were about 1 μ thick, were stained with Azur II and methylene blue or by the von Kossa method, and examined under the optical microscope.

The absence of other species of microorganisms responsible for the bone changes was checked. To do this, autoclaved fragments of human vertebral bodies and fresh bone fragments aseptically removed from rats were used. The autoclave treatment was carried out at 100° or 200° for 20 min. The same procedure was applied for sterilization of the earth in which the bone fragments were buried. Fungal contamination of bone was achieved by using the previously cultivated and isolated mycetes.

The autoclaved human bone was chosen because here the marrow had been lost, so that it could be presumed that the activity of the fungi had been mainly directed against the calcified bone matrix.

A fragment of the *torus palatinus* of the neandertal skull "Circeo I" was used as control material for the "Bohrkanäle". For technical details on the preparation of histological and ultra-thin sections from this fragment see Sergi, Ascenzi and Bonucci (1972).

## Results

*Identification of the Fungi*. The mould which developed spontaneously around and in the bone fragments buried in garden earth consisted of the mycelia of three fungi. They have been identified as belonging to the genera *Mucor*, *Cladosporium* and *Candida*. However, when autoclaved bone or bone aseptically removed from rats was artificially contaminated with a single fungal genus and buried in sterilized earth, only *Mucor* was able to develop, inducing initial bone changes after 15–20 days. In these conditions the development of *Mucor* is limited, while it is luxuriant in the human vertebral fragments buried in unsterilized earth. In microscopic sections *Mucor* was easily recognizable thanks to three main features: absence of septa (coenocytic) in the abundantly ramified jung hyphae, presence of the columella in the sporangium, and pigmentation of the sporangium.

*Optical Microscopy*. As findings were very similar in all the bone specimens examined, a global report is given.

In both fresh and autoclaved bone the marrow is lost and the medullary spaces are completely filled by a mycelium which consists of closely packed, non-septate (coenocytic) hyphae measuring 2–6 μ in diameter and tubular in structure. They branch at right angles or nearly at right angles and only rarely show swellings or enlargements. The hyphae have delicate membranes as walls, which stain fairly well with the haematoxylin in haematoxylin-eosin stain. P.A.S.-positiveness, on the other hand, is rather scanty. The mycelium is concentrated near the bone trabeculae, while at the center of the medullary spaces the mycelial network is lost. Here the hyphae are often swollen and show degenerative changes such as vacuoles and lipid droplets. In the same area and never near the bone trabeculae, the mycelium sometimes gives rise to sporangiophores. These differ considerably in length, branch irregularly and bear terminal globose, spore-filled sporangia.

At the periphery of the medullary spaces near the trabeculae, where the mycelium is very crowded, the hyphae lie very close to the bone (Figs. 1, 2). Characteristically, their presence is accompanied by the notching of the bone surface. This is easily identified at quite low magnifications as saucerlike depressions or deeply punched-out-looking pits, which have sharp borders and to some extent

resemble the lacunae of Howship (Fig. 2). Moreover, at several points hyphae penetrate into the bone trabeculae at different angles, forming canaliculi which are apparently unrelated to the pre-existing bone structures such as the lacunae and canaliculi of osteocytes (Fig. 1).

Two different patterns have been observed at the border between the hyphae and bone matrix. In many cases, the fungi were found very close to a normal calcified bone matrix and there were no findings which suggested that bone resorption was started by a process of bone matrix decalcification (Fig. 2). In these cases, the fungi appeared to be normally structured.

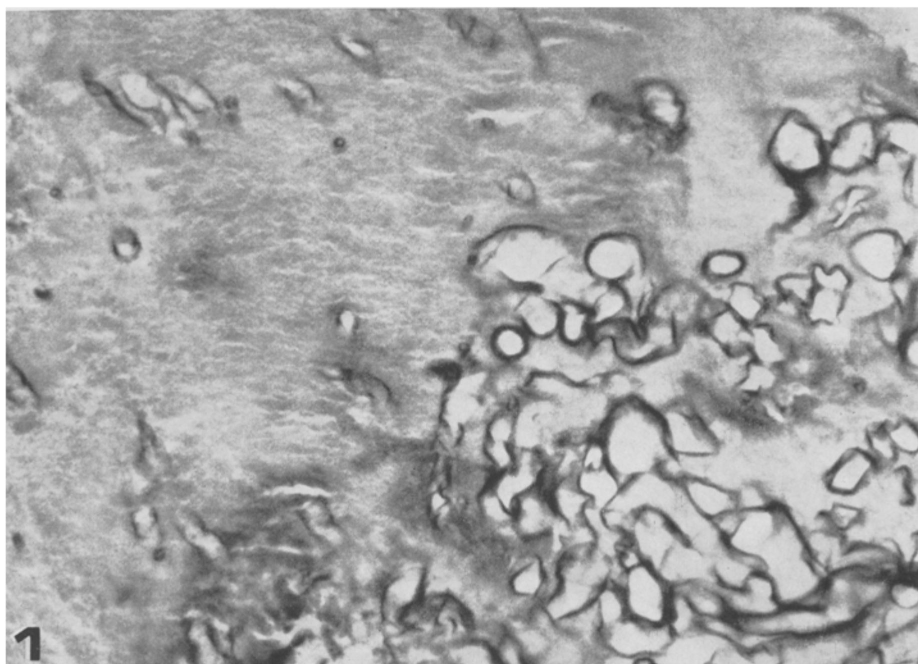
In other cases, the bone matrix which was undergoing fungal resorption was decalcified and the hyphae were placed near to and within a bone matrix in which only a few patchy islands of inorganic substance were left. In these cases, the fungi showed obvious accumulation of lipids in the hyphae.

The feature called "brush" or "striated" or, more recently, "ruffled" border, which is a characteristic hallmark of osteoclastic resorption, was never found at the interface between hyphae and bone tissue in any of the material examined. The sections stained by the von Kossa method did not show calcareous material in the hyphae of the mycetes.

*Electron Microscopy*. Electron microscope examination allows a detailed analysis of fungal morphology and of how mycetes reabsorb the bone tissue. As far as fungal morphology is concerned, the cytoplasm possesses the common organelles, the most prominent and best preserved being mitochondria (Figs. 3, 4). As a rule the number of mitochondrial cristae is rather low. The cell wall lying in contact with the bone is smooth (Figs. 3, 4) and does not, as in osteoclasts, show a series of fine fingerlike processes which fan out from the cell surface to terminate on the bone surface (Fig. 5). Similarly, there is absolutely no system of channels and vacuoles concentrated in the cytoplasm near the membrane proper. No structures possessing the features of lysosomes are demonstrable either. This is evidence that, although bone tissue is eroded, there is no appreciable phagocytic activity at the interface between mycetes and bone.

In agreement with the optical microscope findings, the electron micrographs show that in many cases the actual "edge" of the bone in contact with the fungal hyphae is a normally calcified matrix (Figs. 3, 4). In other words, appearances suggest, that the collagen fibrils are not deprived of their apatite crystals. As a result, there are sharp boundaries between normally calcified bone and fungal membrane. At some points, where sectioning artefacts have produced a break between the fungal membrane and the front where reabsorption of bone is taking place, there is evidence that a very thin layer of bone crystallites sticks to the outer surface of the menbrane (Fig. 4). Even so, no isolated apatite crystallites are visible either in the membrane or in the hyphae, so that there is no reason to suppose that active transfer of crystallites from bone to fungi takes place.

In other cases, the bone matrix in contact with the fungi was more or less decalcified (Figs. 6, 7). Sections of hyphae were found in areas consisting of closely packed collagen fibrils with a clearly recognizable 640 Å period. Small aggregates of crystallites were often visible in these decalcified areas, sometimes appearing as very thin strips surrounding fungal hyphae (Fig. 7).



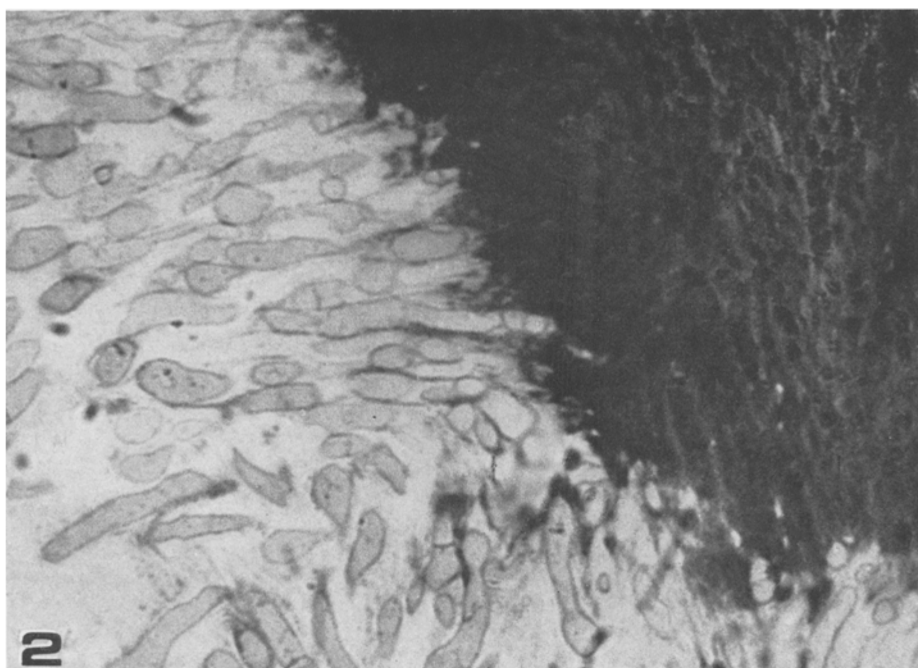


Fig. 1. Section of bone contaminated with *Mucor*. At the right side and at the bottom a medullary space is crowded by fungal hyphae which penetrate into the bone forming canaliculi visible at the top and at the left side. Embedding in paraffin. Haematoxylin and eosin-stained, decalcified section, ×1500

Fig. 2. Semi-thin section of bone contaminated with *Mucor*. Fungal hyphae are penetrating in a trabecula forming deeply punched-out-looking pits in the normal calcified bone. Embedding in Araldite. Kossa-azure II-methylene blue stained, undecalcified section, ×1500

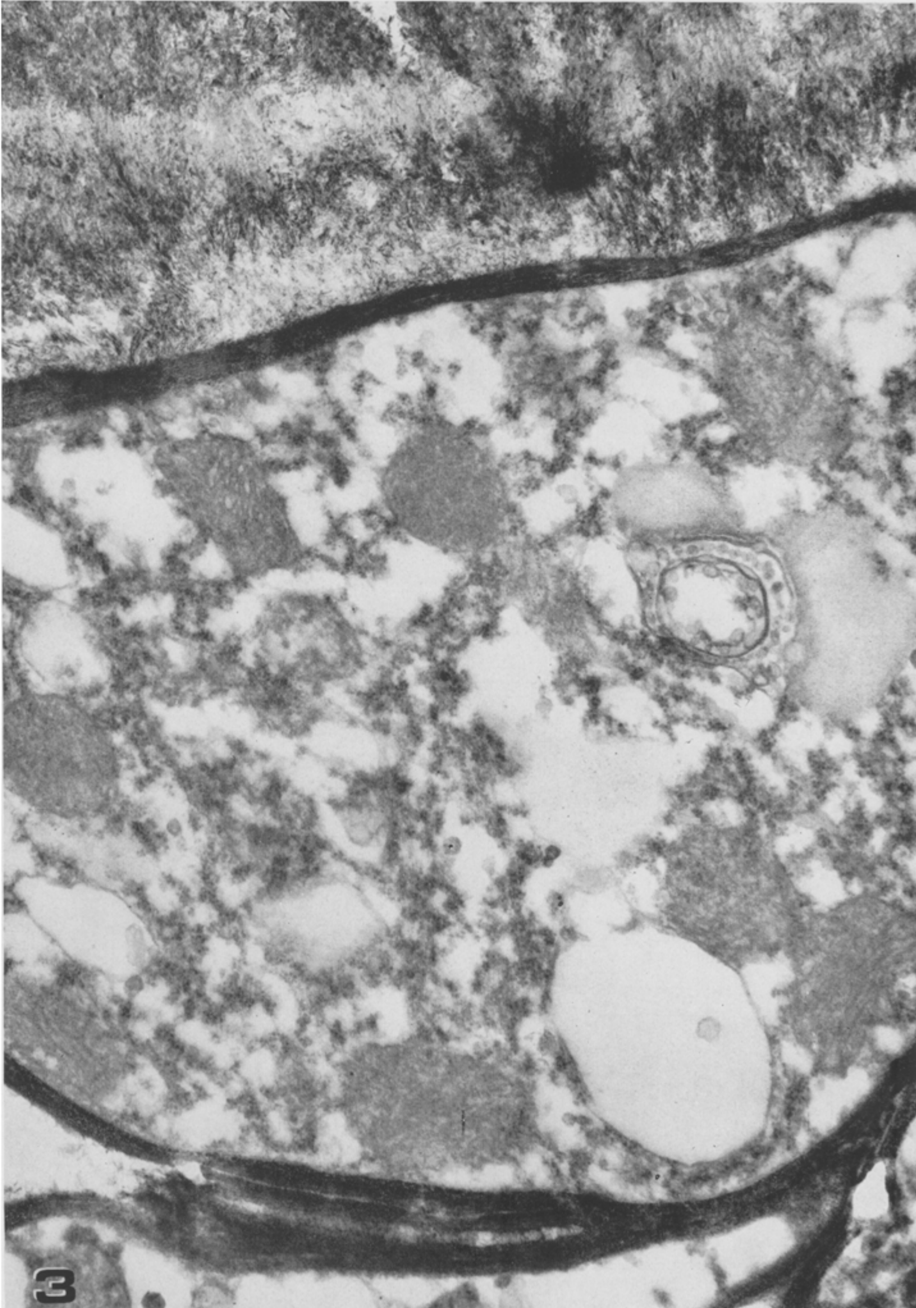
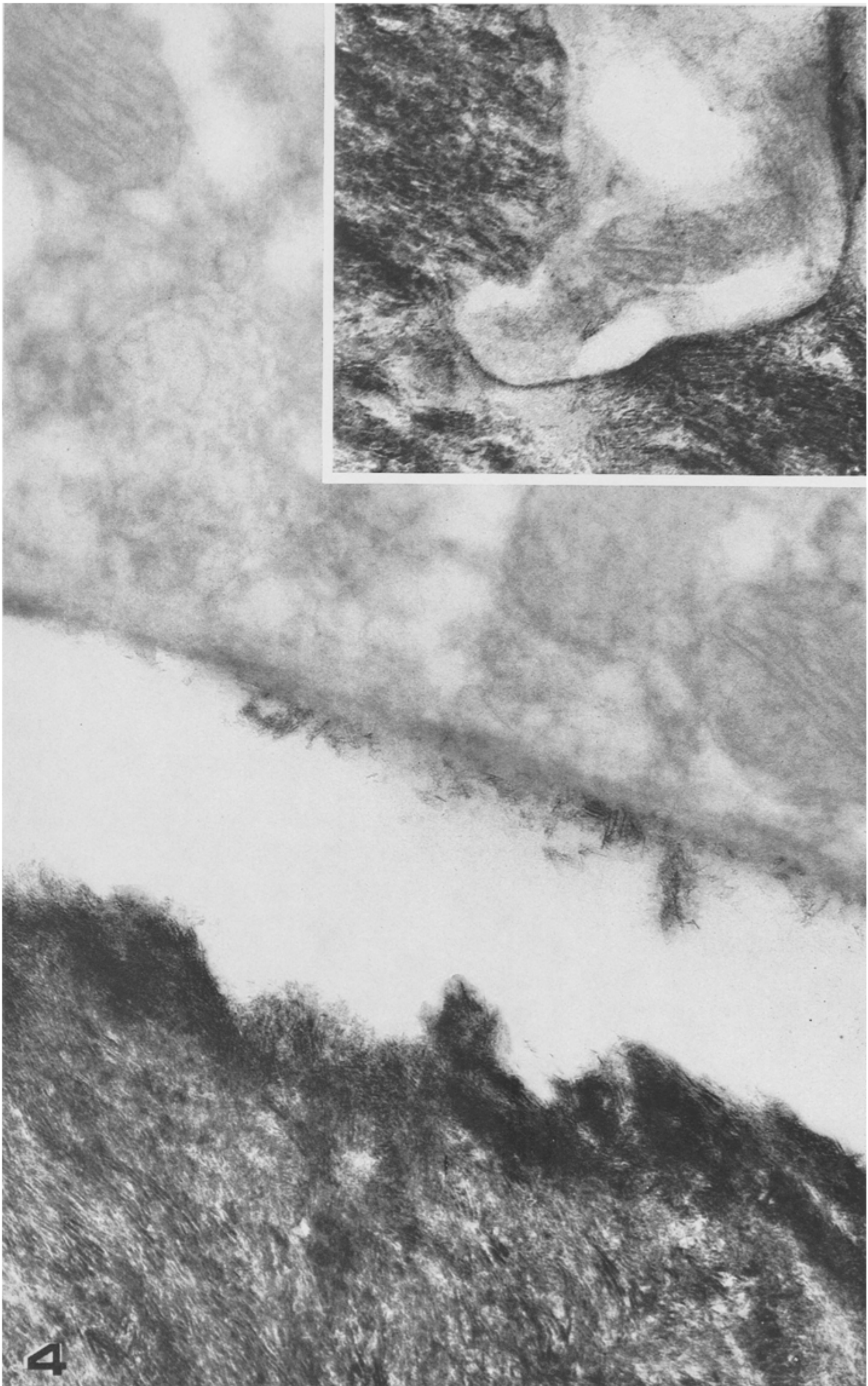



Fig. 3. Electron micrograph. Detail of a hypha having its membrane closely in contact with normal calcified bone. Embedding in Araldite. Uranyl acetate-lead citrate stained, undecalcified ultrathin section, × 33000

Fig. 4. Electron micrograph. A sectioning artefact having produced a break between the fungal membrane and the front of bone reabsorption, there is evidence that bone crystallites stick to the membrane. Embedding in Araldite. Undecalcified and unstained ultrathin section. × 66000. Inset shows the apex of a hypha growing into the bone. Uranyl acetate-lead citrate stained, undecalcified ultra-thin section, × 90000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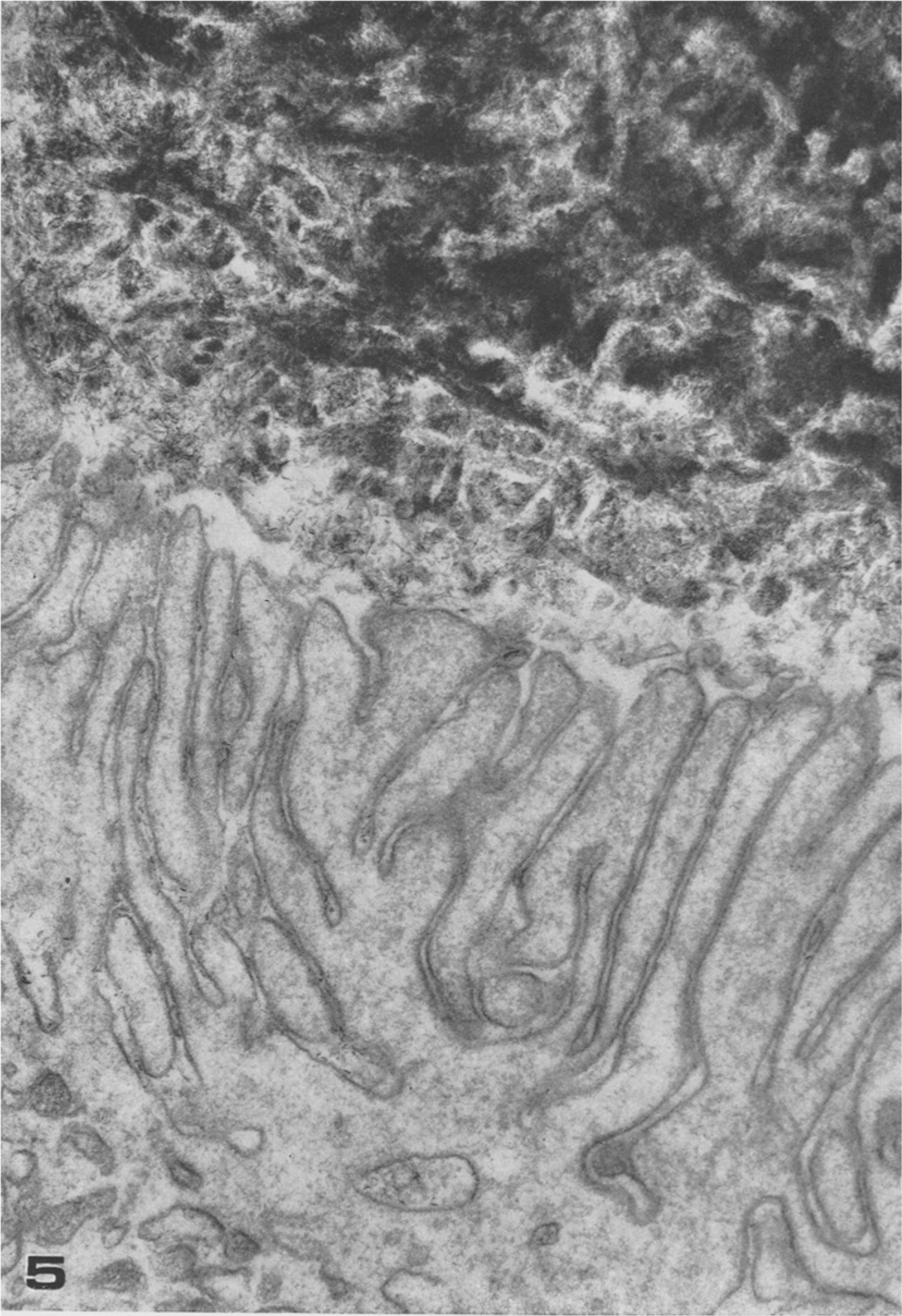



Fig. 5. Electron micrograph. Detail of the ruffled border of an osteoclast and of the adjacent bone matrix. Compare the folds and channels of the osteoclastic membrane with the smooth outline of the fungal membrane. Uranyl acetate-lead citrate stained, undecalcified ultrathin section, $\times 50000$

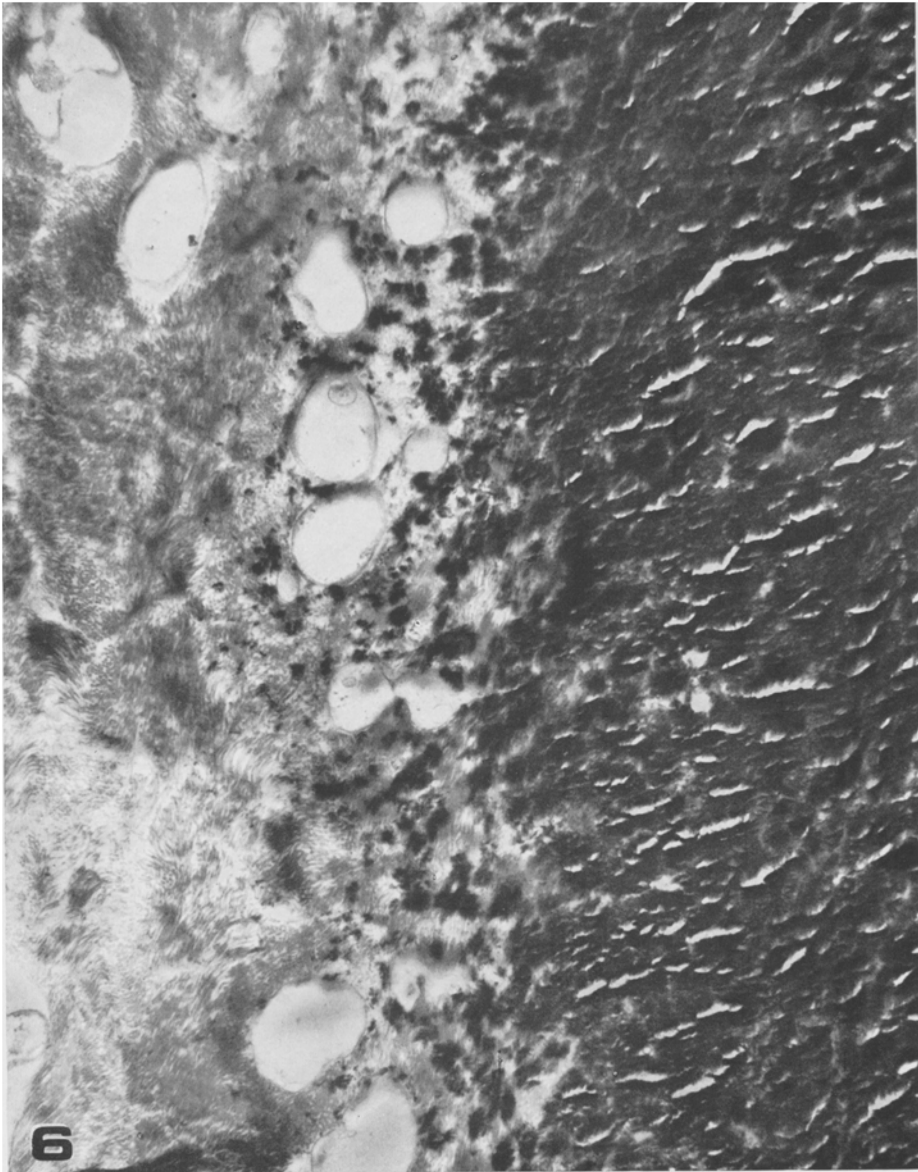



Fig. 6. Electron micrograph. The bone matrix in contact with degenerating fungal hyphae is decalcified. Uranyl acetate-lead citrate stained, undecalcified ultrathin section, $\times 6000$

The fungi present in the decalcified areas were always in an advanced stage of degeneration. Cytoplasmic organelles were almost completely lost and in their place large, often coalescing lipid droplets were present (Fig. 7).

As regards the patterns found in fossilized bone, that is, in the *torus palatinus* of the neandertalian skull "Circeo I", the electron microscope investigation of

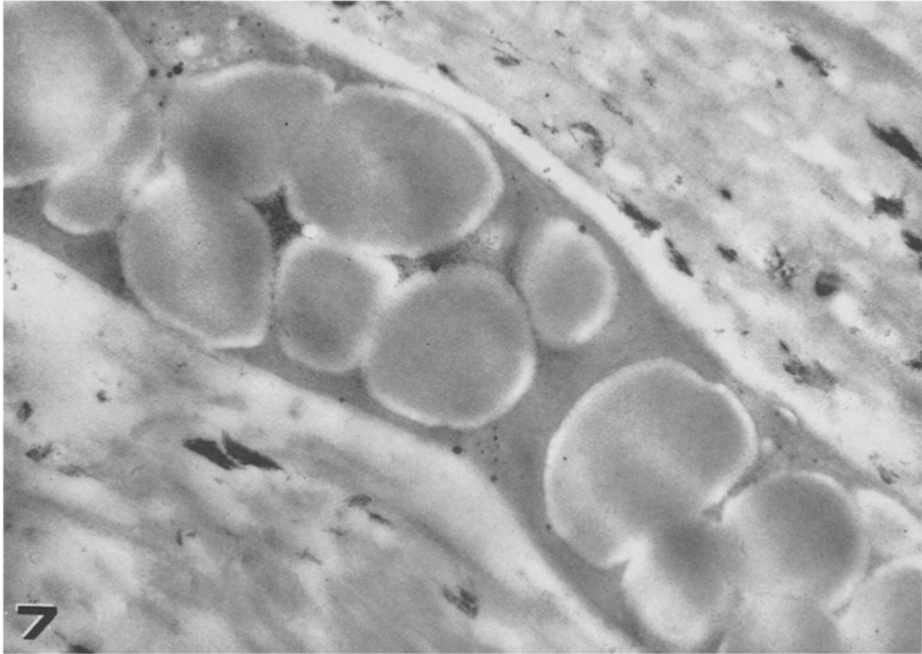
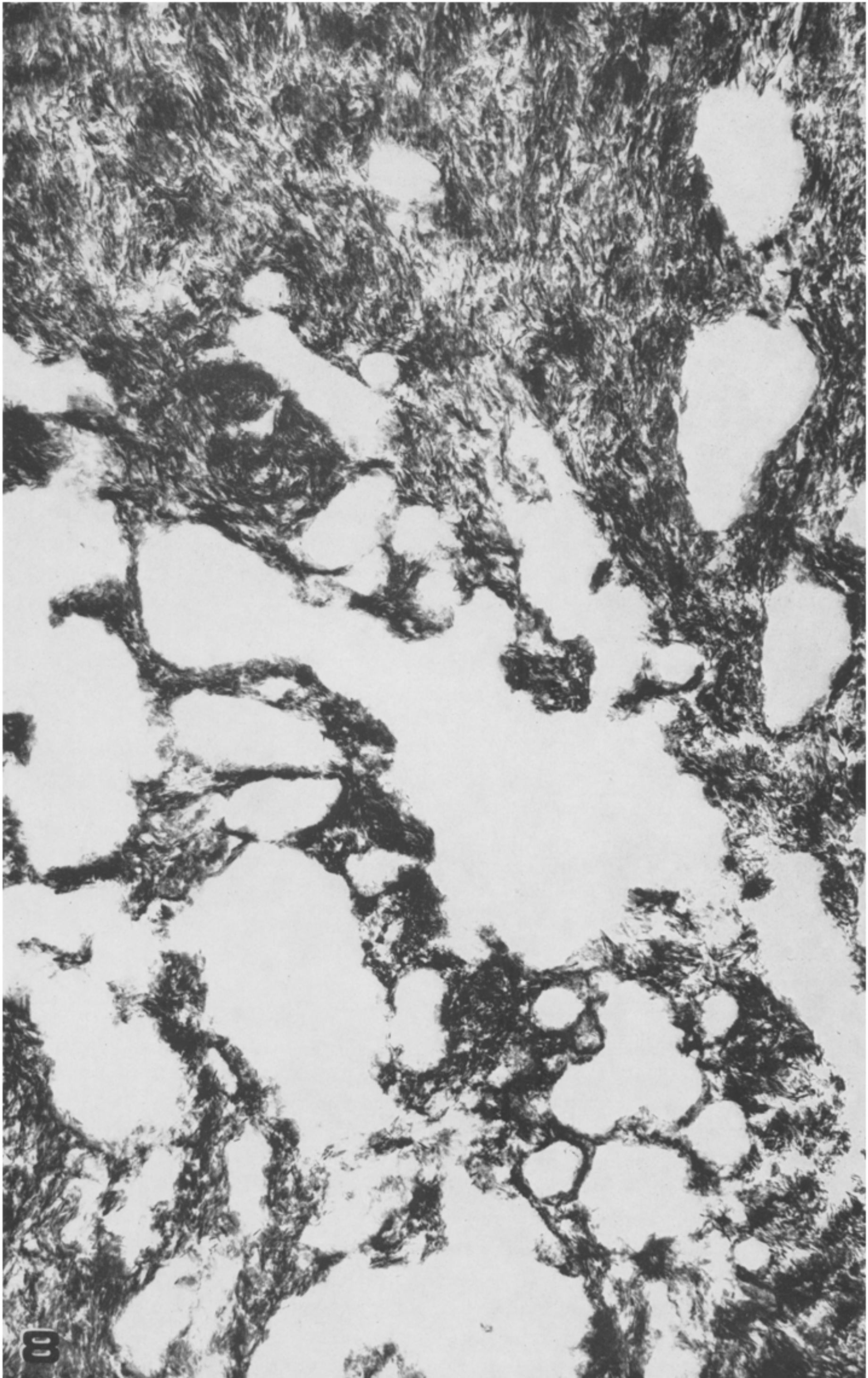


Fig. 7. Electron micrograph. In a decalcified area the cytoplasmic organelles of a hypha are completely lost and replaced by coalescing lipid droplets. Unstained and undecalcified ultrathin section, × 15000

unstained sections has revealed the ultrastructure of the bone mineral together with many roundish or irregular spaces with no structures in them (Fig. 8). Serial sections show that these spaces are tubules or channels with diameters ranging from 2 to 4 μ. They are apparently formed by the confluence of smaller tubules with diameters ranging between 0.3 and 0.8 μ. They are irregularly interconnected and form a complicated labyrinth-like structure. Their walls are so thin that they often consists of only a few irregularly oriented crystals. The channels are usually very close to each other, but some project towards the adjacent bone in an almost radial fashion. The channels appear empty even after uranyl acetate or lead citrate staining. The channel walls are sharply defined and well calcified up to the free edge, suggesting that bone resorption involved organic matrix and crystallites simultaneously. However it appears impossible to exclude the hypothesis that crystallites may have been removed before the dissolution of the organic matrix, leaving a decalcified border, because in fossil bone only the calcified matrix is preserved.

Fig. 8. Electron micrograph of a fragment from the neandertalian skull "Circeo I". Many irregular spaces corresponding to boring channels are present. They are separated by very thin walls and form a labyrinth-like structure. Unstained and undecalcified ultrathin section, × 24000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## Discussion

The present investigation of the ability of *Mucor* to bore into buried and unburied dead bones reveals a fundamentally different situation compared with the changes commonly found in the living skeleton in cases of human mucormycosis. It may be worth recalling that the organism which most frequently causes mucormycosis is a species of the genus *Rhizopus*. This organism is ubiquitous in nature and a saprophyte, growing on bread and fruit and causing spoilage. Species of the genera *Mucor* and *Absidia* have also been reported as causal agents of mucormycosis. All the three genera *Rhizopus*, *Mucor* and *Absidia* belong to the family Mucoraceae of the order Mucorales. Among the 255 reported cases of mucormycosis until 1971 (see Baker) the results of cultures were positive in 43 cases. *Rhizopus* was reported as having been cultured in 32 instances, *Mucor* in 10, and *Absidia* in 2. Mucormycosis, because of its important predisposing host factors is classified as an opportunist fungal infection. Acidotic diabetes, leucemia and debilitating diseases are predisposing conditions. The therapy for some of these diseases is another important – and probably often decisive – factor in many fatal cases. In fact antileukemic drugs, steroids, and bacterial antibiotics are implicated, as may be radiotherapy and splenectomy. Severe neutropenia is the usual background to development of the mycosis. Hyphae grow by direct extension through contiguous tissues and tend to invade blood vessel walls, producing angiitis, thrombi and ischemic necrosis; in addition, emboli establish metastatic infections in many organs. Acute inflammation is characteristically present at sites of infection. Mucormycosis affects bones in the cranial form of the disease. The fungus enters the nose and spreads rapidly, producing vascular thrombosis and infarcts of the nasal mucous membrane, the hard palate, the orbit, the tissues of the face, and the brain and meninges. In cranial cases of mucormycosis and in particular in those due to *Mucor* (Banker, 1961; Lubbe, Amborg and Pennington, 1964) damage to bone is a consequence of vascular thromboses. The fungus has never been shown to take any direct part in bone destruction.

By contrast, in buried and unburied dead bone *Mucor* reveals a striking ability to bore into calcified tissue, suggesting that boring itself may be sometimes, or always, associated with feeding. A very similar case is that of the fungi which spread through dead or living bivalve shells, utilizing the energy present in the organic conchiolin matrix of the shell. The best known instance is provided by the causal agent of Dutch shell disease, which formerly did great damage to European stocks of oysters. According to Korringa (1952) the germinating spores, which are distributed by the water currents, are able to perforate thin parts of the oyster shell and proliferate when they reach the interior surface of the shell. Although oysters die if the shells become heavily infected, this is due to reaction by molluscan tissue. The fungus itself never penetrates the soft tissues.

The hallmark of the erosive bone activity of *Mucor* is the resorption pits and boring channels it produces. However, the main problem to be solved is how the fungus induces destruction of both crystallites and organic matrix. Unlike the situation during osteoclastic activity which irrefutably shows bone material, such crystallites, within the channels and vacuoles of the brush border, here the electron microscope reveals no migration of material from the calcified bone matrix to the fungal membrane and protoplasma. This finding suggests the following inter-

pretation. Resorption is produced by the fungal membrane which is in contact with the bone; the material which enters the fungus has been previously solubilized, so that its migration is not appreciable under the electron microscope. If this is so, there can be no doubt that enzymes and other substances able to attack bone are present inside and outside the fungus membrane and attack crystallites and organic matrix simultaneously.

Little is known about the chemical constituents or structural organization of the walls of the filamentous fungi, although a great deal is known about similar structures in yeast (Phaff, 1963; Nickerson, 1963; Villanueva, 1965). Evidence has been given that there are quantitative chemical differences between cell walls in various species of fungus. Some fractionation of the walls of various species of fungi has been achieved. Most studies of the composition of the cell wall have been qualitative in nature and very few data obtained by quantitative analysis are available (Johnston, 1965). Similarly, little is known of the quantitative variations in wall composition that may exist between closely related species. The ultimate goal in elucidating the properties of the cell walls is to determine to what extent the wall is involved in the physiological and biochemical activities of the cell. Sussman (1957) has drawn attention to the cell surface in fungi, pointing out that it is not an inert envelope, but rather a region where many activities take place. Such activities are seen in the surface localization of certain enzymes. How closely surface enzymes are integrated in the cell wall is not known exactly, but this locus seems to be appropriate to enzymeswhich are present in wall components and those whose main activity is to degrade extracellular nutrients to make them available for absorption. The main characteristic of the second type of enzyme is that they are present on the surface of the cell and can be washed off by water or other solvents without damaging the cell.

Pathogenic fungi excrete pectinases and cellulases (Wood, 1960; Gascoigne and Gascoigne, 1960); these loosen cell walls of plants and facilitate penetration of the parasite into the plant. In *Fusarium* it has been demonstrated that the amount of enzyme produced by mycelium is proportional to its capacity to spread infection (Paquin and Coulombe, 1962). Similar enzymes excreted by soil mould and wood-destroying fungi help to decompose plant material.

Although the enzymes present on and within the walls of *Mucor* are still unknown, there is no difficulty in admitting the existence of enzymes which allow the digestion of collagen, together with a substance able to solubilize apatite crystals. From collagen digestion *Mucor* probably utilize aminoacids, incorporating them directly into proteins. Some phycomycetes (*Blastocladiella emersonii, Sapromyces elongatus, Leptomitus lacteus*) are, in fact, unable to utilize ammonium nitrogen and depend on amino acids.

Two problems arise in connection with the solubilization of apatite crystals: What is the substance responsible for solubilization? Can the fungus use phosphates for its metabolism? The part played by micro-organisms in the weathering of rocks and mineral may help to give an answer to the first question. The role of micro-organisms in this process has been acknowledged for a long time, but very little is known of its specific mechanism, because only a few recent studies have been devoted to it. Silverman and Munoz (1970) report on their investigation of the fungal isolate *Penicillium semplicissimum* WB-28, which solubilized significant

quantities of Si, Al, Fe and Mg in several types of igneous and metamorphic rock and caused changes in the infrared absorption spectra of some of the residual rock material. By culturing this fungus in a glucose-mineral salts medium with basalt, granite, grandiorite, rhyolite, andesite, peridotite, dunite or quartzite the authors came to the conclusion that the mechanism of fungal attack appeared to be one in which the organism produces acid which then attacks the rock. This was supported by the following observations. During growth the medium became progressively acid. The rate of solubilization of the Si, Al, Fe and Mg of basalt was closely correlated with increases in titratable acidity. Spent acid growth medium solubilized basalt when placed in contact with the rock, whereas spent medium neutralized with NaOH showed no such effect. The major acid produced was identified as citric acid. Comparable solubilization of basalt was achieved in spent acid medium and in fresh uninoculated medium containing an equivalent concentration of citric acid. Finally, extracellular enzymes did not appear to be involved, because heated and unheated spent acid mediums were equally effective in solubilizing basalt.

Quite different results are obtained when the ability of fungi to solubilize phosphate is studied, i.e. their ability to attack salts whose composition is like that of the inorganic bone fraction. Out of 149 cultures of soil fungi tested by Mehta and Bhide (1970), 42 showed ability to solubilize tricalcium phosphate in culture. The most efficient solubilizers were cultures of *Penicillium sp.*, *Aspergillus fumigatus*, *Aspergillus niger*, *Pythium sp.*, *Curvularia interseminata*, *Curvularia lunata*, *Chaetomium firneti* and *Humicola sp.* However no correlation was observed between acid production, as judged by the drop in pH of the medium, and phosphate solubilization. In fact, there was a rise in pH in many cases where phosphate solubilization was 50% or more. Similar results had been previously obtained by Sundara Rao and Sinha (1963).

As regards the boring activity of *Mucor*, there is good reason for supposing that a substance spreads freely into bone tissue, inducing decalcification of the matrix when hyphae show the effects of ageing. In this connection it is worth recalling that, unless hyphae can spread continually into a new medium, their environment changes as a result of their own growth, which slows down in an ageing colony. When this happens, the many control substances present cannot fully cope with the new situation, and basic changes take place in the cells. One visible sign of ageing in hyphae is the increase in number and size of vacuoles and the accumulation of the various products of metabolism, such as fat droplets or pigment. Our findings indicate that decalcification speeds up when these degenerative changes are visible in the hyphae; one is therefore led to conclude that metabolic abnormalities in the fungi either lead to the production of certain substances which have decalcifying properties or that they reduce the capacity to control such substances.

As to whether *Mucor* uses phosphates for its metabolism, the literature only furnishes unspecific data. Phosphate is certainly the most important inorganic anion for the yeast cell, and there is a special mechanism for its transfer into the cell. The properties of the mechanism are similar to those of typical carrier-mediated active transport systems, with saturation kinetics, competition, specificity and dependence on metabolism (Rothstein, 1964). In contrast to $K^+$, the uptake of

phosphate proceeds without measurable efflux, and therefore without steady-state relationship (Goodman and Rothstein, 1957). When phosphate is taken up, the level of inorganic phosphate in the cell does not change appreciably. The absorbed phosphate is converted into inorganic polyphosphates of high molecular weights which are aggregated in volutin granules or metachromatic granules (Wiame, 1949). The –P–O–P– bonds in polyphosphates are high-energy bonds. They are formed by a metabolic reaction involving ATP at the cost of metabolic energy. It is not clear whether the sequence of events in phosphate uptake involves the direct formation of ATP and polyphosphates at the membrane, or whether the transport system loads the interior of the cell with inorganic phosphate which is later converted into polyphosphate. Phosphate-transporting systems have not been as carefully studied as some others. Phosphorylation reactions are probably involved at some stage in the process, but as is the case in other transporting systems, the specific sequence of reactions is not known.

There is good reason to suppose that *Mucor* is not the only fungal genus able to bore into dead bone. Although our investigation was limited to this myceted, the bone fragments buried in garden earth were found to contain two other micro-organisms, one belonging to the *Cladosporium* genus and the other to the *Candida* genus. Morgenthaler and Baud (1956) reported a *Dematiacea*. Similarly, it has been recognized that several types of fungus are able to attack rock or solubilize phosphates. This agrees with the data obtained from fossil bones. In fact, the dimensions of the boring channels vary slightly between different bone specimens. In our fossil material the boring channels have a rather smaller diameter than that of the channels induced by *Mucor* in recent bone. The latter have dimensions comparable with those reported by Wedl (1864), Roux (1887) and Schaffer (1889, 1890, 1894) in fossil bones.

Electron microscope furnishes evidence that the walls of the boring channels in fossil bone are sharp and well calcified up to the free edge. In this respect, therefore, they closely resemble those bored by *Mucor* in recent bones.

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