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PALEOPATHOLOGY OF PRE-COLUMBIAN MUMMIES AT THE MUSEUM OF ANTHROPOLOGY AND ETHNOLOGY IN FLORENCE

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SUMMARY

PALEOPATHOLOGY OF PRE-COLUMBIAN MUMMIES AT THE MUSEUM OF ANTHROPOLOGY AND ETHNOLOGY IN FLORENCE

We performed a histopathological study on the mummified tissue specimens of seven pre-Columbian mummies which arrived in Italy in the second half of the 19th century and are housed in the Section of Anthropology and Ethnology of the Museum of Natural History of the University of Florence. The results confirm that the modern techniques of pathological anatomy can be successfully applied on mummified tissues, so as to perform important paleopathological diagnoses.

Among the results obtained from this study there is the only known complete paleopathological study of Chagas' disease (American Trypanosomiasis), comprising macroscopic, microscopic and ultrastructural data, as well as information on atherosclerosis, anthracosis, emphysema and pneumonia.

Introduction

Several pre-Columbian mummies belonging to different periods¹ are stored in the Museum of Anthropology and Ethnology of the

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University of Florence (Fig. 1). These bodies were brought from South America to Italy in the second half of the XIX century by Prof. Ernesto Mazzei², Prof. Enrico Hillyer Giglioli³, and by Dr. Oscar Perrone "di Lima"⁴.

Particularly interesting is the history of transportation of the mummies catalogued as 586, 587 and 588, recovered by Prof. Enrico Hillyer Giglioli.

In October 1865, together with Filippo De Filippi, Director of the Zoological Museum of Turin, went on a journey of circumnavigation of the globe aboard of the Pirocorvetta "Magenta", a sailing ship with a steam engine. The purpose of the trip was to contact the



Fig. 1- Some of the mummies in their glass case at the Museum.

authorities of the visited countries in order to establish diplomatic relations and collect samples of flora and fauna.

During the journey, De Filippi died of dysentery in Hong Kong on February 9 1867, and his place was taken by Giglioli until his return to Italy in 1868. In the catalog of the museum the mummies of Giglioli are classified as coming from Cobija (Bolivia).

Objectives

The aim of our work is to conduct a paleopathological and histological study of seven mummies (catalogued as Fi1 (3071), Fi2, Fi3, Fi4, Fi6 (2993), Fi9 (3076), Fi12)⁵.

Results

Fi1, Fi2, Fi3, Fi4

These mummies were donated by Ernesto Mazzei, and arrived in Italy from Cuzco between 1875 and 1914.

Mazzei, a patriot and volunteer 'garibaldino' in 1866 and 1877, studied both at the University of Pisa and at the University of Bologna, where he graduated in Medicine. He practised ophthalmology in Egypt and then left for South America in 1874. In 1875 he obtained the lectureship in Bologna and, on his return to Peru, he was appointed Professor at the University of Santiago and, in 1889, at the University of La Paz in Colombia. He died in Pisco (Peru) in 1905. *Fil*

This mummy, in fetal position, is well preserved, and wrapped in a red cloth, with the exception of the feet, shoulders and head (Fig. 2). Histochemical staining⁶ shows perfect preservation of the skin layers of the entire body, from the dermis to the subcutaneous fat, along with skin *adnexa*, i.e. the hair follicles and the sebaceous glands and ligaments. Some structures inside the tongue were detected, belonging to a

Some structures inside the tongue were detected, belonging to a phase of the life cycle of two species of necrophages insects, some exuvia of pupa of *Dermestidae* and some pupa of *Diptera*.

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Fig. 2 – Mummy Fi1

Fig. 3 – Mummy Fi6

Some species of *Dermestidae* are attracted by any preserved organic substance (leather, animal fibers, tissues, etc.), so that it is impossible to exclude that the insects had colonized the body when it was still in the Florentine Museum, and therefore not part of the original fauna in the body.

The *Diptera* are the first species of insects that colonize the body, with massive invasions immediately after the death. Instead, they are no longer attracted to the body when the remains have passed a certain stage of decomposition or are mummified. This is an interesting element, as it assures that these insects belong to South America and reached the body shortly after his death⁷.

Fi2, Fi3, Fi4

It was possible only the histological study of small samples of skin. The mummies shows a good preservation of the skin with the *adne-xa* and a massive presence of fungi and ectoparasites.

Fi6

This body was sent to Italy by Dr. Oscar Perrone and was entrusted to Dr. Benjamin Calcagno, ship doctor of the Royal Cruiser 'C. Colombo', and unloaded in Venice on 19 January 1883.

It is the mummy of an adult male, in fetal position and in poor state of preservation, with the exception of the lower limbs which are in almost perfect condition; the left half of the trunk is missing (Fig. 3).

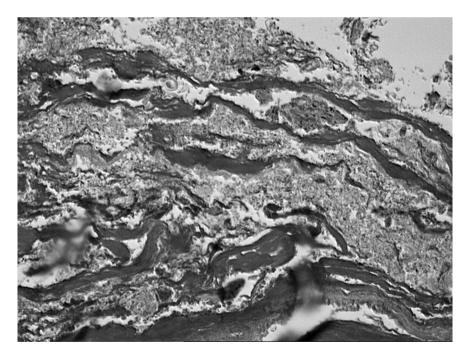


Fig. 4 – Mummy Fi6: alveoli with exudate (E.E. 200X)

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The skull is almost completely skeletonized and devoid of hair, with lack of the superior and inferior incisors and canines for post-mortal events. The chest has already been emptied of its content. It is possible to locate the trachea and esophagus and an arterial trunk, perhaps the aortic arch. The opening in the left hemithorax is definitely artificial. The diaphragm is present. The anus and rectum are intact. The estimated age is about 30 years.

At E.E. staining, the preservation of lung tissue was optimal, with the presence of collapsed alveoli with adjacent areas where it was still possible to recognize the focally expanded alveolar structure, the vases and a massive alveolar exudate (Fig. 4).

There is a large infiltration of ectoparasites in the skin tissue, and there are darker areas, probably consisting of carbonaceous material, as well as residues of the smoke process used in the Inca period to preserve the corpses.

During the autopsy we were able to identify the aortic arch, highlighted thanks to elastic fiber staining (Weigert). Well evident is the presen-

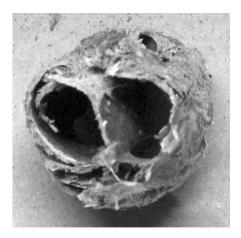


Fig. 5 – Mummy Fi6: Lumen of the trachea (left) and the esophagus (right)

ce of coated lung tissue on the outside wall of the vessel, due to the collapse of the structures and to compaction during the drying process. The pulmonary parenchyma shows traces of anthracosis, i.e. carbon dust inhaled during the life of the subject, for the extensive use of fire for cooking and heating.

The trachea and esophagus, still in contact, were so exceptionally preserved, that we kept all the structures, even in the subsequent phases of sampling (Fig. 5). Microscopically, the zone of contact between the esophagus and the trachea is evident, with the structures that are still distinguishable. The sections of the great vessels of the neck showed excellent tissue preservation, demonstrated by the staining of elastic fibers. Several sections show an infiltration area of vascular layers of amorphous material, probably composed of lipids, i.e. atherosclerotic plaques at different stages of evolution (Fig. 6); in one case, the lesion is in very advanced stage, in which the fatty tissue has invaded all the vascular layers (Fig. 7).

Other sections carried out on the same tissue demonstrate the excellent preservation of the tracheal cartilage and perichondrium hyaline, with areas of anthracosis in the adjacent connective tissue. There are *lacunae* in the array which contain numerous chondrocytes that are still in situ.

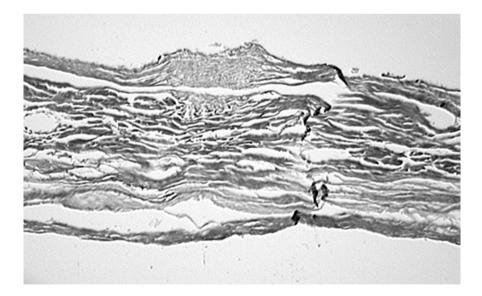


Fig. 6 – Mummy Fi6: atheroma (E.E. 100X)

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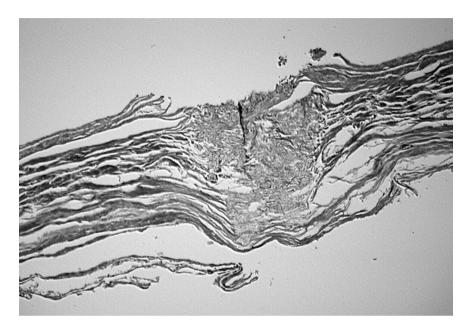


Fig. 7 – Mummy Fi6: atheroma invading all vascular layers. (E.E. 100X)

Fi12

This natural mummy of unknown origin and in bad state of preservation and difficult to date.

It is an adult female aged about 20-25 years, lying in fetal position, with its head flexed forward, upper limbs inflected, hands close to her face and lower extremities inflected (Fig. 8). This particular posture is certainly due to the deposition in a basket, typical of the burials of the Inca period. It has been restored in some of the missing parts, with coloured parchment sheets, especially in the pelvic region.

The only funerary goods present is a bracelet of stony corals, found in the cutaneous *plicae*.

During autopsy both lungs, as well as the upper right bronchus, were easily identified and sampled. The inferior *mediastinum* and the diaphragm had been removed. The intestinal loops and the tran-

sverse colon are present and well represented.

The right lung microscopically shows numerous *alveoli*, in large fibrous areas, with well recognizable vessels and bronchi and widespread areas of pulmonary anthracosis, visible even in Masson's trichrome stain. An important element is the massive presence of alveolar exudate.



Fig. 8 – Mummy Fi12



Fig. 9 – Mummy Fi12: bracelet of stony coral

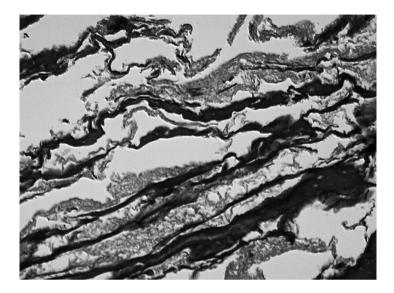


Fig. 10 – Mummy Fi 12: alveolar exudate (E.E. 200X)

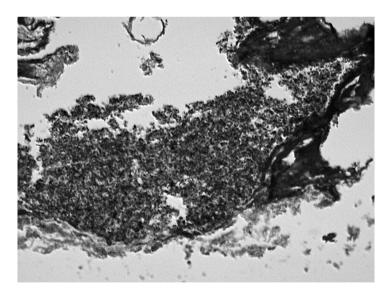


Fig. 11 – Mummy Fi12: anthracosis (E.E. 200X)

In a sample of the same tissue it is possible to detect an area with roundish structures and hyaline PAS positive material, suggestive of thyroid follicles. Tracheal cartilage with well preserved chondrocytes is also present.

In the left lung there are residues of pulmonary fibrous texture; the alveolar pattern is still clearly visible, and we can observe the tissue with *lacunae* containing a dense material. A well preserved nervous tissue and large amounts of ectoparasites and fungal *hyphae* invading the tissue can also be recognized.

The material identified as "intestinal loops" is quite fragmented, despite the wall of the organ and the fecal material of the intestine visible in some areas..

The well-preserved wall of the colon is composed of fibrous material and areas with greater presence of muscle tissue. Four seeds were found in the lumen of the colon, probably of tomato plants, together with vegetal fibers; current paleobotanical studies are designed to determine the belonging species.

Fi9

This 14-15th century mummy from Cuzco, arrived in Italy between 1875 and 1914 and was donated by Prof. Ernesto Mazzei.

It lies in a basket, made of vegetal fibers (Fig. 12), which contains two red drapes covering the body entirely. The drapes are joined by two belts, and there are two colorful



Fig. 12 - Mummy Fi9

patches on the head and the pelvis. A long braid hangs down from the head, which is almost completely skeletonized.

A mummy in fetal position, with ropes tied around the wrists, ankles and hips, appeared after removal of the drapes.

The pelvis displays partial tissue loss, and highlighted the bone junctions, the ischiatic tuberosities, and the intestinal loops full of presumably fecal material. The upper limbs are well preserved, while the lower ones show bilateral dislocation of the small feet bones. The estimated age is about 18-23 years⁸.

A complete autopsy was performed⁹. We practiced an opening in the right posterior hemithorax by cutting the skin tissues and the ribs, which revealed the right lung and the hepatic diaphragm. Once these organs had been removed, the liver, the esophagus and the stomach were visible. The stomach, evidently ectasic, was opened so that it displayed the *pylorus* and *duodenum*. The esophagus seemed to be very enlarged.

The left lung and the heart were then removed in block, revealing a severe cardiomegaly. The right, transverse, left and sigmoid colon

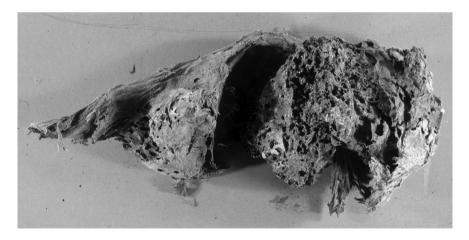


Fig. 13 - Mummy Fi9: megacolon with abundant feces

and spleen were also withdrawn in a single block. A large amount of feces was present in the colon, which looked exceptionally distended (Fig. 13).

These multiple elements (cardiomegaly, megaesophagus and megacolon) immediately suggested a megavisceral syndrome, compatible with Chagas' disease¹⁰.

Chagas' disease (or American trypanosomiasis), is a disease caused by the protozoan *Trypanosoma cruzi*. This organism causes a chronic or acute *parasitemia* and colonizes the parenchymal cells of some organs, in particular the heart, brain, esophagus and colon. As a zoonosis, the man is not necessarily part of the parasite's life

cycle. *T. cruzi* is found only in the American Western hemisphere, where it primarily infects animals and insects. The insect vector, i. e. various species of *Tryatominae* bugs which can transmit the protozoan, can be found in an area stretching from central Argentina to the south of the United States. Chagas' disease is endemic in Mexico and in all the countries of central and southern America, while it is absent in the Caribbean.

The cycle of the parasite begins when the insect vector penetrate in the blood where it turns from trypomastigote in epimastigote. The parasite then moves to the terminal part of

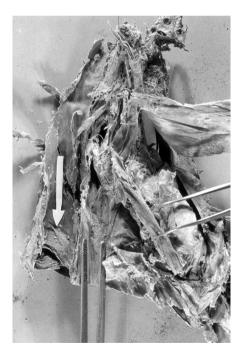


Fig. 14 – Mummy Fi9: the heart with pericardial sac and the esophagus (arrow)

the digestive system of the insect vector, turning into a metacyclic trypomastigote, which is eliminated through the feces during a meal of blood. Transmission can also occur through contaminated blood, from the mother to the fetus, or by the mucous membranes contaminated by the infectious feces of the parasite.

The parasite enters the host, where it transforms into a amastigote. Here the parasite fills the cell, becomes a trypomastigote and breaks down the cell wall, invading the neighboring cells and infecting distant sites by hematogenous spread. It then starts asynchronous cycles within the host, thus maintaining a continuous parasitemia.

The parasite seems to have lived a particular tropism for certain cell types, such as macrophages, nerve and muscle cells.

According to the Pan American Health Organization (PAHO), Chagas' disease is responsible for the deaths of about 20,000 people a year and those infected are about 8 million. However, thanks to the major controls on vector insects and to blood banks, in recent years countries like Uruguay, Chile and Brazil have been certified as free of transmission.

At the level of symptoms, approximately 70-90% of chronically infected people do not develop cardiac symptoms or gastrointestinal problems. Strangely, however, there is a geographically-based differentiation of cardiac and gastrointestinal symptoms: in Colombia, Venezuela, Mexico and Central America the megavisceral symptoms are almost unknown¹¹.

In 1992 mummy Fi9 was studied at the Division of Paleopathology of the University of Pisa by immunohistochemistry and electron microscopy techniques applied on cardiac tissue samples and on the esophagus¹².

The esophageal and cardiac tissue stained with Giemsa showed oval formations of about 1-2 μ m, with small nuclei. These elements supported the diagnosis of Chagas' disease on the basis of the megavisceral syndrome and myocardial gastrointestinal tract fibrosis. The

immunohistochemical study with anti-flagellar *T. cruzi* antibody showed a strong reactivity to immunoperoxidase in these small oval formations.

Electron microscopy of the esophageal wall showed massive clusters of rare irregularly oval formations, adherent to collagen fibers, of a maximum diameter of about 1 μ m characterized by: double membrane and peripheral microtubules (T) of the transverse diameter around 20 nm; a storage of a more dense material of diameter approximately 200 nm, identifiable as nucleus (N); an elongated body, approximately 300X80 nm, identified as kinetoplasto (K); a cilindriforme structure, 300x30-40 nm, with an enlarged dense ovaloid bulk at one end, identifiable as axonema (A) with its basal

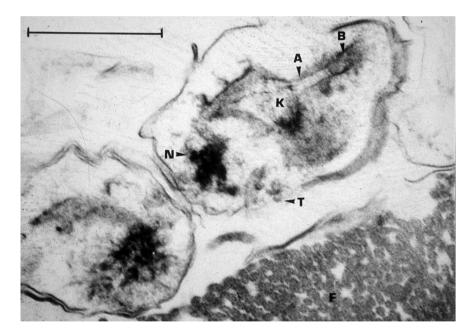


Fig. 15 – Mummy Fi9: two amastigotes of *T. cruzi* (electron microscopy). T=microtubules; N=nucleus; K= kinetoplast; A=axoneme; B=basal body (bar: 0,5 μm.).

body (B). It is the characteristic appearance of amastigotes of the Trypanosomatidae family .

It was therefore concluded that it was a case of Chagas' disease in its chronic phase.

This is the first paleopathological case of direct demonstration of this disease and its causative agent in the American continent, and in particular in the pre-Columbian period¹³.

In 1999 the mummy was employed as ancient positive control sample in a molecular study of aDNA to verify the presence of T. cruzi in mummies from northern Chile¹⁴.

Microscopic anatomy of new sections of the sampled heart were found to be markedly altered by T. cruzi. All histological sections showed partial substitution of the myocardium in fibrous and adipose tissue (*adipositas cordis*), with only residual muscle tissue. Both venous and arterial cardiac vessels, very dilated and tortuous, were visible by elas-

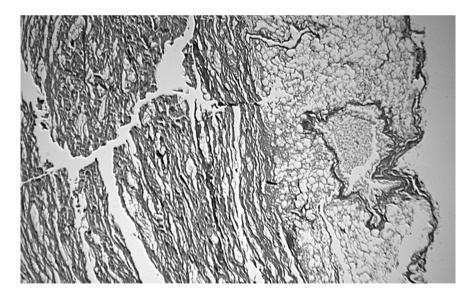


Fig. 16 – Mummy Fi9: adipositas cordis (50X)

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tic fibres staining (Weigert). At a higher enlargement numerous amastigotes cysts of T. cruzi were visible within the tissue. Variegated material, probably a degenerated parasitic thrombus, is seen in a vessel¹⁵. The sample of the esophagus is in good condition: the wall is preserved in different layers, with an important colonization of ecto-parasites. There are also large areas of fatty tissue within the layers. In some sections there are areas with possible colonization of amastigotes of *T. cruzi*. In the stomach there is prevalence of fibro-muscular and of adipose tissue, with traces of the epithelium positive at immunohistochemistry for cytokeratins.

The colic wall, with fibrous structure and areas full of fecal material and colonies of amastigotes of *T. Cruzi*, can be observed.

The alveoli are visible in both lungs, with the presence of fibrosis and anthracosis, and an excellent preservation of the cartilaginous tissue chondrocytes, evidenced by PAS staining.

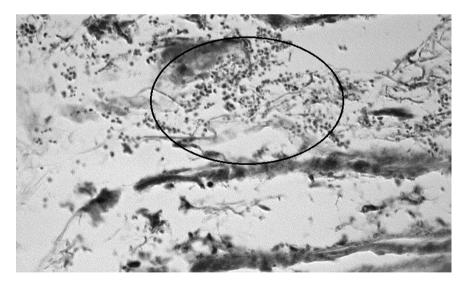


Fig.17 – Mummy Fi9: section of myocardium. The dark and small spots in the circle are the *T. cruzi* amastigotes (Giemsa, 1000X)



Fig. 18 – Mummy Fi9: uterine portio

The liver is almost entirely composed of fibrous tissue, with the presence of abundant trivalent ferric iron detected by Perls' staining, probably the product of hemoglobin degradation.

The sample of the scalp seems to be very damaged and fragmented, but many hair follicles and skin adnexa are still visible.

An organ found in the pelvis was identified as *portio*: microscopical analysis showed rounded structures of dense PAS positi-

ve material, by accumulation of mucus, evidently mucinous ectasic glands of the *portio uteri*, or Naboth's cysts.

The braid is formed by 60 cm long hair. It was entrusted to the Toxicology Laboratory of the Forensic Medicine Division of the University of Pisa, together with the hair of the mummies Fi1, 2 and 12, for toxicological studies on psychoactive substances.

Several insects have been identified in this mummy too, especially during cleaning of the basket: the preliminary report underlines abundant remains of *Dermestidae* and *Diptera*.

Discussion and conclusions

- 1. The case of atherosclerosis in mummy FI6 is very important, considering the young age of death (about 30 years) and the probably poor nutrition.
- 2. In mummy FI6 it was possible to observe the presence of pulmonary alveolar exudate, with a diagnosis of bronchopneumonia and areas with air space expansion and

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Fig. 19 - Mummy Fi9: some Naboth's cysts, filled with mucus (E.E. 100X)



Fig. 20 – Mummy Fi9: the braid of mummy Fi9

destruction of septa, by possible emphysema. Another case of bronchopneumonia is present in mummy FI12.

- 3. Finally, we found clear presence of severe pulmonary and mediastinal anthracosis, easily explicable by life in proximity to fires from early childhood, especially in people who use bonfires to cook and warm the housing environment.
- 4. Survival of the *T. cruzi* woman despite the severe degrees of heart disease, megacolon and megaesophagus, suggests that she was probably aided and perhaps "cured" with drugs. It is hoped that the toxicological analysis, currently underway, will shed light on the use of pharmacologically active substances in Peruvian and Inca culture.

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