HISTOLOGICAL ANALYSIS AND STAINING TECHNIQUES MODIFIED AND VERIFIED ON ANCIENT MUMMIFIED TISSUES TO STUDY MICROORGANISM INFESTATIONS

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INTRODUCTION

The Egyptian Museum of Turin has an important historical collection of mummified human remains from Egypt and collected during the Italian Archaeological Mission directed by Schiaparelli from 1903 to 1920, and by Farina up to 1935. Most mummies come from the necropolis of Assiut and Gebelein, located along the middle course of the Nile (1).

For protection of these historical remains it is important to estimate their preservation and control any likely physical, chemical and biological changes using histological tissue analysis. Histological examinations are indeed useful to assess the general tissue characteristics (type of mummifications and preservation methods). Also, they offer very fine and specific evaluations on any possible tissue degradation and on the presence of any inorganic or organic pollutants. In particular, a careful investigation of infesting agents such as fungi and bacteria is particularly important before deciding on the actions to be taken for specimen preservation and for determining the best preservation environment (2-7).

Mummified, properly rehydrated tissues maintain most of the histological and histoarchitectural properties of fresh tissues, although to different degrees that may vary from case to case. This variability depends on

different types of either natural or artificial mummification, on the quality of final mummification and, finally, in the case of artificial mummification, on the substances employed for mummification.

However, even perfectly mummified tissues have some features that make them different from fresh tissues. In particular, with regard to the skin, bundles of collagen or elastic fibers can be observed in the derma which tend to aggregate into hematoxylinophilic strands. Also, reticular fibers have collapsed into partially fragmented trabeculae of different thickness and, finally, the entire loose connective tissues have condensed into irregular bundles.

Furthermore, the different physical-chemical features of mummified tissue versus fresh tissue lead to a different affinity for the stains normally employed in histochemistry, which visually translates into chromatic aberrations, metachromasia, color deviations. These variations can be both modest and marked and can significantly hinder specimen interpretation. For this reason, a careful selection of histochemical staining methods and materials, and basic ones in particular, is necessary. Also, histochemical stains must be properly applied and adjusted with particular attention to the timing and selection of reagent dilution in order to obtain the best result.

MATERIALS AND METHODS

Our study concerned the mummified human remains kept at the Egyptian Museum in Turin.

Our study focused on the specimens contained in the store-room for mummies. Twenty-one resulted to be suitable for histological testing since they had only partial or sometimes no bandages at all, thus exposing more or less larger areas of mummified tissues to the destructive action of contaminants and/or pollutants. This work has been carried out on a first sample of 16 mummies which were rather heterogeneous as to their preservation. Small pieces of mummified tissue were taken, near extant fractures or lacerations, without damaging the remains.

The tissues were hydrated again, according to Sandison's method (8) and

following modifications (9) and then stained with hematoxylin-eosin, and trichromic Masson's, Mallory's and Van Gieson's (10).
The Giemsa staining method, modified for tissues, was successfully employed. This stain is commonly employed in histochemistry in order to identify the presence of rod-like bacteria. However, before its use on mummified tissues, this method was further modified by some of us: before staining, sections were put in a mildly alkaline solution to lower tissue acid level. This technique offers the best results and is both simple and fast to carry out.
With regard to specific stains for fungi, Grocott silver impregnation was employed as well as PAS reaction. Both techniques are useful to identify fungal hyphae and spores.
The preparations were examined with ordinary bright and polarized light microscope to point out foreign inorganic structures or mineral dust.

RESULTS

Five cases of fungal infestation were observed. In four of them the skin has lost its upper surface layers. One case of bacterial contamination was observed where the skin has lost its epidermis (this subject in particular also has fungal infestation and carbon dust layers).
Contamination is likely to be due to particular conditions (temperature, humidity) that support tissue rehydration and favor the settlement of fungal and bacterial colonies.
After colony identification, it was important to define the viability of infesting agents in order to determine the potential and actual infestation risk. Non-viable bacteria are quickly degraded and therefore cannot be identified with standard stains (except for mycobacteria).
Fungi, conversely, may remain perfectly recognizable for a very long time. As some of us were able to demonstrate (11) cryptococcus, for example, keeps its staining affinity over time.
However, fungi causing mummy decay over time tend to lose some of their original chemical characteristics. Hence two techniques, both Grocott staining and PAS reaction on seriated sections, are proposed here.

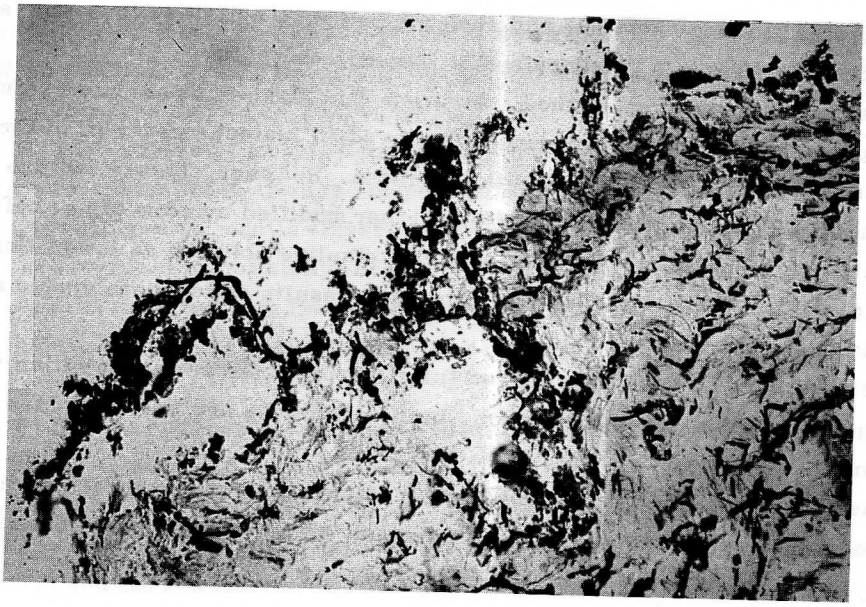

Fig. 1 - Fungal contamination (25x, Grocott staining).

Fig. 2 - Fungal contamination (25x, PAS reaction).

Actually, hyphae and spores may still be identified with silver impregnation techniques, showing the capsule material very well. However, if colonies are biologically dead, their reaction to PAS is either weak or non existent.
With this special staining method on seriated sections, we were able to suspect, at least in one case, the presence of biologically active forms (figs. 1,2). In order to verify this condition, cultures from small fragments of the five specimens under investigation were made in both solid and liquid media. The result was undoubtedly positive in the same case already identified with the staining techniques. The presence of vital microorganism was demonstrated.

DISCUSSION

Our preliminary results show that histological analysis, with particular modifications for mummified tissue, and special staining methods on seried sections can identify the vitality of fungi and their involvement in the production of enzymes responsible of damages and alterations to mummified human remains.
These analyses, with traditional methods, are necessary to assess physical alteration due to the microclimate as well as biologic agents and chemical pollution, also to get more specific information on these issues. Appropriate conservative actions can thus be planned, for each single case.

The purpose of this work is to give a brief account of the possibility to estimate the preservation of human mummified tissues using histological analysis. This method can be useful to identify injuries and to plan qualified conservative actions on ancient human remains.
Some preliminary results are presented here regarding the study on 16 ancient mummies from the Egyptian Museum of Turin. Samples of mummified tissues were taken without damaging the remains; they were hydrated again and dyed with histological techniques which were specifically

modified and verified in same cases. Our research identifies some agents (biological and chemical) that appear to be responsible, among others, of mummified tissue destruction. The microscopic examination reveals features that might refer to fungal and bacterial infestation. Using special staining methods on seried sections we were able to suspect, at least in one case, the presence of biologically active forms. Microbiological assays confirm the vitality of fungi. Histological tissue analysis can then be useful to guide any conservative intervention for preservation and protection of the integrity of biological remains from museum collections.

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