

Histological Investigations to Estimate the Preservation of Mummified Remains.

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Abstract

In order to protect historical remains, it is important to assess their preservation conditions and control any likely physical, chemical, and biological changes using histological tissue analysis. Histological examinations 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.

Communication

The preservation conditions of the mummies are being checked with histological examinations of both surface and deep tissues. Histological examinations are indeed useful to assess the general tissue characteristics (type of mummification 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 (1). In fact, the histological examination allows to control the preservation of mummies much more accurately than the gross examination, which show only great damages, sometimes when it is too late for the restoration. For this reason even superficial sampling could be diagnostic. 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. When speaking about preservation, an operative protocol has to be defined with the preservation of findings as its main priority.

A) Sampling.

Mummies with partial bandages or none at all are suitable for histological testing. Small pieces of mummified tissue (5 mm), removed from pre-existent fractures or lacerations, without damaging the remains, are used in the analysis.

B) Rehydration.

The pieces are hydrated again according to Sandison's method (2) and following modifications (3). In order to

avoid specimen rehydration - a procedure for which a certain amount of tissue is required - a resin embedding technique has been developed (TECHNOVIT 8100 - Kulzer) (4). The visual impression of resin-embedded sections may result less satisfactory, but other considerations are remarkable. First there is a good preservation of cytological and in particular nuclear details. Secondly resin embedding saves the washing of the sample's surfaces and that way it preserves the particles on the surface, which stick during the mummification process or later due to air pollution. It is necessary to underline later that the sample for resin-embedding is really smaller to these necessary for the rehydration, in which a good result depends on the great dimension of the sample.

C) Staining techniques to identify tissues.

Accurately selected and dosed histochemical staining follows. Some changes to the timing and dilution of reagents have been introduced in order to get the best results. Hematoxylin-eosin, Van Gieson, trichromic Masson's and Mallory's, PAS are performed (5).

D) Staining techniques to identify bacteria and fungi.

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. 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.

E) Staining techniques to define the viability of infesting agents.

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. However, fungi over time tend to lose some of their original chemical characteristics. Hence two techniques, both Grocott staining and PAS reaction on serial sections, are proposed here. 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.

Our research identifies some agents (biological and chemical) that appear to be responsible, among others, of mummified tissue destruction. Using special staining methods on serial sections we were able to suspect the

presence of biologically active forms of fungi.

Microbiological assays confirm (on a series of selected and random cases) the vitality of fungi (*Candida parapsilosis*) and the presence of bacteria belonging to the *Serratia* sp., *Streptomyces* sp..

F) Analysis for the identification of mineral and plant substances.

Dust deposits on the surface have been analysed under polarized light microscope. Under this microscope, carbon dust, normally caused by air pollution, has no birefringence properties. Conversely, mixed dust, made up of different minerals and vegetable particles, is generally birefringent under the polarized light microscope. Therefore, useful information can be gathered from these studies on the chemical pollution and on the type of substances employed during embalming.

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