Histological Investigations of Mummified Human Tissue from Qilakitsoq

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Mummified tissue must be rehydrated prior to processing for histological sections. A comparison is made between different rehydrating solutions. The solution described by Ruffer in 1909 gave the best results with regard to preserved structures.

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Mummified, desiccated tissue is extremely hard and cannot be cut by the microtome unless softened by rehydration. When rehydrated the tissue specimens can be treated like common tissue specimens in the laboratory and cut in a microtome after paraffin wax embedding and properly stained.

The aim of rehydration is to make the tissue come as close as possible to its previous size and structure, with the least possible damage in order to be able to examine the finer structures of the tissues.

Mummified tissue from many previous mummy finds all over the world have been submitted to various procedures for rehydration and technical preparation of specimens. Excellent reviews and guidelines have been given by Sandison (1970), Reyman & Dowd (1980) and Allison & Gerszten (1982). The various methods employed by different authors have not always been described in detail, and comparative studies of technical procedures have not been performed to any great extent. The general impression given by the literature is that many authors have used the solution originally described by Ruffer in 1909 (Ruffer 1921) as well as Sandison's modification (Sandison 1955, 1970) with good results.

In order to find the optimal technical method for rehydration of specimens from the Qilakitsoq mummies a comparison was made between different methods mentioned in the literature.

Material

Specimens from the abdominal wall measuring approximately 18 × 5 mm with a thickness of about 3 mm were used. The specimens were dark brown, very hard and the abdominal layers showed some splitting when cut.

Method

Before rehydration gross evaluation was made by measuring the specimens using a micrometer screw and photographing in two dimensions through a dissecting microscope x 40. This procedure was repeated after rehydration/before fixation and after fixation.

The specimens were suspended in the rehydrating solution (see below) one to 25 parts for 24 hours at room temperature, fixed in 10 per cent buffered formalin for 24 hours, embedded in paraplast and stained (see below) before microscopy. Some paraplast blocks were later re-embedded in plastic (JB-4 from Polysciences Inc.), cut in a rotation microtome using a phosphorus tungsten knife and stained before microscopy.

The rehydrating solutions employed were the following:

1: Ruffer's solution (Zimmerman *et al.* 1971, Zimmerman 1975). Five parts water, 3 parts absolute ethyl alcohol, 2 parts 5 per cent sodium carbonate, saline added to make 0.85 per cent.

2: Sandison's modification of Ruffer's solution (Sandison 1955, Sandison 1970). Three parts 96 per cent ethyl alcohol, 5 parts 1 per cent aqueous formalin, 2 parts 5 per cent sodium carbonate, saline added to make 0.85 per cent.

3: 10 per cent aqueous formalin in 0.85 per cent saline.

4: 0.2 per cent Comfort in 0.85 per cent saline (Turner & Holton 1981). Comfort is a commercial fabric softener (kindly supplied by Turner).

5: 0.2 per cent Comfort in 5 per cent aqueous formalin.

6: 0.1 per cent Comfort in 0.85 per cent saline.

7: 0.1 per cent Comfort in 5 per cent aqueous formalin.

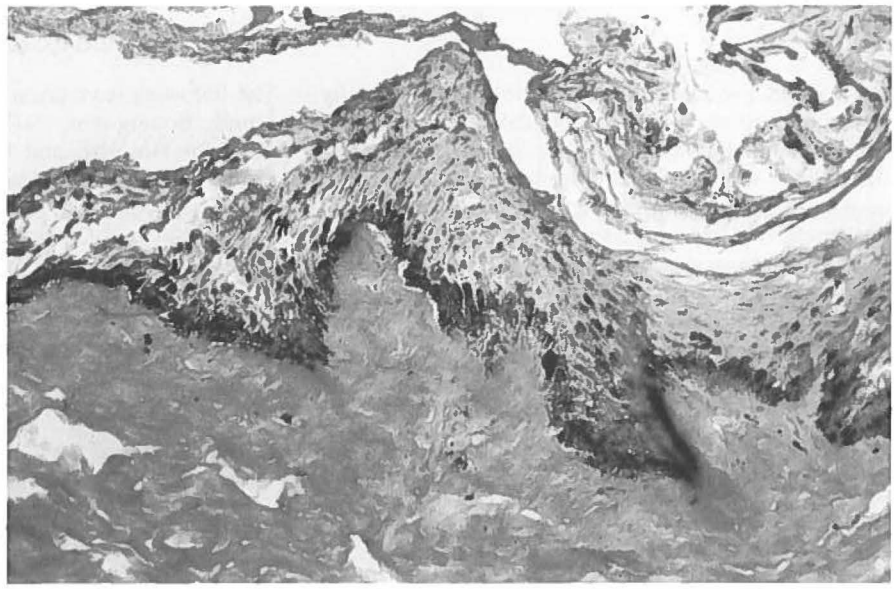

Figure 1. Skin with preserved nuclei in the epidermal cells. In the basal layer of the epidermis deposits of melanin can be seen showing that the person in question was heavily pigmented. Hematoxylin-eosin. x 250.

8: Bierring's solution (Ry Andersen 1979). Eleven parts 40 per cent aqueous formalin, 71 parts 96 per cent ethyl alcohol, 9 parts pure glycerine, and 9 parts water.

9: Direct fixation in 10 per cent buffered formalin without rehydration.

The following stains were employed. The staining time was increased to obtain satisfactory slides.

1: Hematoxylin-eosin.

2: Periodic acid-Schiff (PAS).

3: Alcian blue-van Gieson.

After comparing the rehydrating solutions, further sections of tissue from the Qilakitsoq mummies were prepared with Ruffer's solution according to Zimmerman *et al.* 1971 and Chapel *et al.* 1981: one third of the rehydrating solution was exchanged with absolute ethyl alcohol every day for three days and the specimens were then cleared in xylen and impregnated and embedded in paraplast. The following supplementary stainings were also employed.

4: Verhoeff's method for elastic fibres.

5: Grocott-Gomori methenamine silver method.

6: Gridley's stain for fungal organisms.

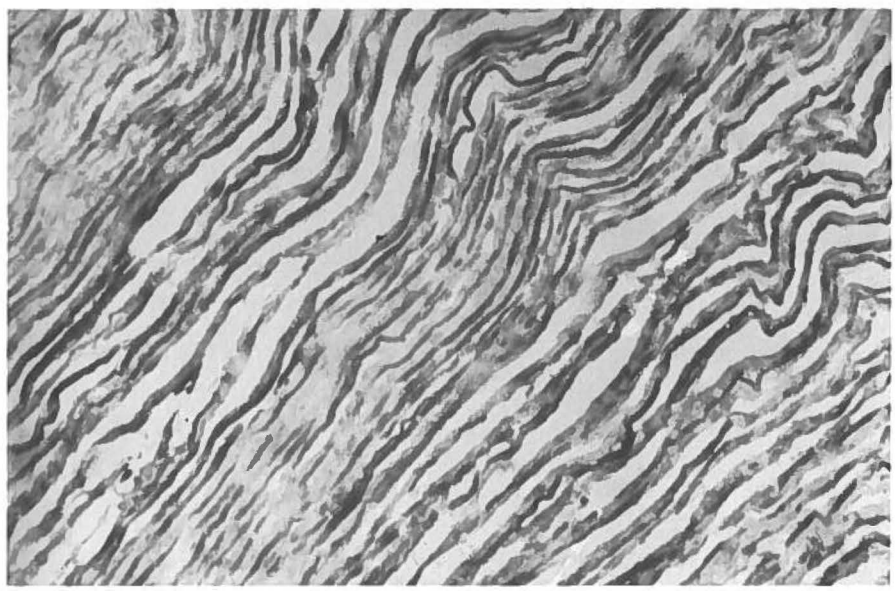

Figure 2. Part of the aortic media showing well preserved elastic fibres. Verhoeff. x 400.

Results

During the process of rehydration specimens rehydrated in solutions 1–7 showed equal swelling and only slight sedimentation. Specimens from solutions 1–2 showed less sediment than those from 3–7. There were no colour changes. All samples floated in the solution. Specimens from solution 8 showed no sediment, did not float and had swelled less than half as much as the previously-mentioned specimens. With regard to cutting in the microtome the technicians described the quality of specimens from solutions 1–2 as good, whereas the other specimens in general were too brittle. Specimens from solutions 3–5 were rather hard, from solution 6 hard as leather.

On histologic examination the slides of the best quality in terms of preserved structures came from specimens rehydrated by using Ruffer's original recipe and formalin fixation as originally described in 1909 (rehydrating solution 1). These slides showed the fewest distortions of the tissue. This procedure was accordingly chosen for the preparation of histologic slides from mummified organs and soft tissues from the Qilakitsoq mummies.

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