

Histopathological Examinations of Eyes

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Mummified tissue from the upper arm of the six-year-old child was processed according to various rehydration and staining schedules. A 0.2% concentration of the wetting agent "Comfort" in a 0.9% saline was found useful. Staining with Eskelund's combined Alcian blue-van Gieson-elastic and haematoxylin-phloxine-saffron made it possible to distinguish between human and non-human structures. Remnants of the eyelids, sclera, choroid, lens and retina with melanine granules were found in the orbits. These structures revealed no sign of intravital lesions.

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The Eye Pathology Institute in Copenhagen has had the opportunity to investigate the two infant Eskimo mummies from Qilakitsoq.

The investigations were in three parts: 1: A study of fungal contaminants, 2: Investigations of various preparation techniques, and 3: Histopathological examination of remnants of eyes.

1: *The study of fungal contaminants* has been published (Bodenhoff *et al.* 1979).

2: *Preparation techniques.* Pieces of skin, muscle, and bone from the right upper arm of the six-year-old child, which had been kept unfixed at +4°C, were rehydrated in five different solutions (Table 1). The tissues were processed for 24 hrs at room temperature, with constant

stirring (rotary mixer, 20r/min). The rehydrated tissues were processed as stated below, and effectiveness of the treatment was considered optimal (+++) when normal anatomical proportions in the tissues were reestablished.

As will be seen from the table, a 0.2% solution of wetting agent "Comfort" in 0.9% saline gave the best results (Turner 1979). However, all solutions containing saline caused some rehydration, in contrast to solutions without saline. Rehydration in solutions without fixatives called for postfixation. Of the solutions tested (Table 1), the 10% buffered formalin provided the best results.

Rehydrated, post-fixed tissues from the arm and orbits were further processed using the routine paraffin technique, but this was carried out by hand with 24 hours between each step.

Table 1. Rehydration, fixation and staining.

Procedure	Reagents	Results
Rehydration	Distilled water	0
	Saline (0.9%)	+
	5% formalin in saline	+
	10% buffered formalin	0
	0.2% Comfort in saline (0.9%)	++
Post-fixation	10% buffered formalin	++
	Sandison's solution, modified*)	+
Staining	Acidic orcein	+
	Alcian blue, 0.2M MgCl ₂	+
	Alcian blue, 1.0M MgCl ₂	+
	Benzidin-nitroprusside	+
	Eskelund's Alcian blue-van Gieson-elastic	+++
	Grocott's modification of Gomori's silver methanamine silver nitrate	+
	Haematoxylin-phloxine-saffron	+++
	Iron-haematoxylin	0
Periodic-acid-Schiff	++	

*) = 1.25 vol 40% formalin, 8 vol 96% ethanol, 1/5 vol potassium nitrate, 1 vol glycerin and 1 vol distilled water.

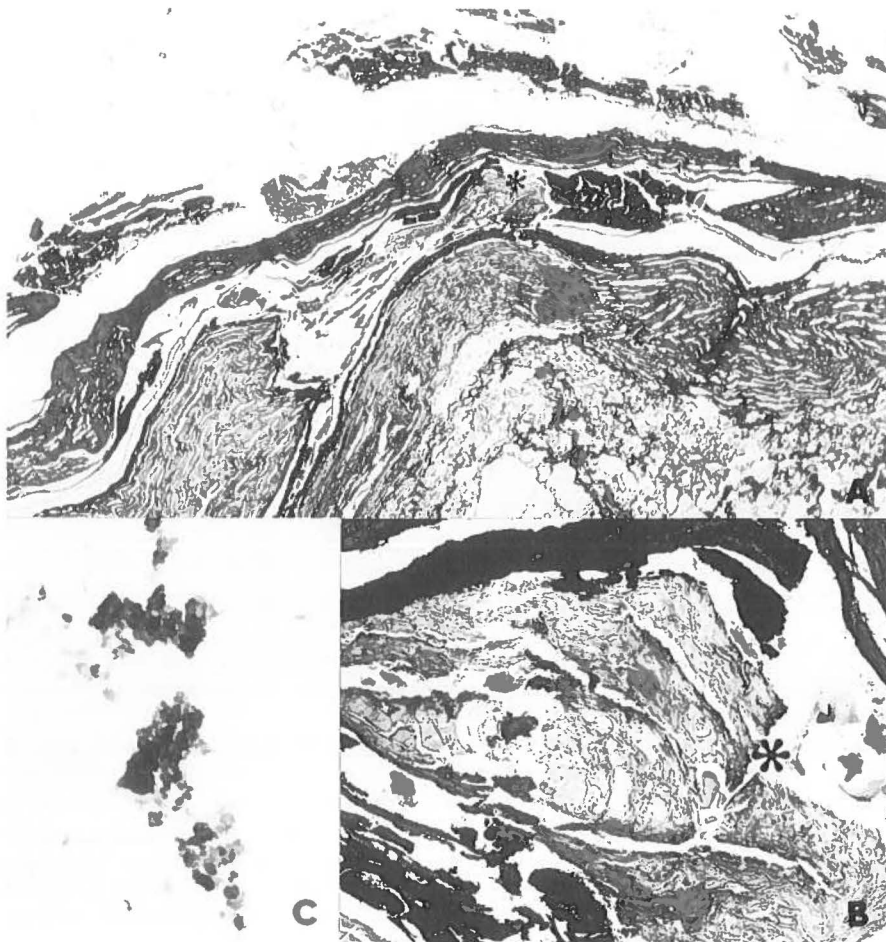


Fig. 1. A. Section of eye tissues from the six-year-old child (Eye Path.Inst.No. 401/78). Choroidal tissue, melanine granules and lens material (asterisk) are to be seen between the two layers of sclera in the collapsed eye. Haematoxylin-phloxine-saffron (x24).

Fig. 1. B. Lens tissue (asterisk) in higher magnification (x140).

Fig. 1 C. Melanine granules of the large retinal size and shape (x560).

A variety of stains was used, with a half, one and two times normal staining times (Table 1). Double staining time was advantageous except with Grocott's modification of Gomori's methanamine-silver nitrate stain, which constantly only gave a faint staining of fungi. In contrast, staining with iron-haematoxylin was always too heavy whatever the staining time.

In line with our earlier experience in preparing tissues from a 2700-year-old human bog body (Andersen & Geertinger 1982), the combined Alcian blue-van Gieson-elastic stain (Eskelund 1957) gave very good results, especially when distinguishing between non-human structures such as fungi and human tissues. Connective tissue was adequately stained with haematoxylin-phloxine-saffron.

3: *Histopathological examination* was performed on tissues from both orbital regions of the 6-years-old child.

Macroscopic remnants of the eyelids were identified. They contained skin and connective tissue, but the tear glands were not seen. A totally collapsed eye was seen at the bottom of each orbit with cornea and sclera readily recognizable.

Microscopy revealed periodic-acid-Schiff-positive fungi both in the skin of the eyelids and in the sclera. These were classified as *Sporotrix fungorum* and *Torulopsis candida* (Bodenhoff *et al.* 1979).

Choroidal tissue and remnants of lens material could be identified in both eyes between two layers of collapsed sclerae (Fig. 1 A-B). In this region, heaps of brownish-black 3-4 μm melanine granules were also observed (Fig 1 C). Their large size and oval shape indicate that they are of retinal origin, choroidal pigment granules being much smaller and more spherical. No other remnants of the retina or of the optic nerve could be identified microscopically and there were no signs of intravital lesions of the eyes or their surroundings.

References

- Andersen, S. R. & Geertinger, P. 1982. Bog Bodies Investigated in the Light of Forensic Medicine. – J. of Danish Archaeology 3: 111–119.
- Bodenhoff, J., Geertinger, P. & Prause, J. U. 1979. Isolation of *Sporotrix fungorum* from a 500-year-old mummy found in Greenland. – Acta pathol. et microbiol. Scand. Sect. B. 87: 201–203.
- Eskelund, V. 1957. Mucin staining with Alcian blue. – Acta pathol. et microbiol. Scand. 40: 107–109.
- Turner, P. J., East Birmingham Hospital, G.Br. 1979. – Personal Communication.