

## A Bleaching Method for Melanin and two Staining Methods

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### SUMMARY

An acidified permanganate method can be used for bleaching melanin in mammalian skin and eyes. It allows good staining to be performed later.

Microfilariae in nitrocellulose sections can be stained by a modified phloxine tartrazine method.

Elastic fibres can be selectively stained by using a diluted solution of synthetic orcein.

### A METHOD FOR BLEACHING MELANIN

THE Mallory type of bleaching method involves the use of a solution of potassium permanganate followed by a solution of oxalic acid. Gomori (1941) used an acidified solution of potassium permanganate followed by a solution of sodium bisulphite as a preliminary to the staining with chrome alum haematoxylin and phloxin. We have found that this method, in a slightly modified form, is useful for bleaching melanin in paraffin and nitrocellulose sections.

(1) Transfer the sections from water to a freshly prepared mixture of equal parts of the following:

- (a) 0.3% potassium permanganate.
- (b) 0.3% sulphuric acid.

Leave the sections in this mixture until the melanin is bleached. This usually occurs in the following times:

skin	2 min
choroid and ciliary processes of the eye	15 min
iris of the eye	30 min

(2) Treat with 1% oxalic acid until the brown colour disappears.

(3) Rinse in two changes of distilled water.

(4) Wash in running tap water for at least 45 min.

(5) Rinse in distilled water.

(6) Stain as desired. Some staining times may be slightly altered.

This method is the only one that we know, which will bleach the melanin of the eye without seriously affecting subsequent staining methods.

### A METHOD FOR STAINING MICROFILARIAE IN NITROCELLULOSE SECTIONS

Lendrum (1947) introduced a phloxine tartrazine method which has been used by Jamison (Kershaw, Jamison, Nugent, and Duke, 1956) to show microfilariae in paraffin sections. This method in a modified form can be used to show them in nitrocellulose sections.

(1) Stain the sections with Weigert's iron haematoxylin for 3 min.

(2) Differentiate in 1% hydrochloric acid in 70% alcohol.

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- (3) Wash in several changes of tap water.
- (4) Stain for 30 min in the following solution:
 

phloxine	0.5 g
calcium chloride	0.5 g
distilled water	1000 ml
- (5) Wash in distilled water.
- (6) Transfer to the following solution for about 1 min or until most of the red colour has disappeared:
 

tartrazine	0.5 g
alcohol, 90%	100 ml
- (7) Remove excess tartrazine with 90% alcohol.
- (8) Clear and mount.

This method reveals microfilariae that would otherwise be difficult to notice. It works well after initial bleaching of the melanin with acidified permanganate solution. We have recommended this method to Dr. F. C. Rodger, who has used it in his studies of the eye in cases of onchocerciasis. We are indebted to him for the provision of the material.

#### A METHOD FOR STAINING ELASTIC FIBRES

Prepare a stock solution of orcein by dissolving 0.5 g of orcein (synthetic, G. T. Gurr) in 100 ml of acid alcohol (70% alcohol containing 1% of hydrochloric acid). Leave overnight and then filter.

Treat paraffin sections as follows:

- (1) Rinse in 70% alcohol.
- (2) Stain overnight in a weak solution of orcein made by diluting 1 volume of the filtered stock solution with 10 to 20 volumes of acid alcohol.
- (3) Rinse in acid alcohol.
- (4) Wash in running tap water for 15 min.
- (5) Counterstain with Weigert's iron haematoxylin if desired.
- (6) Dehydrate, clear, and mount.

More intense staining of the elastic fibres may be obtained by using the acidified permanganate bleach before staining with orcein.

It is essential to use diluted stock solution and not to make up a dilute solution from the solid dye.

With this method, particularly after bleaching, even the finest sub-epidermal elastic fibres of the skin are stained a deep purple to black. Little or no background staining occurs after most fixatives.

#### REFERENCES

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