A Simple Pyronine / Methyl Green Technique

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Summary

Brachet's pyronine / methyl green technique, which gives good results when made with certain foreign dyes, does not work well when British specimens of pyronine and methyl green are used. Instructions are given for making up and using a reliable staining solution containing dyes that are readily available in this country.

FOR his pyronine / methyl green (P/MG) technique, Brachet (1953) recommends the pyronine of Geigy and of Grübler and the methyl green of Anachemia, Grübler, and Ciba. In trying his method we used dyes sold by English firms. Our results were not satisfactory. The pyronine was largely extracted during the 5 minutes' stay in 95 per cent. alcohol, and the methyl green did not stain well except in the internal part of the piece of tissue.

We had been engaged in an attempt to improve the P/MG technique when Brachet's paper was published, and our failure to get good results with English dyes when we tried his technique caused us to revert to this subject. In this work we have used the dyes of Messrs. G. T. Gurr (pyronine G 6104, methyl green 05563).

Our work has been carried out on the following tissues: skin, stomach, intestine, submaxillary gland, liver, pancreas, kidney, and anterior mesenteric ganglion of mammals (mouse, cavy, and rabbit); pancreas, leg-muscle, and cerebellum of the frog; cerebral ganglion of *Helix pomatia*; ovary and ventral ganglia of the earthworm; and transverse sections of the leech, *Glossiphonia complanata*. We have found the pancreas of the mouse the most useful test-object for comparing the effects of variations in technique.

The staining solution that has given us the best result is made thus:

Extract 0·5 per cent. aqueous methyl green solution repeatedly with chloroform until the chloroform is nearly colourless. At least eight extractions are necessary.

Make an acetate buffer at pH 4·8 by mixing 81 c.c. of N/5 acetic acid with 119 c.c. of M/5 sodium acetate.

Prepare the stain as follows:

Pyronine, 0·5 per cent. aq.	37 c.c.
Methyl green, 0·5 per cent. aq. (extracted)	13 c.c.
Acetate buffer at pH 4·8	50 c.c.

This solution, which can be used at once, still stains well four months after preparation.

The pyronine in our solution is considerably weaker than in Unna's (1913)

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and Brachet's solutions, and slightly weaker than in Trevan and Sharrock's (1951). The methyl green in our solution is much weaker than in Unna's and Brachet's (less than half the concentration), though somewhat stronger than in Trevan and Sharrock's.

Our technique is as follows:

I. Fix a piece of tissue not exceeding 3 mm. in thickness in Zenker's fluid for 3 hours.
II. Wash in running water for 24 hours.
III. Embed quickly in paraffin through alcohol and toluene.
IV. Cut sections at 7μ.
V. Bring sections to water in the ordinary way (passing through iodine and sodium thiosulphate solutions on the way, in accordance with the usual practice after Zenker fixation). Wash for a few minutes in running water.
VI. Rinse in distilled water.
VII. Blot with filter-paper.
VIII. Stain for ½ hour in the buffered P/MG.
IX. Rinse for a few seconds in distilled water.
X. Quickly blot nearly dry.
XI. Acetone (to dehydrate), 1 minute.
XII. Pass through 1:1 acetone-xylene into xylene, and mount in D.P.X. (or other neutral mounting-medium).

Result: chromatin, blue, blue-green, or green; nucleoli and basiphil cytoplasm, red. The material stained red can only be interpreted with certainty as ribonucleic acid if tests are made with ribonuclease.

We tried a large number of fixatives, and found that 3 hours in Zenker's fluid gave the best fixation that was compatible with characteristic staining by P/MG.

The method presents no technical difficulties.

Kurnick (1952) found that dehydration in acetone removed pyronine after P/MG staining, and that it was necessary to re-stain in a saturated solution of pyronine in acetone. He used pyronine B. We do not find any restaining to be necessary with the specimen of pyronine G that we have used. It seems doubtful whether pyronine gives characteristic staining results unless it is ionized in aqueous solution.

It is worth remarking that our P/MG solution can conveniently be used for showing the macronucleus of *Paramecium*. Put a drop of water containing the living ciliates on a slide. Add a drop of P/MG. Mix the fluid by drawing it gently into a pipette and pressing out again several times. Leave for 15 minutes. Cover. Result: macronucleus, green; cytoplasm, pink. The method is very suitable for use in elementary classes in zoology.

Rhodamine S can be substituted for pyronine in this technique, as one of us showed many years ago (Baker, 1942). This dye is more powerful than pyronine, and the concentration of the stock solution should be reduced from

0·5 to 0·25 per cent. We have used the product of the British Drug Houses Ltd.

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