J. clin. Path. (1968), **21**, 107

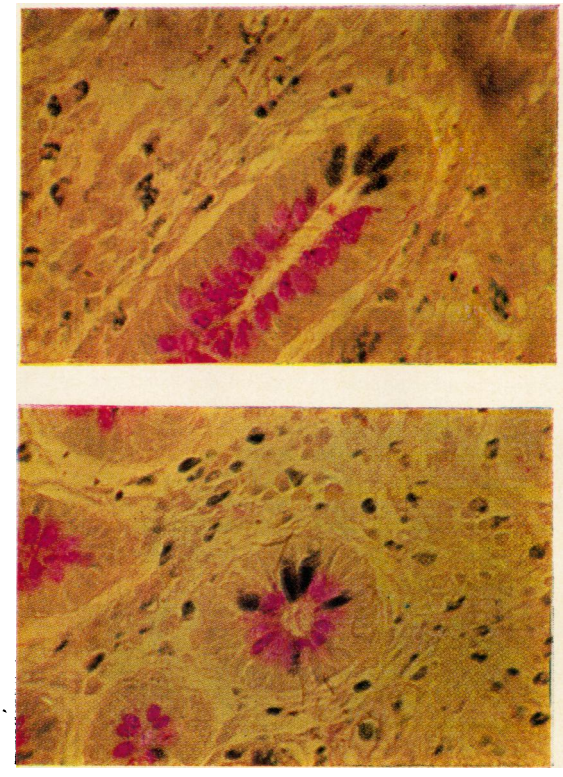
Technical methods

Demonstration of Paneth cell granules using Naphthalene Black

DIANA BOWER AND C. G. CHADWIN *From the Department of Histopathology, Royal Marsden Hospital, London*

Many tissue elements stain with aqueous Naphthalene Black, but after differentiation with a weak solution of lithium carbonate, eosinophils and erythrocytes appear to have a greater affinity for the dye. Assuming it has some specificity for eosinophilic structures, Naphthalene Black was applied to sections of small intestine, and Paneth cell granules were also found to retain the dye after differentiation.

Specificity of Paneth cell granules for Naphthalene Black is increased if 50% propylene glycol is used as the solvent for the dye, and greater contrast is obtained when the mucin-secreting cells are stained by a modified P.A.S. technique. Nuclear counterstaining with haemalum tends to mask the Paneth cells; therefore the nuclei are left unstained to facilitate study of the granules.



TECHNIQUE

MATERIALS Sections, 5μ, of paraffin-wax-embedded 10% formal-saline-fixed tissue.

SOLUTIONS

Naphthalene Black solution Dissolve 0·5 g. Naphthalene Black in 50 ml. distilled water, add 50 ml. propylene glycol and mix thoroughly.

Periodic acid-Schiff solutions These solutions are made up according to the method of Hotchkiss (Pearse, 1960), but are kept at 4°C. when not in use.

METHOD

1 Bring sections to 70% alcohol.
2 Treat with alcoholic periodic acid 3-5 min.
3 Rinse in 70% alcohol.
4 Treat with reducing rinse ½-1 min.
5 Rinse in 70% alcohol.
6 Wash section free of alcohol in running tap water, then rinse in distilled water.
7 Treat with Schiff's reagent 5 min.
8 Wash in running tap water 10 min.
9 Treat with 0·5% Naphthalene Black solution 15 min.
10 Wash in water.
11 Differentiate in 0·01% lithium carbonate until Paneth cell granules are well defined.
12 Wash in water.
13 Dehydrate, clear, and mount in Xam.

Received for publication 19 April 1967.

RESULTS

Paneth cell granules and eosinophilic leucocyte granules stain deep blue; mucin stains magenta, and erythrocytes stain greenish-blue.

COMMENT

Occasionally granules can be seen unstained amongst groups of granules staining strongly with Naphthalene Black. This does not appear, in our experience, to be due to technical variations. In the stained sections the background staining is almost colourless rather than pale yellow as it appears in the photomicrographs reproduced here. The yellowish tinge has also caused the granules to appear brownish black rather than deep blue.

We wish to thank Dr. N. F. C. Gowing, Director of the Department, for permission to publish this technique, and Dr. I. M. E. Hamlin for her encouragement and advice.

REFERENCES

Pearse, A. G. E. (1960). *Histochemistry, Theoretical and Applied.* p. 831. Churchill, London.