Screening methods

Screening for neurofibrillary tangles and argyrophilic plaques with Congo Red and polarized light

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The capricious nature of silver impregnation techniques renders them unsuitable for bulk staining and screening sections for the presence of argyrophilic (senile) plaques and neurofibrillary tangles. For this purpose, we describe a simple, consistent and highly sensitive bulk staining method. It depends on the congophilia and resultant anomalous colour or polarization (Ladewig, 1945) that characterizes amyloid and other fibrillary protein components of plaques and tangles (Terry and Wiśniewski, 1970).

Method

STOCK SOLUTIONS

Solution A
0.5% Congo Red in 50% ethanol.

Solution B
0.15% Gallocyanin is added to a 5% aqueous solution potassium chromium sulphate. The solution is mixed, boiled for five minutes, cooled and filtered before use.

PROCEDURE
Formalin-fixed tissue is used.
1. Paraffin wax sections (8 μ) are taken through xylene to 50% alcohol.
2. Stain in solution A for five minutes.
3. Wash rapidly in tap water.

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A simple lyophilizer for direct line sublimation—continued.

When the preparation time was extended, the plate, H, was prechilled in order to prevent partial thawing of the sample before the vacuum was applied.

References

4. Differentiate in 0.2% potassium hydroxide in 80% alcohol for one minute.
5. Wash in water for 10 minutes.
6. Counterstain in solution B at room temperature overnight.
7. Wash in distilled water.
8. Dehydrate, clear, and mount in Xam or DePex.
9. View by standard light microscope incorporating polarizing equipment and a Lambda (2nd order red) interference filter.

The red congophilic staining of fresh amyloid is usually adequate for its detection by conventional transmitted light microscopy. But recognition of plaques and tangles by this method can be difficult, possibly because of the small content of oriented protein fibrils. Congo Red binds to amyloid and to the protein fibrils of tangles in such a way that an optically active configuration is produced. When viewed by conventional linearly polarized light, a green anomalous (interference) colour of polarization is produced which is often misnamed dichroism. If a Lambda (2nd order red) interference filter is placed in the light path between the polarizer and analyser, correct orientation of the object in relation to the place of polarization, reveals the stained material as bright green or yellow-blue structures standing out against a dark red background.

Comment

Unfortunately, the methods usually employed for the demonstration of plaques and tangles, the silver impregnation techniques, are costly, capricious, time consuming, and not consistent when carried out in bulk. Batch impregnation of large numbers of sections can lead to wide variation in the quality of the final preparations. Nor is the conventional use of metachromatic dyes satisfactory, as small deposits of amyloid and similar fibrillary proteins may fail to show red, despite being silver positive. Polarized light microscopy, however, can, by correct adjustment of the lighting, be made extremely sensitive, the sought-for material standing out by both its brightness and colour from the background.

Of the various techniques using Congo Red (Bennhold, 1922; Puchtler, Sweat, and Levine, 1962) for the demonstration of amyloid, we find that of Highman (Highman, 1946) is most suitable for our bulk staining. Easily prepared stock solutions can be used many times and there is little danger of over-differentiation. Background staining is minimal.

As a nuclear stain, gallocyanin (Einarson, 1951) gives excellent results in conjunction with Congo Red. It gives a precise nuclear stain on a clear background, avoiding the ‘blueness’ often associated with haematoxylin, and yet shows cytological detail.
Thanks to its stable progressive staining it requires no differentiation.

We are indebted to Dr A. D. Dayan for his encouragement to publish this technique.

References

Letter to the Editor

Resistence of *Staphylococcus aureus* to Sulphamethoxazole and Trimethoprim

Nakhla in 'Resistance of *Staphylococcus aureus* to sulphamethoxazole and trimethoprim' (J. clin. Path., 1972, 25, 708-712) states: 'It is not known whether trimethoprim-resistant strains of staphylococci existed naturally before the drug came into use, or whether the resistance has arisen spontaneously and been selected since.'

In December 1963, through the kindness of Dr E. W. Witherspoon, a medical director of Burroughs Wellcome & Co (Australia) Ltd, we received a supply of BW56-72 trimethoprim. In the first quarter of 1964 we tested some hundreds of strains of organisms isolated in this laboratory against trimethoprim by a plate-dilution method. Four hundred and sixty-six strains of *Staph. aureus* were inhibited by a concentration of 1 μg/ml trimethoprim; 40 grew in the presence of 1 μg/ml but were inhibited by 2 μg/ml; eight strains tolerated 2 μg/ml but were inhibited by 5 μg/ml and two strains were not inhibited by 5 μg/ml.

The medium used was blood agar containing lysed horse red blood cells. It was satisfactory for the determination of sensitivity to sulphonamides, but probably was not entirely free of substances that interfered with trimethoprim. However, I believe that these results show that trimethoprim-resistant strains of *Staph. aureus* existed here in those days.

Prior to that time Cooper and Wald had used trimethoprim at the Royal Perth Hospital in Western Australia but I do not know of any being used in this vicinity, certainly not in this hospital.

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Book review

The Bare Facts of Systemic Pathology

To quote from the Preface might suggest, incorrectly, that your reviewer had not read this book.

'Students will find that this book meets their needs in several ways. First, it gives succinct, clear, no-nonsense, up-to-date definitions and descriptions of essential, important concepts of pathology. It contains much relevant, current information not available in any other single textbook, particularly in the areas of molecular pathology, lysosomal diseases and immunopathology.'

These are bold assumptions, and I also doubt if concepts can possibly be transmitted in the staccato telegraphese the author employs. Admittedly the last three areas occupy a lot of space, but they certainly flout Sir Robert Hutchison's advice, 'It is always well, before handing the cup of knowledge to the young, to wait until the froth has settled' (Brit. med. J., 1925).

'Students themselves suggested the novel layout of the book with the text appearing on the left and blank pages for notes on the right. They take lecture notes on the right-hand pages and expand on the "bare facts" with pertinent notes from classic pathology texts, literary articles and other sources.' One real disadvantage of a telegraphic skeleton, as of a railway timetable, is the monotonous uniformity of each gobbet of information. There is neither chiaroscuro nor perspective. It is to be hoped that the students do indeed seek truly literary articles, because this type of textbook indubitably imperils their ability to use the English language.

'A glance at the pages of this book reveal that the key word is "communicate". To that end I have dedicated this book. Alas, the proof reader was inadequately dedicated and the misprints may not always be recognized by the students.'

'Coephorectomy prevents cancer' in Turner's syndrome; 'Urinary bladder carcinoma' etiology: key words: Analine dye workers and schistosomiasis in Egypt'; Fungi 'often cause suppuration with draining sinuses'.

Under carcinoma of cervix we find, aetiology: SEX (too early? too often? etc). This recalls an old school master's definition of *et cetera* as a confession of ignorance; it can here scarcely be said to communicate. Slang, the language of a group, is similarly non-communicating eg, under Grave's (sic) disease ... 'patients are hyperthyroid with "bug" eyes'. His contraction Rx presumably stands for therapy and under benign prostatic hypertrophy it is 'Rx: TUR'. 'N. Gonorrhoea is MOST common cause of PID in USA'. Contraction-slang is the craziest.

There are many quaint bits of information. 'Scar = collagenised granulation tissue with few or no fibroblasts and (sic)—reviewer's (sic) blood vessels 'A scar is a scar, is a scar, is a scar'. Presumably Stein; here is another: 'Lesch-Nyham syndrome is a "touchstone disease",' 'Everybody harbors 20 or more "chronic" viruses'. 'Granuloma inguinale has Pund-Greenblott macrophages'. 'Legally intoxicated—0.15% in blood (3 highballs) some states now 0.10%'. His last entry under AGING KEY WORDS No. 6 is 'Sisson's rule: There are at least 2 pathological diagnoses for every ten years of age,' and so ends this book without a full stop.

I would not commend this book to anyone with less than five years' experience in pathology, or with a taste for style in communication.

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