A modified haematoxylin and eosin stain for histological sections of lymph nodes

JAMES O'DRISCOLL and J. P. RYAN *Histopathology Department, Regional Hospital, Dooradoyle, Limerick, Eire.*

Good cytological detail, especially good nuclear detail, is obviously of value to the histopathologist when examining sections of lymph nodes and, in particular, when examining lymph nodes from patients suspected of having a malignant lymphoma.

In an attempt to improve the nuclear detail of lymph node sections, we have recently established a routine of staining lymph node sections with a modified haematoxylin and eosin stain in addition to the standard haematoxylin and eosin stain and a reticulin preparation.

Material and methods

3 μ sections of formalin-fixed paraffin-embedded tissue are cut and stained with the modified haematoxylin and eosin stain.

Haematoxylin (Harris)
0·5 g eosin Y
0·2 g phosphotungstic acid
1 drop saturated aqueous lithium carbonate
100 ml of 95% alcohol.

Received for publication 23 January 1978

Results

We have found that, using this stain, there is a noticeable gain in nuclear detail when compared with sections stained with the standard haematoxylin and eosin. This modified haematoxylin and eosin is a useful adjunct to the procedures normally used by the histopathologist when dealing with lymph nodes.

Discussion

Slidders (1969) has discussed the effects of an alcoholic solution of phosphotungstic acid on already stained nuclei. He noted an increased resistance of haemalum-stained nuclei to the acid components of several trichrome stains and he produced trichrome stains of 'notable nuclear detail'. We have used an alcoholic solution of phosphotungstic acid in the modified haematoxylin and eosin stain and found that it produces improved detail in nuclear staining.

Reference

Slidders, W. (1969). A stable iron-haematoxylin solution for staining the chromatin of cell nuclei. *Journal of Microscopy*, **90**, 61-65.

Requests for reprints to: Dr J. O'Driscoll, Histopathology Department, Limerick General Hospital, Dooradoyle, Limerick, Eire.

Letters to the Editor

Synergy between sulphonamides and trimethoprim in the presence of pus

As the paper by Edmunds (*Journal of Clinical Pathology*, **31**, 162, 1978) contains some inferences different from those of our own work (*Journal of Clinical Pathology*, **31**, 165, 1978), we would like to offer the following explanations and comments:

1 Edmunds claims to have demonstrated synergy by the application of two different antibiotic disks in proximity and comparing resultant zones with those from the individual disks. The increased zones of inhibition from the combined disks do not necessarily denote synergy but could simply be due to additive effects. Controls consisting of placing two similar disks close together might have strengthened the claims for synergy. It is puzzling why no minimum inhibitory concentrations were performed, and these surely must be included in any paper claiming synergy.

2 The observations of Edmunds are confined exclusively to zones of inhibition that express bacteristatic activity. Our work has included assessment of the bactericidal activities of trimethoprim, sulphamethoxazole, and the combination. We have *never* found any bactericidal synergy exclusive to the combination, that is, the cidal activity of trimethoprim alone is not enhanced by sulphamethoxazole *in vitro*.

3 The diluted pus may have lost some of its thymidine content during preparation of the plates; this possibility is particularly likely for the pus that was filtered where some thymidine might have become adherent to the filter or cellular debris. Many workers have isolated thymidine-requiring mutants from purulent material, so there must be a continuous release of avaiable thymidine in some samples of pus. The use of a particular aliquot of pus will contain only a fraction of the potential thymidine *in vivo* where both necrotic bacteria and leucocytes probably contribute to levels of this substance.

4 Perhaps the greatest difference between our conclusions and those of Edmunds is his claim that synergy may be important where pus is present *in vivo*. Even if synergy had been demonstrated *in vitro*, the relative amounts of sulphadiazine (20 μg) to trimethoprim (1 μg) may be far from the levels of these agents in purulent tissues in man.

It is not clear whether Edmunds believes his findings are relevant to the prescribing of co-trimoxazole (this contains sulphamethoxazole rather than sulphadiazine); the actual tissue or urine levels of active sulphamethoxazole are so low, on account of hepatic acetylation, that there is virtually no prospect of synergy *in vivo*. We have demonstrated this with urine and consider that, for many infections, the level of sulphamethoxazole is subinhibitory.

5 One point that we agree upon is that organisms highly resistant to either sulphonamide or trimethoprim never show synergy with the combination. Because of (1) the high frequency of sulphonamide resistance in many pathogens and (2) the possibility of serious toxicity associated with the sulphonamide moiety, the case for preferring trimethoprim alone (when available) to the combination co-trimoxazole for many infections must be overwhelming.

R. W. LACEY and ANNE STOKES
West Norfolk and King's Lynn General Hospital, London Road, King's Lynn, Norfolk, PE30 5QD

The author comments as follows:

1 In my experience, distortion of zones of inhibition due to additive effects are minimal, and I have seen many such instances over my 28 years as a bacteriologist. They never show the obvious and marked increase shown in the Figure in my article (Edmunds, 1978). I agree that MIC tests should be performed; the fact that they have not been done is due to lack of suitable pus, time, and facilities. I hope that someone with access to more of these commodities will take this matter up. There can, however, be no doubt whatever that synergy occurred under the conditions of my experiments, using basal minimal salts agar medium with pus in up to 50% concentration.

2 I have found only minor degrees of bactericidal synergy in Davis-Mingioli minimal salts broth (Davis and Mingioli, 1950), and I make no claims on this score. However, Bushby (1973, 1975) has shown this to occur *in vitro*.

3 There were in fact sufficient antagonists present even in the filtered pus extract at 10% concentration to abolish zones of inhibition of well-separated disks of sulphonamide (SU) and trimethoprim (TM). The same effect of antagonism, and synergy despite antagonists, was observed in plates containing up to 50% unfiltered pus, whose only treatment was autoclaving. How much more antagonists do Dr Lacey and Miss Stokes want? If they wish, I will be happy to provide them with a photograph of the 50% pus plate, although this does not show enough contrast for publication.

4 I do not know whether synergy is important *in vivo*. According to Bushby (1975), it has been established in animal experiments. I merely point out that synergy occurred in my experiments in the presence of large amounts of pus and that this may be of significance in treatment. As far as urine is concerned, I hold no particular brief for using co-trimoxazole in lower urinary tract infections, except that one is not always sure that the infection has no renal tissue element. My arguments are partly directed to the small concentrations likely to be found in the tissues and also to a possible protective effect of the combination of sulphonamide and trimethoprim against the long-term development of resistance to these two drugs, particularly trimethoprim.

5 Dr Lacey and I agree that co-trimoxazole should not be used against an organism which is completely resistant to sulphonamide. The effect would then be that of trimethoprim alone but with the disadvantage of added sulphonamide toxicity. On the other hand, if trimethoprim is used extensively as a single-drug preparation, any protective effect of sulphonamide for sensitive strains will be lost. At present we in Fife enjoy the favourable position where trimethoprim resistance occurs in 1% of hospital *Escherichia coli* strains and 2% of all urinary coliforms combined, excluding *Pseudomonas aeruginosa*. I fear that this position will be eroded and trimethoprim resistance will rise to or exceed the 8·2% reported in London by Grüneberg (1976).

P. N. EDMUNDS
Department of Medical Microbiology, Fife Area Laboratory, Scotland

References

Bushby, S. R. M. (1973). Sensitivity testing with trimethoprim/sulphamethoxazole. *Medical Journal of Australia*, **1**, Special Supplement 2, 10-18.

Bushby, S. R. M. (1975). Synergy of trimethoprim-sulfamethoxazole. *Canadian Medical Journal*, **112**, 63S-66S.

Davis, B. D., and Mingioli, E. S. (1950). Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. *Journal of Bacteriology*, **60**, 17-28.

Edmunds, P. N. (1978). Synergy between sulphonamide and trimethoprim in the presence of pus. *Journal of Clinical Pathology*, **31**, 162-164.

Grüneberg, R. N. (1976). Susceptibility of urinary pathogens to various antimicrobial substances; a four-year study. *Journal of Clinical Pathology*, **29**, 292-295.

A non-carcinogenic substrate for immunoperoxidase procedures

3,3'-Diaminobenzidine tetrahydrochloride (DAB) is used in immunoperoxidase procedures to render the horseradish peroxidase labelled antibody-antigen complex visible at the light microscope level (Burns, 1978). The recent decrease in availability of good quality DAB could be due to its borderline carcinogenic properties (Hanker *et al.*, 1977). An alternative non-carcinogenic DAB substrate was described by these authors

'7·5-15 mg *p*-phenylenediamine dihydrochloride (1 part) + pyrocatechol (2 parts)-premixed reagent (PDP) (Polysciences) dissolved in
10 ml Tris buffer (0·1 M) pH 7·6
0·1 ml H_2O_2 (1%)'

for the demonstration of injected horseradish peroxidase in tissue sections.

I wish to report that this substrate can also be substituted for the DAB substrate in immunoperoxidase procedures for demonstrating antigens in paraffin sections (Figure). Sections are incubated for 5-20 minutes. The end product, like that of DAB, is insoluble and osmiophilic, can be counterstained with routine histological methods, withstands pro-