Table Immunoglobulin positive cells in undigested and pepsin digested sections of bronchial wall

Immunoglobulin	Number of immunoglobulin containing cells	
	Before pepsin	After pepsin
IgG	79	524
IgA	41	452
Lambda	51	479
Kappa	63	292
Total	234	1747

globulin containing cells may be more apparent than real and is due probably to the ease with which such cells are seen in these preparations.

It is clear from these results that the use of pepsin digestion before the application of the immunoperoxidase technique provides a relatively simple solution to the problem of non-specific background staining in sections of bronchial tissues. We have also shown that this technique is applicable to rectal and prostatic tissue.

The mechanism whereby pepsin abolishes the background staining of connective tissue has not been investigated but it is probably related to the removal of ground substance protein molecules rather than to an effect on collagen fibres *per se* because bacterial collagenase is ineffective in abolishing background staining.

I should like to thank Professor J. O'D. McGee, of the Department of Pathology, Gibson Laboratories, Radcliffe Infirmary, Oxford, for his help and encouragement.

References

- Burns, J. (1975). Background staining and sensitivity of the unlabelled antibody-enzyme (PAP) method. Comparison with the peroxidase labelled antibody sandwich method using formalin fixed paraffin embedded material. *Histochemistry*, **43**, 291-294.
- Piris, J. and Whitehead, R. (1974). An immunoperoxidase technique for the identification of gastrin-producing cells. *J. clin. Path.*, **27**, 798-799.

Letters to the Editor

2-Hydroxyethyl methacrylate for sectioning bone marrow

Dancey *et al.* (1976), in their paper entitled 'Section preparation of human bone marrow for light microscopy', seem to me to have been unfortunate in the difficulties encountered when using hydroxyethyl methacrylate for this purpose. Perhaps they did not read my paper on the subject (Green, 1970). We have used the method given therein for about eight years with good results, without trouble, and without the use of special equipment. I agree with their finding that Ruddell's (1971) embedding mixture containing pyridine as an activator instead of N,N-dimethyl aniline used previously (Ruddell 1967a, 1967b) is unreliable. In fact in our own trials we could not make it work. However, the N,N-dimethyl aniline mixture is entirely satisfactory for aspirated marrow particles and also for large pieces of tissue. We regularly section blocks, measuring up to 2 × 2 cm, of a wide range of tissues including decalcified (by EDTA) bone marrow slabs. The

polymerisation failures experienced by Dancey *et al.* do not occur.

The most important technical hints appear to be the use of a cooling water-bath during polymerisation (which occurs within 30 min), and the fact that impregnation of large tissue blocks by monomer can be prolonged more or less indefinitely at 4°C without partial polymerisation, so ensuring complete impregnation. In sections undue staining of the plastic matrix with basic dyes can be reduced by treating the monomer with basic ion exchange resin beforehand (North, 1971).

Many valuable and relevant points about this easy-to-use plastic embedding medium for 'ordinary' hospital laboratories are given by Sims (1974), who used steel knives for sectioning, and Bennett *et al.* (1976), who present some original and stimulating information on the use of large glass knives and ordinary (rather than ultra-) microtomes. Although we use a base sledge microtome, Murgatroyd (1976) has reported good results with a rotary microtome. The great improvement in histological morphology in

hydroxyethyl methacrylate sections compared with paraffin sections makes its use for selected tissues well worth while.

G. H. GREEN
Department of Pathology
The Royal Infirmary
Worcester WR1 3AA

References

- Bennett, H. S., Wyrick, A. D., Lee, S. W. and McNeil, J. H. (1976). Science and art in preparing tissues embedded in plastic for light microscopy, with special reference to glycol methacrylate, glass knives and simple stains. *Stain Technology*, **51**, 71-97.
- Dancey, J. T., Deubelbeiss, K. A., and Harker, L. A. (with the technical assistance of Greenaway, J) (1976). Section preparation of human marrow for light microscopy. *Journal of Clinical Pathology*, **29**, 704-710.
- Green, G. H. (1970). A simple method for the histological examination of bone marrow particles using hydroxyethyl methacrylate embedding. *Journal of Clinical Pathology*, **23**, 640-643.

Letters to the Editor

- Murgatroyd, L. B. (1976). The preparation of thin sections from glycol methacrylate embedded tissue using a standard rotary microtome. *Medical Laboratory Sciences*, **33**, 67-71.
- North, R. J. (1971). Methyl green-pyronin for staining autoradiographs of hydroxyethyl methacrylate-embedded lymphoid tissue. *Stain Technology*, **46**, 59-62.
- Ruddell, C. L. (1967a). Hydroxyethyl methacrylate combined with polyethylene glycol 400 and water; an embedding medium for routine 1-2 micron sectioning. *Stain Technology*, **42**, 119-123.
- Ruddell, C. L. (1967b). Embedding media for 1-2 micron sectioning. (2) Hydroxyethyl methacrylate combined with 2-butoxyethanol. *Stain Technology*, **42**, 253-255.
- Ruddell, C. L. (1971). Embedding media for 1-2 micron sectioning. (3) Hydroxyethyl methacrylate-benzoyl peroxide activated with pyridine. *Stain Technology*, **46**, 77-83.
- Sims, B. (1974). A simple method of preparing 1-2 micron sections of larger tissue blocks using glycol methacrylate. *Journal of Microscopy*, **101**, 223-227.

Macrophage electrophoretic mobility test

The report by Crozier *et al.* (1976) that the macrophage electrophoretic mobility test (MEM) is ineffective in the diagnosis of cancer is in full agreement with that of our group at Weston Park Hospital, Sheffield (Shelton *et al.*, 1975). While the MEM test may be of value in the hands of highly specialist workers it is certainly of no present value outside such centres. It is interesting that a simple macrophage migration inhibition test to encephalitogenic factor gave similar results to the complex and capricious MEM test. It seems likely that further exploration and refinement of the simpler technique is a more hopeful approach to cancer diagnosis than the MEM test.

IAN CARR

Department of Pathology, University
of Saskatchewan, University Hospital,
Saskatoon, Saskatchewan, Canada
S7N 0W8

References

- Crozier, E. H., Hollinger, M. E., Woodend, B. E., and Robertson, J. H. (1976). An assessment of the macrophage electrophoretic mobility test (MEM) in cancer diagnosis. *Journal of Clinical Pathology*, **29**, 608-609.
- Shelton, J. B., Potter, C. W., and Carr, I. (1975). Cellular immunity to myelin basic protein in man and in animal model systems as measured by the macrophage

migration inhibition test. *British Journal of Cancer*, **31**, 528-534.

Plasma paracetamol estimation

Spooner *et al.* (1976) claim that the method of Routh *et al.* (1968), for the measurement of plasma paracetamol, is relatively insensitive and more susceptible to interference from other drugs than the method of Dordoni *et al.* (1973). Although we agree that the former procedure is less sensitive, we feel that this is not a practical problem in the investigation of suspected paracetamol overdose. In fact, the improved sensitivity which these authors obtain limits the linearity of the method to 1000 $\mu\text{mol/l}$, necessitating some modification to the procedure in order to measure higher levels, which are often encountered in overdosage.

With regard to specificity, we have found no interference by phenobarbitone in the Routh procedure provided that the extraction is performed as originally described, that is, from ether into sodium bicarbonate solution before adjusting the pH. If this is omitted and the ether is shaken with sodium hydroxide solution instead, then barbiturate is extracted and does interfere.

Phenylbutazone is a problem in both procedures, and scanning of spectra is essential to minimise the risk of reporting false plasma paracetamol values (Wiener *et al.*, 1976). Simply reading at one wavelength gives no indication of the presence of interfering compounds. Interference by phenylbutazone does not occur in the rapid colorimetric method described by Glynn and Kendal (1975), several modifications of which are now in use. In fact this method seems relatively free from the effects of other drugs with the exception of some sulphonamide preparations which could interfere at high concentration (Wiener, 1976).

K. WIENER and

M. G. LONGLANDS

Department of Clinical Biochemistry,
The North Manchester General Hospital,
Crumpsall, Manchester M8 6RB

References

- Dordoni, B., Willson, R. A., Thompson, R. P. H., and Williams, R. (1973). Reduction of absorption of paracetamol by activated charcoal and cholestyramine: a possible therapeutic measure. *British Medical Journal*, **3**, 86-87.

- Glynn, J. P. and Kendal, S. E. (1975). Paracetamol measurement (Letter). *Lancet*, **1**, 1147-1148.
- Routh, J. I., Shane, N. A., Arredondo, E. G., and Paul, W. D. (1968). Determination of N-acetyl-p-aminophenol in plasma. *Clinical Chemistry*, **14**, 882-889.
- Spooner, R. J., Reavey, P. C., and McIntosh, L. (1976). Rapid estimation of paracetamol in plasma (Letter). *Journal of Clinical Pathology*, **29**, 663.
- Wiener, K. (1976). Paracetamol estimation: Comparison of a quick colorimetric method with a standard spectrophotometric method. *Annals of Clinical Biochemistry*, **13** (In press).
- Wiener, K., Longlands, M. G., and Tan, B. H. A. (1976). Interference by oxyphenbutazone in paracetamol estimation. *Annals of Clinical Biochemistry*, **13**, 452-453.