Detection of cobalamin deficiency using the urinary methylmalonic acid test by gas chromatography mass spectrometry

In their recent paper Chanarin *et al*¹ state that urinary methylmalonic acid (MMA) concentrations are not an early sign for the detection of cobalamin (Cbl) deficiency. They base their opinion on a study that used gas chromatography as a means to quantify urinary MMA. Methodology using gas chromatography only lacks the specificity and sensitivity to accurately differentiate slightly increased concentrations of urinary MMA from normal amounts of urinary MMA.

Measurement of urinary MMA by gas chromatography mass spectrometry (GC/MS) is a highly sensitive and specific test for detecting Cbl deficiency.²⁻⁵ Norman *et al*² identified 54 consecutive inpatients with Cbl deficiency using the urinary MMA assay by GC/MS and 20% had a normal hematocrit at diagnosis. In a prospective clinical evaluation of the urinary MMA test by GC/MS Matchar *et al*³ studied inpatients with obvious Cbl deficiency and Cbl deficient patients. They determined the assay to have a sensitivity of 100% and a specificity of 99%. Specker *et al*⁴ used the urinary MMA assay by GC/MS to detect Cbl deficiency in a non-anaemic strict vegetarian population. More recently, the high sensitivity of the assay was demonstrated by identifying Cbl deficient non-anaemic persons over the age of 65 of whom 40% had serum Cbl concentrations in the normal range.⁵

False negative results have not been reported for the urinary MMA assay by GC/MS.²⁻⁵ It should be noted, however, that a "gold standard" test for Cbl deficiency does not exist because neither the Schilling test nor the serum total Cbl assay is a functional test. The serum MMA assay lacks specificity because the test can give falsely high values in patients with renal insufficiency or intravascular volume depletion. The urinary MMA test by GC/MS is normalised to urinary creatinine, and falsely high urinary MMA concentrations have not been reported in patients with renal insufficiency.⁵

Thus the urinary MMA test by GC/MS detects early Cbl deficiency, can routinely identify non-anaemic Cbl deficiency and is perhaps the "gold standard" for identifying true functional Cbl tissue deficiency.

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Acanthamoeba keratitis

The case report on *Acanthamoeba* keratitis¹ and subsequent response² require elaboration.

Acanthamoeba is a ubiquitous free-living protozoan which occurs in a wide range of environmental niches including domestic tap water. Inhalation of the organism or its penetration into open wounds can lead to development of granulomatous amoebic encephalitis, a chronic central nervous system infection with a prolonged clinical course which in some circumstances may prove fatal.³ *Acanthamoeba* has been cultured from the nasopharynx of normal, healthy subjects and there are a significant number of people with antibody against the organism.

Acanthamoeba keratitis was first recorded in 1974. A large number of cases have been reported subsequently, perhaps as a consequence of increased recognition of the condition. As was highlighted previously,¹ however, awareness of the role of these amoebae in clinical diagnosis and practice is limited. Earlier reports suggested an association between keratitis and trauma with a foreign body contaminated with *Acanthamoeba*. Currently, most presentations are from contact lens wearers, increasingly with so-called "disposable" contact lenses, where these have been immersed in inappropriate disinfecting systems.⁴ For example, commercially available chlorine systems will fail to kill *Acanthamoeba* cysts⁵ if the latter are present in the reusable contact lens storage case.⁶ The condition is more frequently detected in young immunocompetent persons who wear contact lenses for cosmetic purposes, and who have poor compliance with contact lens disinfection regimens. In one study, about 7% of contact lens cases contained viable cysts of *Acanthamoeba*.⁷ The incidence of *Acanthamoeba* keratitis in contact lens wearers in the USA is of the order of 1:250 000.⁸

Recognition of early ocular disease in contact lens wearers due to *Acanthamoeba* is all important. It initially presents as a punctate or dendriform epitheliopathy and may proceed to stromal invasion. *Acanthamoeba* keratitis is therefore most often misdiagnosed initially as herpes simplex keratitis and treated as such with antiviral agents and possibly corticosteroids. If such treatment fails the next diagnosis is often of fungal infection. Definitive diagnosis of *Acanthamoeba* keratitis from superficial corneal scrapings may prove inconclusive, because the organism can be present deeper within the stroma. In such circumstances corneal biopsy extending deeper into the stromal abscess is required; excised tissue should then be subjected to light and transmission electron microscope examination and cultured for the presence of viable amoebae.

For routine culture, a non-nutrient agar (1.5%), prepared at least 24 hours in advance, is seeded with heat-killed *Escherichia coli* or *Klebsiella aeruginosa*. Corneal scraping or tissue is gently spread across the central area of two separate plates, one to be incubated at 25°C and the other at 33°C. If the specimen is to be forwarded to a reference laboratory, it should be placed in sterile isotonic saline and kept at room temperature before despatch. Often clinical specimens which contain *Acanthamoeba*, and amoebae which have been subjected to topical chemotherapy or

corticosteroids, require supplements to promote growth and development of the amoebae in culture. If necessary, various biochemical or molecular biological methods can be used to provide unequivocal speciation and strain identification of the pathogenic *Acanthamoeba*.

Medical treatment with combined topical treatment comprising propamidine isethionate plus neomycin at an early stage can be successful.⁹ If unrecognised, the infection progresses to a ring abscess when medical treatment is often unsuccessful and a corneal graft, including on occasion, a second graft, is required. Anti-acanthamoebic drugs at this stage may result in emergence of temperature sensitive and drug resistant strains of the organism.¹⁰ Sensitivity testing of cultured clinical isolates must therefore be performed.

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Factors affecting the maintenance dose of warfarin

James *et al*¹ have confirmed the observations of others that elderly patients require less warfarin than younger patients to achieve the same intensity of anticoagulation.²⁻⁴ They suggest that the age dependency of dose should perhaps be taken into account in judging initial dose. We have already studied this question⁴ and have demonstrated that if a flexible induction dose regimen such as that of Fennerty *et al*⁵ is used elderly patients can safely start on the same initial dose as other patients. Should a fixed dosage schedule be used this might not necessarily be so.

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Book reviews

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Diagnostic Ultrastructure of Non-neoplastic Diseases. Ed JM Papadimitriou, DW Henderson, DV Spagnolo. (Pp 728; £200.00.) Churchill Livingstone. 1992. ISBN 0-443-03464-8

Electron microscopy is often regarded by those who are not well versed in the ultrastructural approach as the ultimate method of diagnosis on a tissue sample. It is therefore salutary to read in the introduction that useful ultrastructural information can be obtained in only 1-5% of samples received in a teaching hospital laboratory. The figure is even less if tumours are excluded. Why then do we need such a weighty (3.25 kg; 7.15 lb) tome? It is needed because it fills a gap between the general ultrastructure of the cell (Ghadyally) and the ultrastructural appearance of tumours (Henderson, Papadimitriou and Coleman), and is welcome for that reason.

The editors have gathered a variety of experts who have written concisely or extensively and have illustrated sparsely or profusely. As a consequence the coverage is uneven. For example, *in vitro* fertilisation has 40 references and 31 figures, while the eye has 426 references, eight tables, and only two figures, clearly inadequate for an atlas of ultrastructure. Other chapters are more even.

There are chapters on technique, scanning electron microscopy, general cell pathology, stromal pathology, viruses, infectious agents, parasites, tumour-like disorders, endomyocardial biopsies, respiratory disorders, digestive system (746 references; only 21 figures, liver, endocrine system, kidney, breast, CNS, PNS, muscle, bones, joints, lymphoid system (with light microscopy immunohistochemistry included in the text but not illustrated), and blood and bone marrow. I found the chapter on storage disorders disappointing because this is one area where electron microscopy is most helpful and uses many different tissues for diagnosis. The coverage could have been

more extensive. The chapter on skin is an invaluable source of reference.

The choice of sizes of illustrations is very variable and many pages are nearly half empty. The quality of the illustrations is generally good. References are up to 1990.

As a working atlas and text this book should be available to all those involved in diagnostic electron microscopy, especially if they read the introductory short chapter.

BD LAKE

Cases in Pathology. A Clinical Approach for Students. Nancy Standler and Bernard Klionsky. (Pp 242; £21.95.) Churchill Livingstone. 1991. ISBN 0-443-08759-8

This book consists of 37 clinicopathological case studies prepared for American medical students. Each is presented in "parallel text" form with an account of the patient in one column and discussion and clarification of important points in the parallel column. This section is then followed by some general text on the main disease in the patient and some references. The accounts of the cases include history (recent and previous), examination findings, and the results of investigations. Progress is recorded, sometimes to recovery but mainly to necropsy. Illustrations are mainly in colour and of histological sections, though there are some of macro-specimens and some radiology.

These mini CPCs are generally very good for their purpose, but it is easy to disagree with individual comments and interpretations. The aim of this collection is, however, not to give dogmatic teaching but to involve the students in clinicopathological thinking. Would I recommend this book to medical students in the UK? Yes, as additional reading late in the clinical course—or for post-graduates for professional examinations. The cases do require familiarity with clinical terms and clinical medicine. If it were used early in the clinical course there would need to be considerable input from a tutor (or much work with a medical dictionary). An additional problem for the European student is that haematological and chemical data are not given in SI units. It is the sort of book which ought to be very valuable but, with truncated pathology courses given early in the clinical years, it may find its niche in medicine rather pathology.

DR DAVIES

Lymph Node Biopsy Interpretation. Ed AG Stansfeld, AJ d'Ardenne. (Pp 536; 553 illustrations; £95.) Churchill Livingstone. 1992. ISBN 0-443-04072-9.

Since publication of the first edition in 1985 this book has become one of the standard reference texts for lymph node pathology, certainly in the United Kingdom. Quite apart from the quality of the writing, a major reason for this is that many pathologists are attracted to the terminology and general orderliness of the Kiel classification of malignant lymphomas. Much has happened over the past seven years, however, not least the updating of the Kiel system to accommodate most of the more recently delineated types of T cell neoplasia. Other notable changes include the introduction of more antibodies applicable to paraffin wax sections and the increasing contribution of molecular genetics to the unravelling of

lymphoid neoplasia. The two new chapters dealing with these developments are therefore appropriate as is the recognition of new entities such as monocytoid, angiotropic, and T cell rich B cell tumours. The demise of histiocytic neoplasia as a major entity is acknowledged as is the reciprocal emergence of the concept of anaplastic lymphoma. Within the realm of Hodgkin's disease new concepts regarding the lymphocyte predominance subtype and the subcategorisation of nodular sclerosis are well documented. As before the illustrations are of a generally high quality, and the only minor quibble one might have about the excellent descriptions of disease entities is that even at the risk of duplication they might have included more immunocytochemical data. This apart, however, one can see no reason why this second edition should not continue to occupy its pre-eminent place as one of the most useful and least dispensable reference books both in diagnostic and research laboratories.

FD LEE

Renal Involvement in Systemic Vasculitis Contributions to Nephrology. Vol 94. Ed A Sessa, M Meroni, G Battini. (Pp 201; 86.10.) Karger. 1991. ISBN 3-8055-5422-2

This monograph is a compilation of the proceedings of the first seminar on renal involvement in systemic vasculitis held in Vimercate, Italy, in September 1990. It comprises a series of chapters of variable length and quality, somewhat haphazardly arranged. It would be far better if the editors had tried harder to establish some sort of continuity. The book largely documents our current lack of understanding of the basis of systemic vasculitis with renal involvement.

Several papers deal with observations on the antibodies found in the circulation which react with anti-neutrophilic cytoplasmic antigens (ANCA) confirming that c ANCA antibodies tend to be found in Wegener's granulomatosis and p ANCA antibodies in microscopic polyarteritis nodosa, but there is no clear message as to whether they are primary in the cause of the vasculitis or merely an epiphenomenon which is, perhaps, more likely.

The aim of the text is said to be to identify more effective therapeutic schedules for patients with various forms of vasculitis and, given the relative lack of scientific clarity in this area, it is fortunate that the empirical regimens used continue to improve the prognosis for patients with the vasculitic syndromes.

If you want to read what we don't know about vasculitis this is the book for you.

From the pathologist's point of view *Systemic Vasculitis* edited by Andrew and Jack Churg and published by Igaku Shoin, New York, 1992 is, in my opinion, much better value for money.

DR TURNER

Gynecological Tumors. Recent Progress in Diagnostic Pathology. Ed N Sasano. Current Topics in Pathology. (Pp 378; 168 figs; 28 tabs; DM 275.00.) Springer. 1992. ISBN 3-540-52955-1.

This book aims to highlight recent advances in the diagnosis of gynaecological pathology.