Comment

This method provides a clear distinction between Congo-stainable amyloid and elastic fibres. We have stained elastic fibres in this way in dermis, breast, aorta, the gastrointestinal tract and in veins and arteries from many other organs.

Occasionally the distinction between amyloid and other connective-tissue constituents is not always clear using Congo or Sirius reds. This method helps in their separation, and its rapidity obviates the necessity for overnight staining which is a requirement for many of the elastic-fibre stains in routine diagnostic use. The elimination of lengthy elastic staining is especially advantageous in laboratories where elastic fibres in breast biopsies[6] are stained routinely. The procedure allows haematoxylin and eosin sections to be examined concurrently with elastic-stained preparations without delay. Unfortunately immunochemical demonstration of elastin in frozen[7] or fixed paraffin sections[8] are currently not adapted to such rapid processing.

The histochemical basis of this Congo blue stain is currently obscure. The dye Congo red is an indicator, turning blue in acid conditions. To our knowledge the only previous use of Congo red in acid solutions as a stain for elastic fibres was that described in 1925 by Matsuura.[9] He used phosphomolybdic acid in dilute alcohol instead of the hydrochloric acid-alcohol which we have described. His results were similar, although the distinction of elastic from amyloid was not investigated.

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Letters to the Editor

Formalised yolk sac antigen in early diagnosis of Legionnaires' disease caused by *Legionella pneumophila* serogroup 1

Taylor and Harrison[1] reported that because of the specificity of the formalised yolk sac antigen (FYSA) they were able to examine sera for the presence of antibody to *Legionella pneumophila* serogroup 1 by the indirect fluorescent antibody test (IFAT) starting at a dilution of 16 with only 3% of patients with illness other than Legionella infection showing detectable antibody and this only at a dilution of 16. They further noted that antibody detected at this level could be an early indication of Legionella infection. In view of their observation and since the submission of our report,[3] we have examined 24 sera from cases of Legionella infection taken early in the course of illness and which had been found to be negative at a dilution of 32 in the IFAT using heat-killed polyvalent antigen.[2] These sera were examined in the IFAT at a dilution of 16 using both the polyvalent (serogroups 1–4) heat-killed antigen and the FYSA supplied by the Division of Microbiological Reagents and Quality Control at Colindale. Four sera were found to be positive at a dilution of 16, in each case by both antigens. In view of this finding, we examined a further 500 sera on receipt with a serum dilution series starting at 16. To our surprise we found, as shown in the Table, that only 13 (2·7%) of the 483 sera from patients without a rising or high titre (≥ 256) of antibody to *L pneumophila* serogroups 1–4 reacted to a titre of 16–128. These results are similar to those noted by Dr Taylor and Mr Harrison, but differ from those obtained by us over an earlier period (1978–80) when the proportion of sera from patients with titres falling in the range 32–128 and not coming from known cases ("known cases" were those with a rising titre of antibody or demonstration of Legionella infection by examination of tissue by direct immunofluorescence) was 13%.[3] The reason for this finding is unknown as we have used the same batch of antigen since 1979.

We now screen all sera for the detection of antigen to *L pneumophila* serogroups 1–4 starting at a dilution of 16, in view of the sensitivity of the test and the predominance of *L pneumophila* of serogroup 1 as the cause of legionellosis. Although, as Taylor and Harrison note, infection with *L pneumophila* of serogroups other than 1 is rare in their experience, we have reported that six of 67 patients with serologically

Numbers of sera reacting in the indirect fluorescent antibody test with polyvalent antigen prepared from L pneumophila serogroups 1–4

Antibody titre					*Total*
< 16	*16*	*32*	*64*	*128*	
470	6	4*	2	1	483

*These sera came from two patients.

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proven Legionella infection due to serogroups other than 1 did not show a serological response to the serogroup 1 antigen. These cases could well have been missed if polyvalent pools of Legionella antigens other than sergroup 1 had not been used.

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Aluminium-induced dialysis eosteomalacia

We were interested to read the paper by Buchanan *et al* in your issue of December 1981 concerning the use of Aluminon (aurine tricarboxylic acid) to stain aluminium in the bone of haemodialysis patients.[1]

Over the past few years we have successfully applied this technique (together with other standard methods for staining aluminium such as solochrome azurine and naphthochrome green) to demonstrate aluminium in formalin or alcohol fixed undecalcified bone sections after embedding in our standard resin (Bondaglass, Polymaster 1209 AC).

The stains are positive in experimentally-induced peritoneal aluminium granulomata, in the bones of rats given aluminium chloride and in the bones of patients with chronic renal failure dialysed with water containing aluminium and seem to provide a reliable indication of the presence and localisation of aluminium in bone. Thus these patients and the experimental animals have raised serum aluminium concentrations, increased amounts of aluminium in bone as estimated by neutron activation analysis and recently it has been confirmed by microprobe analysis that the aluminium is located in the region of the mineralisation front[2,3] precisely where the positive staining reaction occurs.

In our experience the staining reaction appears mainly in the site of the mineralisation front as a narrow finely granular line at the interface between mineralised bone and osteoid and just spreading on to the osteoid itself corresponding to the position and appearance of a toluidine blue stained normal mineralisation front. In addition there is frequently a narrow line of positive staining at the bone surface not covered by osteoid or in cement lines. In some patients treated for years with a dialysate containing aluminium cement lines giving a positive reaction may be seen deep in bony trabeculae and in cortical bone. Less frequently the stain is positive in finely granular patches in osteoid where there are attempts at abnormal mineralisation. In the past we have noted this type of positive reaction in the osteoid and surrounding osteocyte lacunae particularly in the bones of patients with aluminium-induced osteomalacia who had been treated with 1α-hydroxy vitamin D3 and phosphate supplements before the cause of the osteomalacia was fully appreciated.

The locations described probably represent the true sites of accumulation of aluminium in bone. Incorporation of aluminium in the mineralisation front with subsequent interference with normal mineralisation would provide a convenient explanation for the development of osteomalacia in the dialysis patients and aluminium-treated experimental animals. It could be argued however that the aluminium is located elsewhere in the bone or marrow in life and diffuses during the processing of the bone to become secondarily "fixed" in the region of the mineralisation front. Studies of rapidly frozen fresh bone by microprobe analysis will be needed to resolve this problem.

In some haemodialysis patients initially without osteomalacia we have noted the accumulation of aluminium in bone and development of osteomalacia in serial iliac bone biopsies and any osteitis fibrosa initially present tends to resolve. However there are some patients with increased amounts of bone aluminium, as judged by the staining reaction and confirmed by neutron activation analysis, who do not develop osteomalacia. These patients tend to be amongst those with progressively severe hyperparathyroidism and osteitis fibrosa and interestingly we have observed that osteomalacia may develop in some of these patients following parathyroidectomy. It seems that severe degrees of hyperparathyroidism may provide some protection against the adverse effect of aluminium on bone mineralisation.

Since the importance of aluminium in inducing osteomalacia was appreciated the disease has been eliminated in most centres by using appropriate water treatment (deionisation, reverse osmosis). In Newcastle, which was formerly a centre with crippling dialysis osteomalacia, no new cases have occurred in recent years. We still see occasional referred examples from centres elsewhere and wish to stress the value of using these simple staining techniques when examining iliac bone biopsies from dialysis patients.

This type of osteomalacia does not heal following the use of the newer vitamin D metabolites such as 1α-hydroxy vitamin D3 or 1,25 dihydroxy vitamin D3 and troublesome hypercalcaemia with soft tissue calcification often ensues.[4] The aluminium stain provides a simple means of distinguishing this particular group of patients.

The main source for the aluminium is the water used for the preparation of the dialysate, particularly in those regions where aluminium is added to the water supply to flocculate colloidal suspensions. However increased serum aluminium and deposition of stainable aluminium in bone can result from the use of oral aluminium-containing phosphate binders even in predialysis patients with chronic renal failure. In addition we have studied bone biopsies from several patients who developed multiple fractures after treatment by a closed loop haemofiltration system (Redy cartridge) in which there has been osteomalacia and characteristic deposition of aluminium. In such instances the aluminium is released from the cartridge.

Dialysis encephalopathy is another complication of aluminium intoxication and may accompany dialysis osteomalacia. The chelating agent desferrioxamine has been used to treat this condition and with some success.[5] Recently we have observed healing of aluminium-induced dialysis osteomalacia in serial iliac bone biopsies from a patient treated with desferrioxamine and the amount of stainable aluminium in the bone was apparently reduced. With the exception of renal transplantation, it has been our experience that other forms of treatment are usually ineffective in this type of osteomalacia.

Finally, it is worth mentioning that the aurine tricarboxylic acid stain is reliable and reproducible provided one ignores false-positive reactions which may occasionally occur at the fragmented and fibrillated ends of traumatised trabeculae or cortical bone at the margins of the bone biopsy.

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