

chains in his urine 0.2 g/24 hours. Further chemotherapy was given and his peripheral blood stem cells were harvested with a view to autologous transplantation. As his paraprotein and Bence-Jones protein have since both disappeared with the VMCP regimen, high dose treatment has not been given.

Discussion

Median survival in myeloma is 36 months,¹ and although about 2% of patients survive longer than 10 years, most of these long survivors never had advanced disease.² IgD myeloma is rare, accounting for 1–2% of cases.³ It was first described in 1965⁴; the median survival is 13.7 months.³ It occurs in a younger age group than other types of myeloma and it has a male predominance.³ Our patient was unusual in that he did not have features typical of IgD myeloma, such as hepatomegaly, lymphadenopathy, extraosseous lesions, hypercalcaemia or renal failure. He also did not have Bence-Jones proteinuria, which is almost universal in IgD myeloma, until after his relapse. His prolonged survival after rapidly entering remission is also unusual as a rapid response to treatment is said to be a poor prognostic sign.⁵

The diagnostic criteria for myeloma usually include an assessment of numbers of plasma cells in the bone marrow aspirate.¹ However, in 48% of cases the aspirate will underestimate the degree of plasma cell infiltration.⁶

The disparity between the number of plasma cells in the aspirate and the trephine may reflect clumping of plasma cells. This disparity is worrying in view of the reliance many myeloma trials place on the number of plasma cells in the aspirate for diagnosis and trial entry. The simplicity of the immunohistochemical method and its value in this case suggest that it should be adopted more widely.

Finally, the prolonged survival of this patient emphasises that IgD myeloma need not always have a poor prognosis and argues well for more aggressive forms of treatment, currently under trial. The attainment of remission using a relatively non-aggressive protocol, as in this case, may prolong survival.

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Effect of collagenase on nerve fibre teasing

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Abstract

Teased fibre preparations are important in the investigation of peripheral nerve disorders, but can be a rather tedious procedure. A technique is described in which pretreatment of the nerve sample with collagenase before osmication substantially simplifies the actual teasing procedure. The technique also does not alter the morphology or interfere with any further investigations required on teased fibres—for example, fluorescent antibody studies.

Teasing of nerve fibres is perhaps the best way of studying individual nerve fibres. It permits measurements of internodal lengths of myelin sheaths and fibre diameter, which can be used to differentiate between segmental demyelination and axonal degeneration. Irregularities in the myelin sheath can also be visualised. Teasing of individual fibres can be very time consuming and considerable practice is required to separate long lengths of individual fibres without causing damage. These aspects prompted us to determine whether pretreatment with collagenase would make the procedure easier.

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Methods

Conventionally, 1–2 cm lengths of nerve are fixed in 2% buffered glutaraldehyde for two to four hours after which 1 mm in diameter fascicles are postfixed in 1% osmium tetroxide for two to four hours. These are then dehydrated in alcohol and propylene oxide as for electron microscopy and then soaked overnight in Araldite (from which the accelerator has been omitted), to soften the nerve and facilitate separation of individual fibres.¹

We modified this technique and used the following procedure. (1) Lengths of nerve (1–2cm) were fixed in 2% glutaraldehyde in 0.06M phosphate buffer for a minimum of one hour, after which they were washed in two changes of phosphate buffer for 10 minutes. (2) Nerve was then placed in colla-

nase type II (Sigma) 300 units/ml physiological saline at 37°C for one hour after which the nerve was washed in three changes of phosphate buffer for 15 minutes. (3) Nerve was then postfixed in 2% osmium tetroxide in 0.1M phosphate buffer for four hours, then washed in two changes of phosphate buffer for 30 minutes. It was then placed overnight in 66% glycerol in water at 4°C, after which it was stored in 100% glycerol at 4°C for teasing. This preparation can be kept at 4°C for 6 months or longer without any alterations in the tissue. (4) This preparation was then used to determine whether it was suitable for fluorescent antibody studies. For this, overnight incubation at 4°C was carried out using a primary antibody against the myelin sheath. After two washes in phosphate buffer for 15 minutes, incubation for 30 minutes with a fluorescein conjugated secondary antibody (Dako) was carried out.

Results

Collagenase did not interfere with the usual postfixation technique with osmium tetroxide, and lengths of individual nerve fibres could easily be separated without damaging the myelin sheath. It was possible to recognise pathological conditions like Wallerian degeneration (fig 1), demyelination, and remyelination with the remyelinated segment showing a thin myelin sheath (fig 2). It was also possible to recognise fluorescence staining along the myelin sheath by fluorescent antibody staining.

Discussion

The fundamental unit of collagen is the collagen molecule (tropocollagen), which is made up of three separate polypeptide chains wrapped together in a triple helix.² Collagenase cleaves this triple helix³ and causes digestion of the collagen. Each fibre when teased apart carries with it its own reticulin sheath (type IV collagen), with a variable proportion of the fibres immediately adjacent to larger diameter interstitial collagen (types I–III). Collagenase therefore digests the interstitial collagen and permits easy separation of individual nerve fibres. Interestingly, this digestion is specific and does not digest any other structural component of the nerve fibre. It is therefore useful in the investigation of peripheral nerve disorders.

The primary antibody against the myelin sheath was raised by staff at the Neurology Department, Institute of Neurological Sciences.

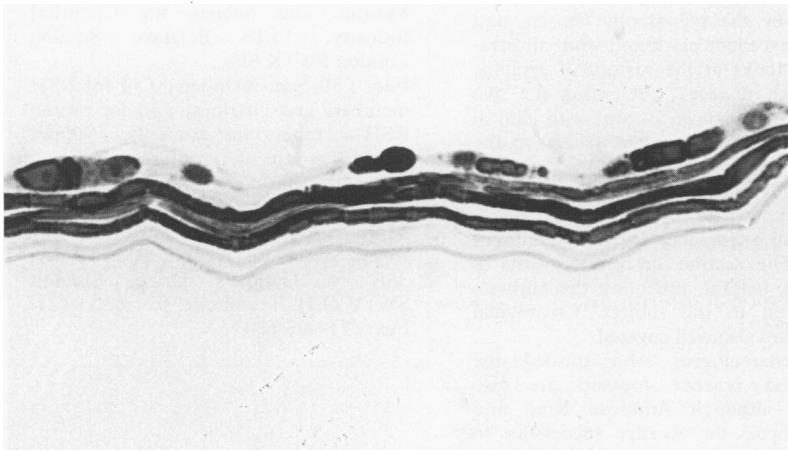


Figure 1 Osmicated teased peripheral nerve fibres pretreated with type II collagenase showing fragmentation of myelin indicating Wallerian degeneration.

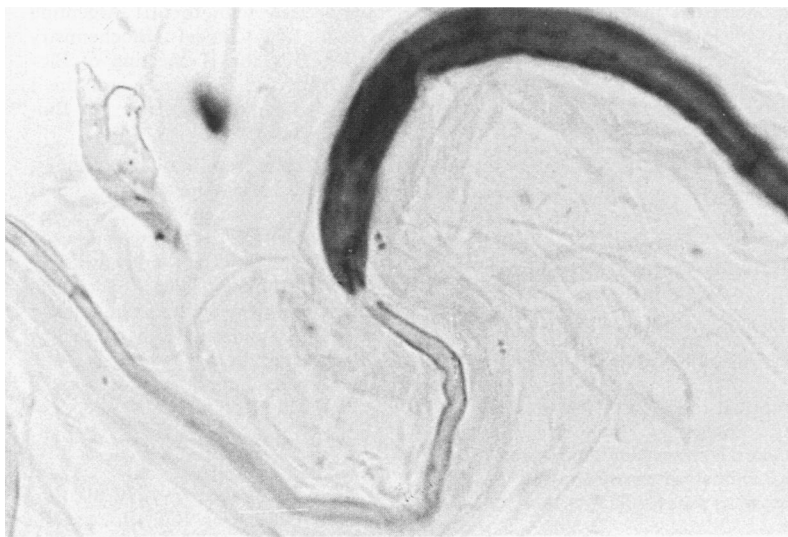


Figure 2 Single osmicated teased fibre pretreated with type II collagenase showing demyelination and subsequent remyelination of a segment. Remyelination is indicated by the thin myelin sheath.

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