the duodenum, "neuromuscular and vascular hamartomas", and gangliocytic paragangliomas may be associated with many of the former lesions that are undoubtedly associated with mucosal damage such as that seen in Crohn's disease.¹⁰ Furthermore, the occurrence of areas of neuromuscular hyperplasia, which may include ganglion cells, is well recognised in Crohn's disease. The histology of the tumours presented shows well circumscribed lesions with no adjacent mucosal abnormality. Case 1 seems to have arisen in the 12 month period between sigmoidoscopic examinations and may be directly related to the site of previous surgery. Although a mucosal lesion may seem to have healed, the chronic inflammatory reaction within the tumour may be evidence of previous surgery.

The identification of a gastrointestinal ganglioneuroma necessitates the clinical and histological consideration of further lesions or associated pathology. Indeed, in several patients ganglioneuromatous lesions of the gut have been the presenting features of multiple endocrine neoplasia syndrome type 2b.

Addendum

Since the preparation of this paper the

occurrence of diffuse neuronal hyperplasia, in some cases including ganglion cells, has been highlighted in von Recklinghausen's disease by C E Fuller and G T Williams: Gastrointestinal manifestations of type 1 neurofibromatosis (von Recklinghausen's disease). *Histopathology* 1991;19:1-11.

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Rapid differentiation of *Mycobacterium xenopi* from mycobacteria of the *Mycobacterium avium-intracellulare* complex by pyrolysis mass spectrometry

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Abstract

Thirty four cultures of slow growing, Tween-80 negative mycobacteria were analysed by pyrolysis mass spectrometry. The results showed that pyrolysis mass spectrometry could positively distinguish strains of *Mycobacterium xenopi* from those of the *Mycobacterium avium-intracellulare* (MAI) complex. Pyrolysis mass spectrometry may be a useful technique for the rapid characterisation of non-tuberculous mycobacteria in such clinical settings as their isolation from immunocompromised patients—for example, those with AIDS.

Pyrolysis mass spectrometry is a rapid and simple technique for comparisons of strains of micro-organisms that has been successfully

applied to a wide range of bacterial species.¹ Pyrolysis mass spectra vary with the age and cultural conditions of the organisms before pyrolysis² so that pyrolysis mass spectrometry cannot assign permanent type designations. However, if organisms are prepared under identical conditions and then examined by pyrolysis mass spectrometry within a single machine batch, the system is highly discriminatory. Mycobacteria form a highly disparate group of organisms with a wide range of different growth cycles and optimal growth temperatures. Consequently, pyrolysis mass spectrometry is only likely to be useful when analysing within groups of mycobacteria of closely similar growth pattern. We recently adopted this approach in analysing members of the *M tuberculosis* complex. Pyrolysis mass spectrometry was able to distinguish *M tuberculosis* from *M bovis*, and also showed the close

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similarity between *M bovis* and *M africanum*.³

An emerging clinical problem is the differentiation of mycobacteria isolated from immunocompromised patients, particularly those with AIDS. In most cases the organism is likely to belong to the *Mycobacterium avium-intracellulare* (MAI) complex, but recent reports suggest that other species such as *M xenopi* can also infect these patients.^{4,5} An ability to distinguish rapidly *M xenopi* from organisms of the MAI complex may become important in epidemiology and patient management.

Conventional differentiation relies on relative sensitivity to isoniazid, growth on tellurite containing media and thermophilicity. All these tests require subculture, take some time to complete, and are not always definitive. Detection of 2-Docosanol has been suggested as a rapid positive test for the identification of *M xenopi*, but requires access to gas chromatography.⁶

On primary isolation both MAI complex and *M xenopi* appear as slow-growing, Tween-80 negative mycobacteria. We have done a preliminary study to assess the potential of pyrolysis mass spectrometry to distinguish rapidly and positively *M xenopi* from organisms of the MAI complex in this context.

Methods

Thirteen clinical isolates of *M xenopi* (designated xen 1-xen 13), all of which had been

characterised by conventional tests, were cultured in duplicate on Middlebrook 7H10 agar slopes (Difco Laboratories), together with a type strain of *M xenopi* (NCTC 10042). All of the clinical isolates were obtained from different patients. Similar cultures, also in duplicate, were set up for one type strain (NCTC 8559), two reference strains (NCTC 8552 and 8562) of *M avium*, and one type and one reference strain of *M intracellulare* (NCTC 10682 and 10425). These cultures were incubated together with 15 clinical isolates of *M avium-intracellulare*, six from AIDS sources and nine from non-AIDS sources (designated AI 1-AI 15), at 35°C for nine days, at which time a confluent growth was present on all the slopes.

Material from each subculture was smeared on to Ni-Fe pyrolysis foils (Horizon Instruments Ltd, Heathfield, Sussex) in triplicate. The foils were inserted into pyrolysis tubes (Horizon Instruments) and heated at 80°C in a hot air oven for five minutes. The sample tubes were then processed on a Horizon Instruments PYMS 200X pyrolysis mass spectrometer. Each tube was pyrolysed for four seconds at a Curie point of 530°C and the ion counts were collected on to floppy disk and stored as previously described.⁷

After normalisation of the ion counts, discriminant analysis was performed as previously described.⁷ The triplicate spectra from both subcultures of each isolate were labelled as distinct groups and analysed for intergroup

Figure Dendrogram showing percentage similarities between 14 isolates of *Mycobacterium xenopi* (13 clinical isolates and one type strain) and 20 isolates of the *Mycobacterium avium-intracellulare* complex (15 clinical isolates, three reference strains, and two type strains). The clustering method was UPGMA.

variation. Principal component and canonical variate analyses were then performed using the 30 mass ion peaks showing the greatest discrimination between groups, resulting in a table of Mahalanobis distances. These data were then used in a UPGMA analysis to produce a dendrogram.

Results

The dendrogram showed the relatedness of all 34 isolates of mycobacteria (figure). The 13 clinical isolates of *M. xenopi* closely resembled each other, 12 of the 13 clinical isolates showing 88% similarity, and all 13 being encompassed by 79% similarity. The most dissimilar isolate was the reference strain (NCTC 10042), which is included at 74% similarity.

None the less all the *M. xenopi* isolates were clearly distinguished from the MAI clinical and reference isolates, these two groups only being related at 68% similarity. There was more heterogeneity within the MAI isolates than that seen within the *M. xenopi* isolates, but all of the clinical isolates and reference strains were included within a 77% similarity.

Interestingly, the type strain of *M. avium* (NCTC 8559) was as different by pyrolysis mass spectrometry analysis from the wild and reference isolates of MAI as from the *M. xenopi* isolates. NCTC 8559 was linked to the whole of the remaining isolates, whether MAI or *M. xenopi*, at 41% similarity.

Discussion

This preliminary study suggests that pyrolysis mass spectrometry can clearly distinguish isolates of *M. xenopi* from organisms of the MAI complex. The distinction is a positive one, the *M. xenopi* isolates being closely related one to another and distinct from the MAI isolates. In taxonomic terms *M. xenopi* is seen to be a "good species". It is likely, therefore, that pyrolysis mass spectrometry analysis of a collection of slow growing, Tween-80 negative mycobacteria will permit a rapid positive characterisation of *M. xenopi*. Isolates excluded from the *M. xenopi* cluster can be characterised as "not *M. xenopi*" and therefore likely to belong to the MAI complex, but not positively identified as *M. avium* or *M. intracellulare*.

Pyrolysis mass spectrometry is a phenotypic comparative technique based on analysis of total cell content. Although results are valid and reproducible within machine batches, it is essential that the isolates to be examined are prepared under identical cultural conditions before pyrolysis. Such factors may have a

bearing on the apparent discrepant result obtained with NCTC 8559, the type (not reference) strain of *M. avium*. Type strains are likely to differ in phenotype from fresh clinical isolates as a result of prolonged serial subculture. We noted a similar phenomenon in relation to the H37 RV strain of *M. tuberculosis* in a pyrolysis mass spectrometry study of the *M. tuberculosis* complex.³ When using pyrolysis mass spectrometry for rapid distinction between closely related groups of mycobacteria, it will be important to use both reference strains and well characterised recent clinical isolates in the comparison.

It is interesting to note the results of a recent study using capillary gas-liquid chromatography analysis of fatty acid, secondary alcohol, and mycolic acid content of mycobacteria in which characteristic results permitted direct positive identification of *M. xenopi* and *M. malmoense*; MAI complex organisms gave much more variable patterns.⁸ The differences in the pyrolysis mass spectrometry spectral data may be related to the presence or absence of these or similar compounds.

As only a few colonies of each organism are required for pyrolysis mass spectrometry analysis it can be performed on primary isolates of slow growing, Tween-80 negative mycobacteria, provided that appropriately grown cultures of *M. avium*, *M. intracellulare*, and *M. xenopi* are available for comparative purposes. Pyrolysis mass spectrometry is simple, relatively cheap, requires no special expertise in sample processing and provides definitive results on the same day.

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