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Assaying dapsone in mouse diets

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Summary A simple colorimetric method is described for checking that dapsone-containing diets have been correctly prepared for mouse foot-pad evaluation of the dapsone sensitivity of strains of *Mycobacterium leprae*.

Introduction

The mouse foot-pad technique was first used by Pettit and Rees in 1964 to demonstrate that lepromatous patients could relapse after many years of sulphone treatment due to the emergence of dapsone-resistant strains of *Mycobacterium leprae*.[1] Numerous other strains of *M. leprae* with acquired dapsone resistance have since been isolated from relapsed lepromatous patients from many different parts of the world.[2–9] Furthermore, strains have recently been isolated with primary dapsone resistance from previously untreated patients in an area where acquired dapsone resistance is now common.[10] In order to estimate the sensitivity of strains of *M. leprae* to dapsone, bacilli are inoculated into mice fed with normal diet and to others given dapsone at concentrations of 0.0001%, 0.001% and 0.01% in the diet. Multiplication of *M. leprae* in both the control mice and in those fed with dapsone-containing diets indicates that the leprosy bacilli are dapsone resistant, since strains from previously untreated patients were inhibited by feeding 0.0001% dapsone in the diet in the original investigations when dapsone-resistant leprosy was very rare and primary resistant strains had not yet been isolated.[3] In view of the serious threat to hopes of controlling leprosy with chemotherapy based on dapsone-containing regimes posed by the spread of dapsone-resistant strains of *M. leprae*,[9] studies of the prevalence of primary and acquired dapsone resistance are currently being undertaken in a number of countries. As a consequence we have recently had requests from several newly-established mouse foot-pad laboratories for help in assaying their dapsone-containing mouse diets to check that these had

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been correctly prepared and that mixing of the drug in the powdered diet was uniform. We therefore believe that a description of the simple colorimetric method that we have used over the past 6 years to assay mouse diets might be of value to other workers contemplating such estimations. This method, which is based on previous adaptations[11, 12] of the Bratton and Marshall procedure,[13] is somewhat simpler than that described by Levy and Peters.[14]

Method

Two gram amounts of powdered diet are extracted by shaking for 1 minute with 20 ml ethyl acetate in a stoppered centrifuge tube on a vortex mixer. After centrifugation and filtration, 10 ml of the ethyl acetate extract is then extracted by shaking with 2 ml 2N HCl. Prior to extraction with HCl, ethyl acetate extracts from the diets containing the highest concentrations of dapsone are first diluted with ethyl acetate to give a calculated concentration of about 1 μg/ml dapsone (e.g. 10-fold for diets supposedly containing 0.01% dapsone). Aliquots of the 2N HCl extracts (1 ml) are then diluted with an equal volume of ethanol and reacted by the successive addition at 5-minute intervals of 2 drops (0.01 ml) of freshly prepared 1% (w/v) aqueous sodium nitrite, 10% (w/v) aqueous ammonium sulphamate and 1% (w/v) N-l-naphthyl-ethylene-diamine-dihydrochloride in acetone/water (1 : 1 by volume). Fifteen minutes later the extinction of the reaction product is measured at 570 nm and the concentration of dapsone in the mouse diet calculated by comparison with the results obtained by extracting 10 ml ethyl acetate containing 1 μg/ml dapsone with 2N HCl and reacting in the same way. Results should be corrected for any Bratton–Marshall positive compounds extracted from normal unmedicated diet. The amounts of such compounds present in the powdered 41B diet used in the studies of Rees and his colleagues were extremely small and equivalent to less than 0.00002% dapsone.

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