SPECIAL ARTICLE

Tracing *Salmonella typhimurium* infections

Since the low point of 1977 the incidence of infection attributed to *Salmonella typhimurium* in England and Wales has shown a steady increase with a total of 4956 cases reported in 1982 (OPCS, 1985). Although the number of general outbreaks and family outbreaks increased by 24 % and 54 % respectively between 1981 and 1982, the greatest increase (55 %) was in the number of sporadic cases reported (PHLS, 1984). The cause of the recent increase in human infection is not known but may be associated with bovine infection and an explanation for the rise in sporadic cases could be that they comprise unrecognized outbreaks due to common food products distributed over wide geographical areas for long periods of time because of deep freezing (Galbraith, 1985). This hypothesis has prompted the Communicable Disease Surveillance Centre to mount a case-control study comparing sporadic cases of *S. typhimurium* infection with an unaffected control group in several parts of the country to find the vehicles of infection. Such an approach requires precise strain identification and detailed epidemiological information.

The serotype *S. typhimurium* is an ancient one which has had time to diversify in chromosomally determined characters by mutation and in extra-chromosomally determined ones by the acquisition of phages and plasmids. Several methods based on these different approaches have been developed for typing cultures of this serotype. An indication of the likely source of infection and its spread can be reached by the use of a single typing method and for this purpose the phage typing scheme as developed by Anderson *et al.* (1977*a*) and used internationally has proved invaluable. The recognition of over 200 phage types allows considerable type discrimination and is useful in the initial screening of a large number of isolates. When those isolates come from an outbreak circumscribed in time and place and are found to be of the same phage type it is likely that they originated from a common source (Anderson, 1971). However, due to the continuing process of type conversion by a change in lysogeny, by gain or loss of a plasmid or by chromosomal mutation (Threlfall *et al.* 1980), dependence on a single typing method may prove misleading when searching for a common source for isolates retrieved from sporadic and widely dispersed cases of infection.

More recently, plasmid profile analysis has been used to identify related isolates from *S. typhimurium* outbreaks (Bezanson, Khakhria & Lacroix, 1982). Plasmid analysis is rapid and reproducible and, in a comparison with phage typing and antimicrobial sensitivity testing of *S. typhimurium* isolates with adequate epidemiological data, proved as specific as phage typing in identifying epidemiologically related isolates as being the same or differentiating them from control specimens (Holmberg *et al.* 1984).

For multiple typing of *S. typhimurium* another method based on 15 biochemical characters has been developed (Duguid *et al.* 1975). In this two-tier scheme 32

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potential primary biotypes were recognized by the possible combinations of positive and negative reactions in the five most discriminating tests using the substrates D-xylose, *meso*-inositol, L-rhamnose, *dextro* and *meso*-tartrates and subtypes within these primary types were defined by the reactions in ten additional biotyping tests. Full biotypes were designated by primary biotype numbers with appended letters to indicate results in the secondary tests. The typing characters were found to be stable in stored cultures and reproducible in repeated tests on the same strain. Transduction studies, reviewed by Old (1984), have demonstrated relationships between strains and allowed construction of a phylogenetic tree postulating a strain of biotype 1a (i.e. one in which all genes for all 15 biotype characters were unmutated) as the archetypal strain. Reference to this tree shows which biotypes differ in one character and are liable to interconversion and which are separated by mutations in two or more characters and are therefore unlikely to arise at a single step.

Three collections of biotyped strains of *S. typhimurium* included representatives of 22 primary biotypes and 184 full biotypes. They came from 2092 cultures collected world-wide and at random from human, animal and other sources between 1920 and 1975 (Anderson *et al.* 1978); from 2010 cultures isolated in medical and veterinary laboratories throughout Scotland between 1974 and 1976 (Barker, Old & Sharp, 1980) and from 175 cultures isolated from animals and birds in northern Japan between 1970 and 1978 (Ishiguro & Sato, 1981). In a fourth study biotyping, using the phenotypic markers of the Duguid scheme and characterization of the R plasmids carried by strains of *S. typhimurium* phage type 10, allowed Khakhria *et al.* (1983) to implicate poultry as the source of human infection on a farm outbreak.

Two collections were phage-typed strains. That of Anderson *et al.* (1978) differentiated the 1937 cultures belonging to one of 204 definitive phage types and 142 full biotypes into 574 different phage type/biotypes. That of Barker, Old & Sharp (1980) differentiated 2010 cultures into 137 different phage type/biotype groups. In this series of Scottish isolates strains in 20 of the 45 full biotypes were subdivided by phage typing. More importantly when surveillance depends on phage typing as the single typing method, 21 of the 58 phage types that were definitive or related contained strains that differed in at least one primary biotype character and in 15 of these phage types the biotypes of the strains were distantly related in phylogenetic terms, i.e. it is unlikely that they would have arisen by a single-step mutation during spread from a common source. This Scottish series included strains belonging to 9 of the 10 phage types that were found to be the commonest in England and Wales in 1982 (PHLS, 1984). Phage types 18 (4 strains of biotype 1a), 104 (55 strains of biotype 2a), 170 (26 strains of biotype 3a), 49a (13 strains of biotype 26a) and 204 (56 strains of biotype 26a) were homogeneous in biotype. Phage type 49 contained 247 strains of biotype 26a, one of biotype 26f and 7 of biotype 26i. These strains of subtypes 26f and 26i probably represented biotype mutant lines that had arisen from strains of biotype 26a. Phage type 10 (4 strains) was subdivided into 2 biotypes, phage 12 (4 strains) was subdivided into 4 biotypes and phage type 193 (46 strains) into 8 subtypes of 6 primary biotypes.

Analysis of the findings from phage typing and biotyping shows that strains in several phage types can be subdivided by biotype. This probably reflects the

acquisition of the same phage type-determining character by different biochemical lines of the serotype and is the most probable explanation for the diversity of biotypes found in strains of phage types 10, 12 and 193. Another well-documented instance of strains of one phage type, PT 141, being subdivided into three biotypes, 1f, 9f and 31bd, has been reported (Barker & Old, 1979). Alternatively, in some instances strains of one biotype have diversified in phage type by the acquisition of different phage and plasmid type-determining characters. Characterization of the extra-chromosomal elements carried by representative strains has demonstrated interconversion among some phage types, e.g. PTs 49, 204 and 193 (Threlfall, Ward & Rowe, 1978; Willshaw *et al.* 1980); PTs 135, 170, 208 and Untypable (Anderson *et al.* 1977*b*); PTs 3, 10, 66 and Untypable (Frost *et al.* 1982). Other phage type interconversions have been suggested from combined phage type–biotype studies, e.g. PTs 8, 9 and 64; 29 and 44; 14 and RDNC (Anderson *et al.* 1978); PTs 42 and 147; 82 and RDNC (Barker, Old & Sharp, 1980); PTs 141 and 193; 56 and 193 (Barker & Old, 1980) and from transduction studies, e.g. PTs 9 and 61; phage types found in association with FIRN strains (Old & Duguid, 1979); PTs 6, 16, 46, 49, 73, 76 and 135 (Barker & Yousuf, 1985).

For the purpose of surveillance of epidemic strains and their possible interconversions they should be typed by more than one method. This is true not only of strains without plasmids and those which are phage untypable, as suggested by Holmberg *et al.* (1984), but also of strains belonging to recognized phage types. The D-xylose, inositol, rhamnose and *meso*-tartrate characters in the primary scheme of Duguid *et al.* (1975) and most of the secondary characters are known to be chromosomally determined (Sanderson & Roth, 1983) and have been found to be stable in stored cultures and during epidemic spread (Duguid *et al.* 1975; Anderson *et al.* 1978; Barker & Old, 1979; Barker, Old & Sharp, 1980). All strains are typable and can be assigned to one of the 32 primary biotypes. The tests are reproducible and easy to perform (Duguid *et al.* 1975). For these reasons, biotyping should be considered as a second method ancillary to either phage typing or plasmid analysis to determine the relatedness or unrelatedness of isolates. In the longer term, monitoring by multiple typing will give an indication of the degree of interconversion, both in phage type by changes in lysogeny and plasmid content and in biotype by mutation, that is continually taking place in naturally occurring strains and should reveal which lines eventually die out and which become successfully established. When more is known of the properties that contribute to persistence we may be in a better position to predict the characters that determine virulence of *S. typhimurium*.

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Department of Medical Microbiology,
University of Dundee Medical School,
Ninewells Hospital,
*Dundee DD*1 9*SY*

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