Types of *Salmonella paratyphi B* and their phylogenetic significance

RUTH M. BARKER, GABRIELLE M. KEARNEY, P. NICHOLSON,
ANDREA L. BLAIR, R. C. PORTER and PAMELA B. CRICHTON

Department of Medical Microbiology, University of Dundee Medical School, Ninewells Hospital, Dundee DD1 9SY

Summary. The substrates inositol, rhamnose, *d*-tartrate and *m*-tartrate used in fermentation tests with 338 cultures of *Salmonella paratyphi B* differentiated strains in some phage types to give information that could be used in epidemiological investigations. Xylose in Bitter's medium, the fifth substrate by which 13 of a potential 32 biotypes were identified, differentiated few cultures with the negative character. The possession of a specific type of outer-membrane protein receptor for colicin M or bacteriophage ES18 and the particular type of ribosomal ribonucleic acid present, defined three groups among the phage-typed and biotyped cultures. The possibility that the serotype *S. paratyphi B* contains clones of different phylogenetic origin and the consequent implications for nomenclature are discussed.

Introduction

Salmonellae within a biochemically defined subgenus are, at present, divided into serotypes on the basis of a particular association of O- and H-antigenic factors and nomenclature is based on this serological definition. Although it is now accepted that serotypes of *Salmonella* do not have the taxonomic rank of species and, therefore, that their serotype names should not be printed in italics (Le Minor *et al.*, 1985), the use of italicised names remains the convention for clinical and epidemiological purposes (Farmer *et al.*, 1985). For bacteria of serotype 1,4,5,12:b:1,2, the name *S. schottmuel-leri* has precedence and is the one given in the latest edition of Bergey's Manual of Systematic Bacteriology (Brenner, 1984; Le Minor, 1984); nonetheless, the name *S. paratyphi B* is the one recognised by clinicians and, hence, is likely to remain in common use.

The taxonomic status of *d*-tartrate-positive strains of this serotype, designated *S. java* by Kauffmann (1955), and their relationship to *d*-tartrate-negative strains of *S. paratyphi B* that have the same somatic and flagellar antigens has been something of an enigma since Kristensen and Kauffmann (1937) noted a difference in clinical manifestation. In man, *d*-tartrate-negative strains were considered to produce a typhoid-like illness

that was generally more severe than the gastroenteric type of infection produced by *d*-tartrate-positive strains. Pathogenicity, however, is not usually considered a reliable criterion for taxonomic purposes and biotype variants with an antigenic formula identical to that of an established serotype have not been given different names (Le Minor *et al.*, 1982).

Phage typing is the recognised method of differentiating strains of *S. paratyphi B* for epidemiological investigations (Anderson, 1964). The existence of biochemical variants, other than those distinguished by *d*-tartrate, has been known since Kristensen and Bojlén (1929) examined the action of 1021 strains on 13 substrates. Strains of some phage types were differentiated by biotyping (Brandis and Thomsen, 1956), as were phage-typed strains of *S. typhimurium* (Anderson *et al.*, 1978; Barker, 1986). Because the biotyping scheme developed for *S. typhimurium* (Duguid *et al.*, 1975) incorporated several of the substrates known to differentiate strains of *S. paratyphi B*, it seemed an appropriate method by which to type a collection of strains belonging to the group comprising *S. paratyphi B* and *S. java*.

Differences in several characters observed in strains of *S. paratyphi B* and *S. java* have suggested that there may be considerable genetic heterogeneity within this serotype. In *Escherichia coli*, the gene *tonA* codes for an outer-membrane protein determining sensitivity to colicin M and phage T5 and

in *S. typhimurium* the allelic gene *sidK* codes for the receptor for phage ES18 (Braun *et al.*, 1977). Graham and Stocker (1977) found that the majority of *S. paratyphi B* strains examined were similar to *E. coli* in being sensitive to colicin M but not to phage ES18 whereas one exceptional strain of *S. java* gave the alternative pattern characteristic of *S. typhimurium*, i.e., sensitive to phage ES18 and resistant to colicin M. A second variable character, based on the observation by Winkler (1979) that *S. typhimurium* strains differed from those of *E. coli* in lacking intact 23S ribosomal ribonucleic acid (rRNA), provided further indication of heterogeneity. In an extended investigation of Enterobacteriaceae, Smith *et al.* (1988) found that strains of most serotypes of *Salmonella* possessed a "coli" type of rRNA pattern; the rRNA of some strains of *S. paratyphi B* was of the "coli" type and that of others was of the "typhimurium" type. We have determined the sensitivity type of 338 strains and the rRNA type of 77 strains of *S. paratyphi B* and correlated the findings with the biotype reactions of each strain.

Materials and methods

Bacterial strains and bacteriophage

The 338 cultures of *S. paratyphi B* included representatives from eight collections and had been isolated in the United Kingdom (150 cultures, including 92 in Scotland), in seven countries in Europe (134, including 88 in France), in South America (7), the Middle East (7), Africa (5), Australia (3) and India (1) or in an unspecified country (31). Seventy-three strains were isolated before 1965 and the remaining 265 strains between 1974 and 1983. They were obtained from man (87 from faeces, 16 from blood, 11 from other body fluids and 96 from an unspecified source), from birds (5), a reptile (1), sewage (50), water (39), food (21) and an unspecified source (12).

A further 54 sequential cultures came from a long-term carrier resident in Dundee who had excreted *S. paratyphi B* of phage type 1 since 1956 (Jamieson *et al.*, 1968). The cultures were isolated from faeces (52) and urine (2) between 1978 and 1985.

Cultures had been phage-typed in the country of origin and information about each culture was supplied by the donor. Eleven of the strains that could not be typed with the *S. paratyphi B* set of phages were monophasic phase 1 (Dr L. Le Minor, personal communication).

E. coli strain K12 W3110 PAP 1370 pColM (from Dr A. P. Pugsley), *E. coli* strain K12 W3110 BZB2102 pColB-K260 (Pugsley, 1985) and *S. typhimurium* strain S3399 (Barker, 1980) produced, respectively, colicin M, colicin B, and colicins B and M.

Bacteriophage ES18, supplied by Dr L. Le Minor, was propagated on *S. typhimurium* strain S375 and stored at

$c. 1 \times 10^{10}$ plaque-forming units (pfu)/ml in Nutrient Broth No. 2 (Oxoid).

Biotyping

Stock cultures were maintained on Dorset's egg slopes at ambient temperature. Each was plated on DCA (Oxoid) incubated overnight at 37°C and single colonies were picked for biotyping.

Each culture was assigned a primary biotype number (1–32) according to reactions in five primary tests with the substrates D-xylose, *meso*-inositol, L-rhamnose, *d*-tartrate and *meso*-tartrate according to the criteria of Duguid *et al.* (1975). Utilisation of *d*-tartrate was assessed by the turbidity test of Alfredsson *et al.* (1972), the lead-acetate test terminated at 48 h and the anaerobic plate test (Barker, 1985). Subtypes (designated by the letters a–j, x, y and z) within primary biotypes were defined by reactions in ten secondary tests (see Duguid *et al.*, 1975).

Colicin production

Each strain was examined on Nutrient Agar (Oxoid) by the overlay method (Ozeki *et al.*, 1962) with the colicin-sensitive strains of *E. coli* K12 14R519 (Anderson, 1975) or CL104 (Ozeki *et al.*, 1962) as an indicator of colicin production.

Sensitivity to colicin M

Cultures were examined by the overlay method (Ozeki *et al.*, 1962) on colicin-producing strains grown as spot inocula on plates of nutrient agar. A zone of inhibition of growth of the test culture over the producing colony indicated sensitivity. Some cultures were tested by the cross-streak method on nutrient agar containing mitomycin C 0.1 µg/ml (Pugsley, 1985).

Sensitivity to bacteriophage ES18

A drop of phage lysate ($c. 10^8$ pfu) was spotted on to a lawn of a 4-h broth culture of the test strain spread on the surface of a plate of nutrient agar. After overnight incubation, cultures were examined for confluent or semi-confluent lysis.

Determination of RNA type

Ribosomal RNA was extracted and analysed by electrophoresis in denaturing formaldehyde-agarose gels as described by Smith *et al.* (1988).

Results

Phage types

The series included 280 strains belonging to the ten phage types recognised by Felix and Callow

(1951) (1, 2, 3a, 3aI, 3b, Jersey, Beccles, Taunton, BAOR and Dundee), to subtype variants of types 1, 2, 3a, 3aI, 3b, Beccles, Taunton and Dundee and to the four additional types Stirling, Scarborough,

Battersea and Worksep (Anderson, 1964). In all, 37 phage types were represented (table I). Of the further 58 strains, nine reacted with the typing phages but gave patterns not conforming with a

Table I. Phage types and biotype reactions of 338 cultures of *S. paratyphi B*

		Number of cultures of primary biotype												Total	
Phage type	Substrate*	1	2	9	13	10	12	26	3	11	19	23	27	7	
	B Xyl	+	+	+	+	+	+	—	+	+	—	—	—	+	
	Inl	+	+	—	—	—	—	—	+	—	+	+	—	+	
	Rha	+	+	+	—	+	+	+	+	+	+	—	+	—	
	d-t	+	+	+	+	+	—	+	—	—	—	—	—	—	
	m-t	+	—	+	+	—	—	—	+	+	+	+	+	+	
Dundee		7	1	0	0	0	0	0	16	22	1	0	0	0	47
Dundee var. 1		4	0	0	0	0	0	0	2	0	0	0	0	1	7
1		0	2	0	0	0	0	0	7	0	0	0	0	13	22
1 var. 1		1	0	0	0	0	0	0	0	0	0	0	0	2	3
1 var. 4		0	12	0	0	0	0	0	1	0	0	0	0	0	13
Jersey		0	1	0	0	0	0	0	0	0	0	1	0	0	2
Battersea		0	0	0	6	0	0	0	1	2	0	0	0	0	9
Worksep		0	0	0	6	0	0	0	0	0	0	0	0	0	6
1 var. 3		11	6	1	0	0	0	0	0	0	0	0	0	0	18
1 var. 8		0	1	0	0	0	0	0	0	0	0	0	0	0	1
1 var. 9		1	0	0	0	0	0	0	0	0	0	0	0	0	1
3b var. 3		2	0	0	0	0	0	0	0	0	0	0	0	0	2
3b var. 9		2	0	0	0	0	0	0	0	0	0	0	0	0	2
Beccles var. 1		2	0	0	0	0	0	0	0	0	0	0	0	0	2
Beccles var. 4		4	0	0	0	0	0	0	0	0	0	0	0	0	4
Beccles var. 5		3	0	0	0	0	0	0	0	0	0	0	0	0	3
Beccles var. 6		4	0	0	0	0	0	0	0	0	0	0	0	0	4
Stirling		3	0	0	0	0	0	0	0	0	0	0	0	0	3
Scarborough		1	0	0	0	0	0	0	0	0	0	0	0	0	1
2		0	0	0	0	0	0	0	0	0	0	0	0	1	1
2 var. 1		0	0	0	0	0	0	0	0	0	0	2	0	2	4
Taunton		0	0	0	0	0	0	0	10	32	0	0	1	0	43
Taunton var. 1		0	0	0	0	0	0	0	0	2	0	0	0	0	2
BAOR		0	0	0	0	0	0	0	6	0	0	0	0	0	6
3a		0	0	0	0	0	0	0	19	2	0	0	0	0	21
3a var. 1		0	0	0	0	0	0	0	0	1	0	0	0	0	1
3a var. 2		0	0	0	0	0	0	0	4	0	0	0	0	0	4
3a var. 4		0	0	0	0	0	0	0	1	3	0	0	0	0	4
3aI		0	0	0	0	0	0	0	7	4	1	0	0	0	12
3aI var. 1		0	0	0	0	0	0	0	1	8	0	0	0	0	9
3aI var. 4		0	0	0	0	0	0	0	0	1	0	0	0	0	1
3b		0	0	0	0	0	0	0	8	1	0	0	0	0	9
3b var. 2		0	0	0	0	0	0	0	1	0	0	0	0	0	1
Beccles		0	0	0	0	0	0	0	3	0	0	0	0	0	3
Beccles var. 2		0	0	0	0	0	0	0	1	1	0	0	0	0	2
Beccles var. 3		0	0	0	0	0	0	0	2	0	1	0	0	0	3
1 var. 6		0	0	0	0	0	4	0	0	0	0	0	0	0	4
RDNC		1	0	0	0	0	0	0	7	0	0	0	1	0	9
Not typable (NT)		2	2	1	7	3	0	7	5	8	0	0	0	1	36
Unspecified		9	1	0	0	0	0	0	1	1	0	1	0	0	13
All		57	26	2	19	3	4	7	103	88	3	4	2	20	338

*Reactions were positive (+) or negative (-) in the following tests: B Xyl = xylose in Bitter's medium incubated at 37°C for 24 h; Inl = inositol in peptone water incubated at 37°C for 24 h; Rha = rhamnose in peptone water incubated at 37°C for 24 h; d-t = d-tartrate in peptone water turbidity test; m-t = meso-tartrate plate test.

recognised type (RDNC), 36 strains were resistant to all phages and classified as not typable (NT) and the phage type of 13 strains was not determined.

The two commonest phage types, Dundee and Taunton, contained, respectively, 13.8% and 12.4% of the cultures examined, followed by phage types 1, 1 var. 3, 1 var. 4, 3a and 3aI, each containing between 3.5 and 6.2% of the cultures. Seven phage types (1 var. 8, 1 var. 9, 2, 3a var. 1, 3aI var. 4, 3b var. 2 and Scarborough) were represented by single cultures.

Primary and secondary biotypes

Each culture was tested for its reaction with *d*-tartrate by the three methods described above. Provided that the turbidity test was performed with ideal conditions of pH, i.e., ≤ 7.2 (see Barker, 1985), the *d*-tartrate reaction was the same for each culture by each of the different test methods. Of the 338 strains, 224 were *d*-tartrate-negative (*d*Ta⁻) and 114 *d*Ta⁺. This latter group included eight strains that gave an intermediate reaction with *d*-tartrate and classified as biotype 1. Both *d*Ta⁺ and *d*Ta⁻ strains were differentiated by other substrates, indicating that there was no direct correlation between ability to utilise *d*-tartrate and fermentation of xylose, inositol and rhamnose or utilisation of *m*-tartrate.

The reactions with xylose in Bitter's medium, with inositol in peptone water at 37°C and with *m*-tartrate were usually clearly positive or negative at

the definitive recording time. These substrates differentiated, respectively, 16, 125 and 40 strains with the negative character. Nineteen rhamnose non-fermenting strains of biotype 13 did not utilise that substrate in peptone water within 77 days whereas the other 24 cultures negative in rhamnose peptone water at 24 h gave a reaction that was usually positive within 72 h; these strains were of biotypes 7 and 23. In all, the 338 strains were differentiated into 13 primary biotypes by the reactions with the five substrates defining primary biotypes (table I).

Eight of the ten secondary tests differentiated cultures in 12 of the 13 primary biotypes so that 56 full biotypes were represented (table II). The rocked-tile test for haemagglutination of guinea-pig erythrocytes (Duguid *et al.*, 1955) detected mannose-sensitive haemagglutinating activity (i.e., type-1 fimbriae) on bacteria of 228 strains. The 110 non-fimbriate strains (subtype b) were found in eight primary biotypes, namely, 1, 2, 3, 7, 9, 11, 13 and 27 (table II). Detection of type-2 (i.e., non-haemagglutinating) fimbriae, known to be produced by some of the strains examined (Duguid *et al.*, 1966; Old and Payne, 1971; Old, 1982), requires examination by electronmicroscopy and was not a part of this study.

Stern's test for aldehyde production from glycerol (subtype g) frequently gave an intermediate colour change at the definitive reading time of 48 h and was clearly positive shortly afterwards, whereas other cultures that were clearly negative at that

Table II. Distribution of 338 cultures of *S. paratyphi B* among the observed primary biotypes and subtypes

Primary biotype number	Number of cultures	Subtypes (letters) observed in the primary biotype (and number of cultures in subtype)	Number of full biotypes
1	57	a(33), b(3), d(7), g(10), z(1), bd(1), dh(2)	7
2	26	a(5), g(14), bg(7)	3
3	103	a(5), b(4), d(1), g(23), h(11), bg(7), bh(12), gh(20), gz(1), bcg(1), bdx(1), bgh(10), bgz(1), ghz(2), bghz(4)	15
7	20	a(4), b(3), g(10), x(1), gz(2)	5
9	2	a(1), bi(1)	2
10	3	i(2), dgi(1)	2
11	88	i(3), bi(2), gi(5), hi(5), iz(1), bci(1), bgi(7), bhi(5), ghi(21), bghi(35), bghiz(3)	11
12	4	i(3), iz(1)	2
13	19	i(18), bi(1)	2
19	3	cg(1), gh(2)	2
23	4	g(3), cgi(1)	2
26	7	i(7)	1
27	2	bg(1), hi(1)	2
All	338		56

time gave reproducible results in replicate tests on cultures of the same strain.

Of the 125 strains unable to ferment inositol at 37°C, 123 were unable to do so at 25°C (subtype i) and, therefore, lacked the temperature-sensitive *m*-inositol phenotype described by Old (1972). Several groups of strains, particularly of biotypes 3 and 11, were weak fermenters of rhamnose (subtype h). This character was found frequently in strains that were also non-fimbriate (subtype bh), or unable to break down glycerol (gh) or that were inositol negative (11hi) or possessed these characters in combination, e.g., 3bgh and 11bghi.

Four strains were non-motile (subtype c) and one was anaerogenic (j); and no strain was found that was unable to ferment xylose (e) or trehalose (f) in peptone water. Eighteen strains were auxotrophic (x, z) with requirements for arginine (4 strains), methionine (1), nicotinamide (2), phenylalanine (1), purine (2), tryptophane (5), vitamin pool (1) and two for which the growth factor was not identified.

Biotypes of cultures from one patient

Each of the 55 cultures isolated from one patient utilised *m*-tartrate and failed to utilise either *d*-tartrate or rhamnose in peptone water within 24 h. Two cultures were auxotrophic, one with a requirement for cysteine (y) and the other for serine (z). The reaction with xylose in Bitter's medium and with inositol in peptone water at 37°C was not reproducible in independent tests on the same stock culture. On some occasions the reaction was positive at 24 h; on others it was negative at 24 h and required incubation for up to 72 h to give a positive reading. The biotypes of these cultures were designated 7a, 23a or 31a depending on the reaction pattern when tests were read at 24 h. The biotype of 12 cultures isolated from 1978 to Mar. 1979 was 7a or 7y; that of 25 cultures isolated between Apr. 1978 and June 1983 was 7a or 23a; that of 18 cultures isolated between Aug. 1983 and Dec. 1985 was 23a, 31a or 31z.

Phage type/biotype groups

The reaction in *d*-tartrate differentiated strains in seven phage types (PTs Dundee, Dundee var. 1, 1, 1 var. 1, 1 var. 4, Jersey and Battersea, table I) and segregated strains in 30 phage types. Twelve phage types (Worksop, 1 var. 3, 1 var. 8, 1 var. 9, 3b var. 3, 3b var. 9, Beccles var. 1, Beccles var. 4, Beccles var. 5, Beccles var. 6, Stirling, Scarborough) contained strains that were *d*Ta⁺ and 18 (2,

2 var. 1, Taunton, Taunton var. 1, BAOR, 3a, 3a var. 1, 3a var. 2, 3a var. 4, 3aI, 3aI var. 1, 3aI var. 4, 3b, 3b var. 2, Beccles, Beccles var. 2, Beccles var. 3, 1 var. 6) contained *d*Ta⁻ strains only (table I). Further differentiation was achieved with the other four substrates of the primary scheme leading to the recognition of 65 PT/BT groups of strains. The largest single groups were 31 strains of phage type/biotype Taunton/11, 22 strains of Dundee/11 and 19 strains of 3a/3.

The 15 monophasic strains were of PT/BT: 1 var. 6/12i or 12iz (4 strains), or NT/9bi (1), NT/10i or 10dgi (3), or NT/26i (7). Thus, all monophasic cultures were In1⁻ at 37°C and at 25°C and, apart from the only strain of BT9, were unable to utilise *m*-tartrate.

Correlation of biotype with source

The 224 strains of *d*-tartrate-negative biotypes 3, 7, 11, 12, 19, 23 and 27 were from 153 cases of human infection (23 specified as isolated from blood or other body fluids) 58 from sewage or water, one from food, two from an avian source and ten for which the source was not specified.

The 114 strains of *d*-tartrate-positive biotypes 1, 2, 9, 10, 13 and 26 were from 57 cases of human infection (four from blood or other body fluids), 31 from sewage or water, 20 from food, four from an avian or reptilian source and two of unspecified origin.

Colicin production

Strains producing colicin active on the indicator strains used were not found.

Sensitivity to colicin M and phage ES18

Tests for sensitivity to colicins showed that no strain was sensitive to colicin B produced by *E. coli* strain BZB2102. Cultures of 260 strains were sensitive to colicin M produced by *E. coli* strain PAP1370 and, by inference from the observation of insensitivity to colicin B, to the M component of the colicin BM complex produced by *S. typhimurium* strain S3399. The strains with this "coli" type of sensitivity pattern were of BTs 1 (12 strains), 2 (26), 3 (96), 7 (19), 11 (80), 13 (19), 19 (2), 23 (4) and 27 (2). Cultures of 62 strains were sensitive to phage ES18. The strains with this "typhimurium" type of sensitivity pattern were of BTs 1 (45 strains), 9 (2), 10 (3), 11 (1), 12 (4) and 26 (7). Cultures of 16 strains were not sensitive to either colicin M or phage

ES18 and no culture was sensitive to both colicin and phage ES18.

rRNA type

rRNA was extracted from cultures of the 18 strains belonging to BTs 9, 10, 12, 19, 23 and 27, and 59 strains representative of phage types and full biotypes within groups of strains of primary biotypes 1, 2, 3, 7, 11, 13 and 26. When analysed by electrophoresis in denaturing formaldehyde-agarose gels, the preparations from 50 strains gave a pattern that included bands representing the 23S and 16S components of rRNA, i.e., the "coli" (C) type of pattern and 27 gave the "typhimurium" (T) type of pattern which lacked the comparable 23S component and possessed two bands flanking the 16S rRNA. Several preparations classified as being of the "coli" type of pattern, particularly those from strains of BTs 7, 10, 12, 13 and 23, showed in

addition to the 23S band extra bands above or below the 16S band that were absent in preparations from the nine strains of BT1 and seven strains of BT2 that were examined.

Groups defined by biotype, phage type, sensitivity type and rRNA type

When the four characters of biotype, phage type, sensitivity type and rRNA type were considered in conjunction, it was possible to discern three groups of strains (table III). Strains in group 1 were those with the "typhimurium" type of rRNA pattern. Most strains had the "coli" type of sensitivity pattern and, apart from one strain of biotype 2, they belonged to the *d*-tartrate-negative biotypes 3, 11, 19, 23 and 27.

Strains in groups 2 and 3 possessed the "coli" type of rRNA pattern and were distinguished by their sensitivity pattern. Strains of group 2 (with

Table III. Primary biotype and phage type of cultures of *S. paratyphi B* defined by sensitivity type and ribosomal RNA (rRNA) type

Group	Primary biotype	Phage type*	Number of cultures of				
			Sensitivity type†			rRNA type‡	
			C	T	NS	C	T
1	2	1v4	1	0	0	0	1
	3	T, 3a, 3av2, D, Dvl, Bec	7	0	1	0	8
	11	T, 3aI, 3aIvI, D, Bat, NT	10	1	0	0	11
	19	3aI, D, Becv3	2	0	1	0	3
	23	Jersey, Unsp.	2	0	0	0	2
	27	T, RDNC	2	0	0	0	2
2	1	D, RDNC	4	0	0	4	0
	2	1, 1v3, 1v4, Jersey	6	0	0	6	0
	3	1, Bat	3	0	0	3	0
	7	1, 1v1, Dvl	7	0	1	8	0
	11	T	1	0	0	1	0
	13	Bat, Worksop, NT	6	0	0	6	0
3	23	2v1	2	0	0	2	0
	1	1v1, 1v3, 3bv3, Becv1, Dvl, S	0	6	0	6	0
	9	1v3, NT	0	2	0	2	0
	10	NT	0	3	0	3	0
	12	1v6	0	4	0	4	0
	26	NT	0	5	0	5	0

* Phage types were: T = Taunton; D = Dundee; Dvl = Dundee var. 1; Bec = Beccles; Bat = Battersea; S = Stirling; RDNC = not conforming to recognised definitive type; NT = not typable; Unsp. = not specified.

† Sensitivity types were: C = similar to *E. coli*, i.e., sensitive to colicin M and does not support growth of bacteriophage ES18; T = similar to *S. typhimurium*, i.e., supports growth of bacteriophage ES18 and not sensitive to colicin M; NS = not sensitive to colicin M and does not support growth of bacteriophage ES18.

‡ rRNA types were: C = pattern similar to *E. coli* with 23S and 16S bands; T = pattern similar to *S. typhimurium* with a 16S band and without a 23S band.

the "coli" type of sensitivity pattern) comprised all strains of biotypes 7 and 13 examined and some strains of biotypes 1, 2, 3, 11 and 23. Strains of group 3 (with the "typhimurium" type of sensitivity pattern) comprised some strains of biotype 1 and all strains of biotypes 9, 10, 12 and 26 examined.

Discussion

The five substrates used by Duguid *et al.* (1975) to define the primary biotypes of *S. typhimurium* also differentiated strains of *S. paratyphi B*. Thus, the primary types 1-32 of the scheme for *S. typhimurium*, defined by the reaction in Bitter's xylose, inositol, rhamnose, *d*-tartrate and *m*-tartrate, were also applicable to *S. paratyphi B*. Among the 338 strains were representatives of 13 primary biotypes with most strains found in BTs 1, 2, 3, 7, 11 and 13. The few strains that gave a negative reaction in Bitter's xylose (biotypes 19, 23, 26 and 27) accounted for only 4.7% of the strains (table I). The *d*-tartrate reaction, considered to be the most reliable of the characters used by Kristensen and Kauffmann (1937) to distinguish strains of *S. java* (*dTa*⁺) from *S. paratyphi B* (*dTa*⁻) (Barker, 1985), separated the 338 strains into 114 that were *dTa*⁺ and 224 that were *dTa*⁻. The *dTa*⁻ strains were received from eight collections with a wider geographical spread than the *dTa*⁺ strains that came from only three collections. The distribution of strains between biotypes found in this survey may, therefore, not represent a natural distribution of types and other types in existence may not be represented. Most of the 338 strains, however, came from separate incidents and may be considered representative. They belonged to one of 37 phage types and one of 13 biotypes; they also exhibited one of two sensitivity patterns and one of two rRNA patterns. The diversity of character combinations suggested considerable genetic heterogeneity among strains of the serotype *S. paratyphi B*. This finding is in marked contrast to the homogeneity of the same characters found in *S. typhimurium*.

Data from transduction experiments with mutant strains of *S. typhimurium* (Old, 1984), the observation that strains of different biotypes were similar in rRNA type (Smith *et al.*, 1988) and that they possessed the same type of colicin/phage receptor (Graham and Stocker, 1977; R. M. Barker and A. L. Blair, unpublished observation) provide further evidence for the theory proposed by Duguid *et al.* (1975) that strains of *S. typhimurium* of all biotypes have evolved from a common archetypal bacterium

of BT1 as a result of a series of mutations in different genes. Strains of the group designated as *S. paratyphi B*, on the other hand, exhibited variety in rRNA type and in sensitivity type and showed such discontinuity in the combination of biochemical characters defining the primary biotypes that it is difficult to postulate any mutational pathway that could accommodate all the observed biotypes. If the serotype *S. paratyphi B* (1,4,5,12:b:1,2) has arisen only once, as has been suggested for the serotype *S. typhimurium* (1,4,5,12:i:1,2), the descendants of that original bacterium must have undergone mutations in many genes to produce bacteria with the variety of characters found today in that serotype.

If we consider the alternative hypothesis, viz., that bacteria with the same antigenic structure do not necessarily have a common origin (Kauffmann, 1953), it would not be surprising to find grouped together in one serotype strains with very different characters because each strain would have retained many of the characters of its archetypal bacterium. Thus, the several clones comprising the serotype should not be any more closely related to each other than are members of different serotypes. The *d*-tartrate reaction was the main character by which Kauffmann (1955) differentiated strains of *S. paratyphi B* from those of *S. java*. Definition by rRNA type and sensitivity type delineated three groups which to some extent accorded with their *d*-tartrate reaction and may reflect differences in origin (table III). Strains of *S. paratyphi B* with the "typhimurium" type of rRNA pattern formed one group possibly derived from a *dTa*⁻ ancestor of BT3 (*Bxyl*⁺, *Inl*⁺, *Rha*⁺, *dTa*⁻). By analogy with accepted routes of descent found among strains of *S. typhimurium* (Old, 1984), "loss" mutations in the *inl*, *xyl* and *rha* genes might have led to the appearance of strains of BTs 11, 19, 23 and 27. Although we did not have sufficient clinical information to draw conclusions on any possible correlation with pathogenicity, we noted that 23 of 27 isolates specified as coming from blood or other body fluids of human patients belonged to BTs 3 and 11. This "clone" of strains of biotypes 3, 11, 19, 23 and 27 probably represents the *d*-tartrate-negative strains with enhanced pathogenicity for man and for which Kauffmann (1955) reserved the name *S. paratyphi B* and suggested that they should be recognised as "classical" strains of *S. paratyphi B*. Whether *dTa*⁺ strains with the "typhimurium" type of rRNA pattern, e.g., strains of BT2, should be included in this group, having descended from an ancestor of BT3 by gain in ability to utilise *d*-tartrate, or whether, apart from the rRNA pattern,

they have other properties that more closely ally them with strains of group 2, requires more detailed genetic analysis. The strain isolated from faeces and urine of the long-term carrier, on the other hand, was of BT7 or of the related BTs 23 and 31. This strain and others of BTs 3, 7, 11 and 23 with the "coli" type of rRNA pattern may represent a second clone of independent origin within the *dTa*⁻ group.

Among *dTa*⁺ bacteria it was possible to recognise several groups of strains with similar characters. Monophasic strains of BTs 9, 10, 12 and 26 were representatives of one such clone for which either a bacterium of BT1 with the "typhimurium" type of phage receptor or a monophasic bacterium of another serotype might have been the progenitor. *Inl*⁻, *Rha*⁻ bacteria of BT13, having the "coli" type of rRNA pattern and sensitivity pattern and belonging to phage type Worksop or Battersea (or phage untypable), formed another group that appeared to be unrelated to other groups of *dTa*⁺ bacteria. The existence of these distinct groups within one serotype can best be explained by postulating that they arose independently from a number of archetypal bacteria and that each acquired the antigenic determinants by which the serotype is now defined. A full understanding of the phylogeny of these bacteria and the status of rare strains with an unusual combination of characters requires investigation with new techniques such as multilocus enzyme analysis and genetic fingerprinting now available.

For the purpose of epidemiological analysis, the value of additional information provided by biotyping phage-typed cultures depended on the group to which the isolate belonged. *d*-Tartrate-negative strains with the characters typical of "classical" *S. paratyphi B* were distinguished from those of BT7 by their reaction in rhamnose. Inositol was the most useful test for differentiating the "classical" strains, separating them into the biotypes 3 (*Inl*⁺) and 11 (*Inl*⁻), as was found by Brandis and Thomsen (1956) in their survey of phage-typed, *d*-tartrate-negative strains. Secondary tests for the presence of type-1 fimbriae (subtype b), for ability to produce aldehyde from glycerol (g) and for utilisation of rhamnose in Bitter's medium (h) provided additional subdivision (table II). Each of the substrates xylose, inositol, rhamnose and *m*-tartrate differentiated *dTa*⁺ strains. Although some phage types (e.g., 3a, 3aI and 3b) appeared to be specifically associated with "classical" strains of BTs 3 and 11, others, (e.g., Dundee and 1) contained both "classical" and "non-classical" strains. In districts where the incidence of infection with *S. paratyphi B* is

high, the recognition of biotype variants within a phage type may assist in tracing epidemic strains.

Concerning the recognition of subtypes within a serotype, Le Minor *et al.* (1982) considered that, as long as serological properties continue to form the basis for classification and nomenclature, it is inconsistent to give strains with the same antigenic formula different names. They suggested that strains designated as *S. java* would be better named as biotype variants of the serotype *S. paratyphi B*, e.g., *S. paratyphi B* var. *d*-tartrate⁺. But why should one biotype variant be singled out when others are known to exist? Whilst discontinuation of the use of the name *S. java* is to be recommended, it is important to consider why we require subtype recognition. In the case of *S. paratyphi B*, the distinction was made because of the observed correspondence with clinical symptoms and the desire to indicate the epidemiological implications of infection with bacteria having the properties of "classical" *S. paratyphi B* compared with other bacteria of the same serotype. The *d*-tartrate character distinguishes bacteria belonging to the "non-classical" biotypes 1, 2, 9, 10, 13 and 26 from *d*-tartrate-negative bacteria of biotypes 3, 7, 11, 12, 19, 23 and 27. Evidence presented in this paper, however, has indicated that bacteria of BT7 have properties excluding them from the "classical" category and that, even within what should be regarded as "classical" biotypes, i.e., 3 and 11, convergent evolution has possibly brought together bacteria that may have arisen independently. The *d*-tartrate reaction alone does not, therefore, adequately define a subtype, yet detailing of each subtype character would render nomenclature cumbersome. Strains of the serotype *S. paratyphi B* would perhaps best be defined by phage type and biotype, together with other characters such as sensitivity pattern and rRNA pattern, enabling them to be categorised as "classical" or "non-classical".

Although the question as to whether the serotype should be *S. schottmuelleri* or *S. paratyphi B* remains unresolved, we favour the practice of Le Minor *et al.* (1982) in rejecting *S. java* for *d*-tartrate-positive strains of *S. paratyphi B*. Within the serotype we found three groups defined by rRNA character and sensitivity pattern and, to some extent, they correlated with the biotype and phage type of the constituent strains. The characters present in combination indicated that the serotype *S. paratyphi B* is complex. Our findings provided evidence for the hypothesis proposed by Kauffmann (1953) that the serotype is composed of strains of different ancestral origin grouped by convergent evolution

in antigenic structure. Genetic fingerprinting may eventually resolve the number of different lines now in existence and may indicate the archetypal bacteria from which they have arisen.

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