J. Hyg., Camb. (1978), **81**, 203
Printed in Great Britain

Correlation of phage type, biotype and source in strains of *Salmonella typhimurium*

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(*Received* 3 *January* 1978)

SUMMARY

A series of 2092 cultures of *Salmonella typhimurium* isolated from human, animal and other sources in 57 countries were differentiated into 204 phage types and 19 primary and 147 full biotypes. Different biotypes belonged to the same phage type and different phage types to the same biotype, so the combination of typing methods differentiated strains more finely than either method alone: 574 different 'phage type/biotypes' were distinguished in 1937 cultures belonging to the 204 recognized phage types.

The combination of biotyping with phage-typing was valuable in studying the phylogeny and spread of epidemic strains by distinguishing clones of different biotype within the same phage type and by confirming the relationship between cultures isolated from widely dispersed clones and that between cultures isolated before and after a clone had undergone variation in phage type, biotype, colicin type or antibiotic-sensitivity pattern.

A widespread outbreak of infection with *S. typhimurium* phage type 141 in Scotland comprised independent dissemination of three clones of different biotypes, 1f, 9f and 31bd. During its epidemic spread in cattle in Britain between 1962 and 1969, another strain underwent variations in phage type (type 44 to type 29), biotype (type 26a to types 26d, 26bd, 26dgi, 26dz and 26i) and antibiotic sensitivity. A group of 275 non-fimbriate, non-inositol-fermenting and non-rhamnose fermenting (FIRN) strains, particularly associated with avian infections and thought to be clonal in origin, contained 27 phage types and 22 full biotypes in the primary biotypes 29–32.

INTRODUCTION

Although *Salmonella typhimurium* is only one of over 1500 salmonella serotypes it is the commonest type throughout the world and is so widespread that the epidemiological investigation of outbreaks requires discrimination of 'subtypes' within the serotype. Such discrimination requires the identification of genetic markers by which individual strains can be recognized when isolated from different hosts and vehicles of infection. Probably the best method of sub-

division is the phage-typing system of Felix & Callow (1943, 1951) which, as extended by E. S. Anderson *et al.* (unpublished observations) distinguishes over 200 phage types of *S. typhimurium*. There are, however, other methods of subdivision, such as biotyping, colicin typing and the identification of drug resistances, which can be used in addition to phage-typing to sharpen the precision of identification (Anderson, 1971).

A scheme of biotyping by fermentation characters developed by Kristensen, Bojlén & Faarup (1937), Hansen (1942) and Harhoff (1948) distinguishes 21 biotypes of *S. typhimurium*. Different biotypes of this scheme have been found in the same phage type, so that the combination of biotyping with phage-typing has improved the identification of epidemic strains (Kallings & Laurell, 1957; Rische & Kretzschmar, 1962; Lewis & Stocker, 1971; Scholtens & Rost, 1972).

A new biotyping scheme for *S. typhimurium*, developed from that of Kristensen *et al.* (1937), has introduced additional methods and identified a larger number of types (Duguid *et al.* 1975). Thirty-two *primary biotypes* were recognized by all the 32 possible combinations of positive or negative reactions in five primary tests with Bitter's xylose medium, *m*-inositol, L-rhamnose, *d*-tartrate and *m*-tartrate, and subtypes were defined within the primary types by ten secondary tests, including observations of motility and haemagglutinating fimbriae. *Full biotypes* were designated by the primary-type number followed by letters indicating the results of the secondary tests. In this way, 144 full biotypes were distinguished in 2030 cultures.

We now report the correlation of biotype with phage type in the same series of cultures, and 62 additional cultures, and the very fine discrimination of strains achieved by the combination of the two typing methods. Observations on the correlation of certain phage types and biotypes with the source of the cultures are included.

MATERIALS AND METHODS

The 2092 cultures of *S. typhimurium* included the 2030 examined for biotype (Duguid *et al.* 1975), over 600 of which had been examined for fimbriae and Kristensen biotype (Duguid, Anderson & Campbell, 1966), 20 of phage type 44 and 42 of phage types 196–209. About two-thirds of the cultures were collected at random on the basis that only one isolation was taken from each distinct epidemic incident, but there were also groups of cultures collected from different patients or animals in the same outbreaks.

Most of the cultures had been received at the Enteric Reference Laboratory for phage-typing, but the series also included collections supplied by colleagues (Duguid *et al.* 1966). The 2092 cultures had been isolated between 1920 and 1975 in 57 countries (Table 1) and their sources were man, mostly patients with gastro-enteritis, 40 other animal species, foodstuffs and sewage (Table 2). Stock cultures were kept on Dorset egg slopes at ambient temperature during the several years of the investigation.

Phage-typing was performed by the techniques routinely practised in the Enteric Reference Laboratory (Anderson & Williams, 1956; Callow, 1959; Anderson, 1964). Phage types of *S. typhimurium* have been progressively designated

Table 1. *Countries of origin of* 2092 *cultures of* Salmonella typhimurium

Country	Number of cultures	Country	Number of cultures
England	820	Algeria	2
Scotland	341	Egypt	10
Wales	34	Ethiopia	4
Northern Ireland	15	Kenya	13
		Malawi	2
Belgium	27	Morocco	4
Denmark	21	Rhodesia	1
Eire	5	Senegal	3
Finland	9	South Africa	1
France	50	Tanzania	3
Germany	12	Tunisia	4
Greece	5	Zaire	4
Hungary	3	Zambia	2
Italy	3		
Netherlands	46	Argentina	7
Poland	54	Barbados	2
Spain	1	Brazil	1
Sweden	51	Canada	17
Switzerland	7	Curacao	2
		Guyane	2
Aden	2	Jamaica	1
China	2	Mexico	37
Hong Kong	3	Peru	1
India	8	Tahiti	1
Israel	2	U.S.A.	135
Kuwait	1	Venezuela	5
Malaysia	13		
Mauritius	1	Australia	222
North Vietnam	2	New Zealand	54
South Vietnam	2		
Sri Lanka	4		
Turkey	1	Unspecified	7

according to the original scheme of Felix & Callow (1943) and Felix (1956) and the revised scheme of Callow (1959). Further development has resulted in the definition of many more types than the 34 described by Callow, and the number now exceeds 200. The final type symbols, designated 'definitive types', have been used throughout this paper. Their equivalence to the previously designated 'provisional types' has been indicated by Anderson, Ward *et al.* (1977).

Biotyping was done by the methods of Duguid *et al.* (1975). Particulars of the tests with *d*-, *l*- and *m*-tartrates have been given by Alfredsson *et al.* (1972).

RESULTS

The phage types and biotypes of the 2092 cultures of *S. typhimurium* are shown in Table 3. Phage-typing divided the series into 204 definitive phage types and biotyping into 147 full biotypes, but the two methods together divided it into 574 different combinations of phage type and biotype.

The series included 1937 cultures belonging to 204 phage types, 31 cultures

https://doi.org/10.1017/S0022172400025031 Published online by Cambridge University Press

Table 2. *Sources of* 2092 *cultures of* Salmonella typhimurium *in non-FIRN and FIRN biotypes*

Source	Number of cultures in non-FIRN biotypes (1–28)	Number of cultures in FIRN biotypes (29–32)	Source	Number of cultures in non-FIRN biotypes (1–28)	Number of cultures in FIRN biotypes (29–32)
Man	1171	121	Budgerigar	1	0
			Canary	0	3
Cat	3	0	Duck	15	2
Cattle	242	9	Fowl	58	59
Chinchilla	0	1	Goose	1	0
Coypu	0	1	Greyhen	1	0
Dog	9	1	Gull	1	3
Elephant	1	0	Macaw	1	1
Guinea-pig	10	5	Owl	1	0
Hare	1	0	Parakeet	1	1
Horse	10	1	Parrot	2	2
Kangaroo	2	0	Partridge	0	1
Marsupial	2	0	Penguin	0	1
Mink	2	1	Pheasant	1	1
Monkey	6	0	Pigeon	10	1
Mouse	10	1	Sandgrouse	1	0
Pig	32	5	Sparrow	0	5
Rabbit	7	0	Toucan	0	1
Rat	3	0	Turkey	14	16
Sheep	18	2			
Zebra	1	0	Toad	1	0
			Turtle	2	0
Unspecified	176	30			

that reacted with the typing phages but gave patterns not conforming with those of any definitive type (RDNC), and 124 cultures resistant to all phages and classified as untypable (U). The commonest phage types, each of which included more than 1 % of the cultures examined, were types 1, 2, 3, 4, 6, 9, 12a, 13, 14, 29, 44, 49, 80, 99, 135, 141, 143 and 170. Together, these 18 common types formed 54 % of the total of 2092 cultures.

Nineteen primary and 147 full biotypes were represented. The commonest full biotypes, each of which included more than 1 % of the cultures examined, were types 1a, 1f, 2a, 3a, 9f, 17a, 17g, 19a, 25a, 25x, 26a, 26d, 26i and 31b. Together, these 14 common biotypes comprised 78 % of the 2092 cultures.

The biotypes were widely distributed among the phage types, many phage types containing more than one biotype and many biotypes more than one phage type. Thus, all but one of the 18 commonest phage types contained cultures of several different biotypes. The 37 cultures of phage type 1 included 14 biotypes; the 72 of phage type 2, 23 biotypes; and the 21 of phage type 170, 3 biotypes.

Altogether, 574 different combinations of phage type and full biotype were represented among the 1937 cultures belonging to the 204 phage types. Over half (352) of the combinations were represented by only one culture. Of the remainder, only twelve combinations comprised more than 1 % of the cultures. These twelve

Table 3. *Correlation of phage type and biotype in* 2092 *cultures of* Salmonella typhimurium. *Where more than one, the number of cultures in a phage type/biotype is given in brackets*

Phage type	Biotype (and number of cultures)
1	1a, 1f, 2a(7), 2f, 2h, 3a(14), 3f, 9i, 17f, 26a, 26f, 31b(5), 31bg, 32b
2	1a, 1cf, 1f(3), 1z, 3a, 17a(17), 17cf, 17d(2), 17dx, 17f(9), 17fg(3), 17fxz, 17g(2), 17j(2), 17x(2), 18a(2), 25a(9), 25d, 25f, 25g, 26a(5), 26f(5), 27dfgi
3	17a(12), 17b(2), 17bf, 17bj, 17f, 17fj, 17j(9), 25a(2)
4	1cf(2), 1df, 1f(24), 2a(4), 2f, 3df, 4df, 17a, 17fz, 17fghz, 17g
5	1a(9), 1bf, 1z, 25a
6	1a, 1f(5), 2a(3), 3f, 17a(2), 25f, 25x(5), 25ix, 26a(7)
7	1x
8	17a, 17g(11), 17exy
9	17a, 17b(5), 17bg, 17fg(6), 17g(7), 17gh, 17h(14), 17hy
10	3a(4), 3f(2), 17a, 17bf, 17d, 17df(2), 17fz, 19a(3), 25b
11	1a(2), 2a(2)
12	1a(9), 1f(3), 3a(4), 3b, 17a, 26a
12a	1a(26), 1f, 18a
13	23by, 29b(5), 29bc, 29bcf, 29bf(4), 31b(28), 31bf(2), 31bz, 31bgz
14	2a, 18a, 31b(83), 31bc(13), 31bd, 31bf(2), 31bg(3), 31bgz, 31bi, 32b
15	1a, 1f
15a	1a(5), 1f(2), 2a, 17a(2)
16	17a, 25a(3), 25d(8), 25x(2), 25gz, 26a(3)
17	1a(14)
18	1a(7), 9a, 9i(3), 9ix
19	1a(3), 1b, 1f(2)
20	1a(10), 1f(2), 9a, 17a
20a	1a(3), 1d, 1f(2), 1z, 3a, 9i(4)
21	1a(4), 1f, 17a
22	1a(2), 1f, 1g, 9b, 17e, 17ef, 17g(5)
23	1f, 7a, 7f, 17j
24	3a(17), 3f, 3g
25	1b, 3a(4), 7a(10), 7f, 7z, 31b(3)
27	1a(13), 1g, 3a(2), 17j
28	1a(5)
29	1f(7), 1bf(2), 25a, 25f, 26a(31), 26bd, 26d(34), 26dgi, 26dz, 26f, 26fg, 26fi, 26gi(2), 26i(7), 31b(3)
30	17g(9)
32	1a(12), 1f(2)
35	3a, 7a(2), 26a
36	17a, 17g
37	1a
38	25bcg
39	1a, 17a
40	1a(3), 29bc, 29bf, 31b(7), 31bf, 32b, 32bi(5)
41	1a(3), 1d, 1f, 1h(2), 3a(2), 3b, 17a, 17j, 25a, 31b(4), 31by
42	2a, 3a(10)
43	3a(2)
44	1f, 26a(11), 26d(3), 26f, 26i(32)
45	3a, 9a
46	17a(8), 25a, 25i
47	19a(2), 19h
49	1a, 1f, 3a, 9f(2), 17a(4), 25a, 25fx, 25gx, 25h, 25x(17), 26a(61), 26b(2) 26f(10), 26fj, 26i(2), 26iz, 26jz, 26y(3)
50	3a
51	1a(2), 1z(2)
52	1a, 17a, 17g(2)
53	1f
54	2d, 3a(2)
55	1a, 7a(3)
56	17g(17), 29bd
57	3a, 26a
58	1f(2), 3a, 25a, 31bg
59	9i(4)
60	17a, 17b(2), 17g
61	17b, 17g(4), 17gz
62	1a
63	1a(3)
64	17a(4), 17b, 17d(3), 17f, 17g(7), 17h(4)
65	12bdhx
66	17hz, 19a(2), 25b(2)
67	1a(2), 25b
68	1f(6), 7a(2), 9f
69	1bc, 3a
70	1a, 18e, 25a
71	1a, 3a, 31bg
72	2g, 17a
73	2a, 3a, 25x
74	1a(2), 2a(7), 2h(2), 3a
75	17a(2), 19a
76	1f(2), 25x
78	3a, 17a, 19a
79	17a(2)
80	1a, 1f, 2a, 30bfy, 30by(9), 32b(5) 32bf(3), 32by(7)
81	1f(2), 32b
82	1f, 17a
83	1a(2), 2a, 3a, 17f, 25a(2)

https://doi.org/10.1017/S0022172400025031 Published online by Cambridge University Press

Table 3 (*cont.*)

Phage type	Biotype (and number of cultures)
84	3a, 17a, 19a, 26i
85	17a(2), 25a, 31b(3), 31bg(2)
86	31bi
87	1a(2)
88	3a(2), 17a(2), 19a, 25b, 25d
89	1d, 2a(5), 2f(2), 29b(2), 29bf(3), 31b
90	3a, 17a, 19a
91	1f, 25b
92	26a, 31b
93	17a(2), 26a, 31b(10)
94	9i, 17a, 17d, 25f, 27ei
95	2a
96	17a, 17j, 19a(3)
97	1a(7)
98	3a
99	25bhi, 25fghi, 25ghi, 25hi(7), 25hix, 26a, 27fhi, 27hi(6), 27hiz(2)
100	17a, 19a
101	1a(9)
102	1a, 3a
103	1a, 1f, 1x(2), 17a
104	2a(3)
105	1a(6)
106	1a(4), 1fz, 3a(2), 3b, 9i
107	1a, 3a
108	3a(2)
109	1a
110	1g
111	2a, 9i, 26a
112	1a, 1f(2)
113	1a, 1f
114	3a, 17a, 25a, 25g, 26a(4)
115	1a, 1f
116	1a(2), 17a
117	17be(2), 26a
118	1a(6), 1f(3)
120	1a(2), 1h, 3a(2), 31b
121	17g
122	1g
123	1f(3), 31b(4), 31bd(2)
124	1a(3)
125	17fg
126	25a, 31b
127	2a
128	3a
129	29b
130	3a(2), 3b, 3h
131	3a
132	17a(5), 26z
133	17a
134	3a
135	1a, 1f, 1j, 2a(2), 17f, 25a(12), 25d, 25dx, 25e, 25f(3), 25fx, 25hx, 25ix, 25x(49), 25xz, 26a(9)
136	2d
137	1g
138	1a
139	2a
140	25a
141	1f(41), 1fz, 9f(196), 31b, 31bd(13)
142	19a
143	17a(43)
144	1f(3)
145	2a, 3a, 17a
146	32bef
147	3a
148	17a
149	1a
150	17g
151	1a
153	25a(5), 25f, 25fz, 25z
154	3a(3)
155	2a, 18e(2)
156	3a
157	17a
158	3a, 17dg, 25a
159	19a
160	21b, 32b(2)
161	1a(2), 29b(2), 31b, 32b(2)
162	1h
163	1a
164	3a
165	27i
166	29b
167	3a(4)
168	1a(2)
169	17a
170	1a, 3a(19), 17a
171	3a
172	1a
173	2a(3), 25a
174	3a
175	2a, 26a(2)
176	3a
177	1a
178	1cf, 3a(4), 17a(8), 17f
179	1a
180	1a
181	1f
182	1a
183	7b
184	17g
185	26a(2)
186	3a
187	1a
188	2a
189	17fg
190	19a(2)
191	17a
192	19a

https://doi.org/10.1017/S0022172400025031 Published online by Cambridge University Press

Table 3 (*cont.*)

Phage type	Biotype (and number of cultures)	Phage type	Biotype (and number of cultures)
193	1f	206	3a(3)
194	1f	207	26a(3)
195	31b	208	26e, 26ei(2)
196	1a(3)	209	25g(3)
197	1a(2), 9a	RDNC	1a(4), 1f, 1x, 2a(3), 17d, 17f(2),
198	3a(3)		19a, 19f, 25fi, 25x(6), 26a(5), 26f,
199	2a, 3a(2)		31b(3), 31bf
200	26a(3)	U	1a(23), 1f(10), 1g, 2a(5), 2f, 3a(45),
201	21a(3)		3f(3), 3fg, 9di, 17a(8), 17e, 17f(2),
202	1a, 3a(2)		17g, 19a, 25a, 25b, 25f(2), 25fx(2),
203	3a(3)		25x(2), 26a(8), 26i, 29b(2), 31b,
204	26a(3)		32bz
205	2a(2), 3a		

commonest phage type/biotypes were phage type 4/biotype 1f (4/1f), 12a/1a, 13/31b, 14/31b, 29/26a, 29/26d, 44/26i, 49/26a, 135/25x, 141/1f, 141/9f and 143/17a. The last two of these common phage type/biotypes consisted exclusively of cultures isolated from widespread epidemics in Britain, but the others included cultures of diverse and international origin.

DISCUSSION

The main use of a typing procedure is to determine whether cultures from different sources belong to the same epidemic strain (clone) or to different strains. In routine investigations a single, convenient method of typing should be used, and for *S. typhimurium* the preferred method is phage-typing. However, most typing characters, including those of phage-typing, are subject to occasional variation, so the difference in a single character does not show with certainty whether cultures belong to different strains or have recently diverged by variation from the same strain. The true relationships between cultures are most likely to be revealed by an assessment of the results of as many typing methods as possible. Thus, in our study, the combination of biotyping with phage-typing proved to be particularly useful for three purposes: (i) for distinguishing strains of different biotype within the same phage type, (ii) for confirming the clonal relationship between cultures isolated from widely dispersed sources of a single strain, and (iii) for confirming the clonal relationship between the cultures of an epidemic strain isolated before and after it had undergone a variation in phage type.

Differentiation of biotypes within the same phage type

The finding that cultures of 204 phage types were further subdivided by biotyping into 574 phage type/biotypes shows the great discriminating potential of the combined typing method. Such subdivision is particularly useful for strains of the common phage types.

Phage types 2 and 135 are examples of common types. They comprised,

https://doi.org/10.1017/S0022172400025031 Published online by Cambridge University Press

respectively, 72 and 86 of our 2092 cultures. Until recently, their phage-typing similarity led to both being designated phage type 1a. Lewis & Stocker (1971), using five biochemical tests, divided type 1a into eight biotypes. One of these biotypes, termed 'group 4', consisted of 144 cultures that required nicotinamide as a growth factor (Nic$^-$, our biotype symbol 'x') and did not ferment inositol (Inl$^-$).

Our method of biotyping with 15 tests was even more discriminating. It divided 72 cultures of phage type 2 into 23 biotypes and 86 cultures of phage type 135 into 16 biotypes. Inl$^-$ Nic$^-$ cultures corresponding to Lewis & Stocker's group 4 occurred only in phage type 135. Of 54 such cultures examined, 49 were in biotype 25x and single cultures in biotypes 25dx, 25fx, 25hx, 25ix and 25xz, which were probably variants from the common 25x strain.

Stocker & Edgar (1959) found that 15 Nic$^-$ cultures isolated in Britain, Australia, Italy and South Africa had their Nic$^-$ mutations at identical intragenic sites and so probably were phylogenetically related to one another. Our 54 cultures of biotype 25x and its variants, which were isolated in nine countries between 1952 and 1967, included representatives of Stocker & Edgar's cultures, and most of them may have belonged to the same strain.

Phage type 141 outbreak

In 1972 and 1973 there was a widespread outbreak of infection in Britain with organisms of phage type 141, a type that had previously been uncommon. In the absence of biotyping, the outbreak would have been attributed to the dissemination of a single strain, but biotyping revealed that three strains, of types 1f, 9f and 31bd, were responsible for different parts of the outbreak. The organisms studied were 241 cultures isolated from patients, cattle, sheep, milk, foodstuffs and other sources in 16 farms and 14 towns in Scotland and 16 towns in England and Wales.

Phage type/biotype 141/9f

The isolations of type 141/9f were 192 from a multi-centred epidemic in southern Scotland and 4 from patients in England. The course of the epidemic has been described in the Communicable Diseases Scotland Weekly Report 73/11 of the Scottish Home and Health Department. There were outbreaks in cattle, sheep and man in farms in six counties, sporadic human infections in nearby towns and two large outbreaks of food-poisoning, one due to eating chicken in a restaurant in Edinburgh and the other, milk-borne, in the town of Penicuik (Maclachlan, 1974).

The 9f set of biotyping characters was present in every culture from each component outbreak in the 9f epidemic: e.g., in all 53 cultures from the restaurant outbreak, all 17 from the milk-borne outbreak and all cultures from each farm outbreak. Both the phage type and the biotype were completely stable during the spread of the strain in 1972 and 1973. Bovine and human infections continued to take place in 1974 and 1975, and, although the cultures isolated in these years are excluded from the present series, only 2 out of 179 showed a variation in biotype; one variant was non-fimbriate and the other non-flagellate.

The 141/9f strain was variable in colicinogeny; 46 of the 192 Scottish cultures

produced colicin Ib, one produced colicin Ia and the rest were non-colicinogenic (Col$^-$). Both Col$^+$ and Col$^-$ cultures were obtained from the same localized outbreaks. Thus, of 11 isolations from a Midlothian farm, 3 produced colicin Ib and 8 were Col$^-$, and of 53 from the restaurant outbreak, 5 produced colicin Ib and 48 were Col$^-$. The earliest isolations were Col$^-$ and it is thought that some members of the clone acquired a colicinogenic factor while they were resident in the intestine in particular hosts. After becoming Col$^+$, the strain could still spread in an epidemic fashion. One farm outbreak in cattle and man was caused exclusively by a Col Ib line of the organism.

Phage type/biotype 141/1f

Thirty-two of the 241 cultures of phage type 141 from the 1972–3 outbreak were biotype 1f. Nine of these 1f cultures were isolated in Scotland in 1973, but none was obtained from any source on a farm or in a food-poisoning outbreak in which the 9f strain was present. Six of the nine cultures were isolated from patients in or near Glasgow, where no 9f cultures were isolated in 1972 and 1973. Three others were from Australian meat imported into Glasgow and this meat may have been the source of the human 1f infections. One of the meat cultures produced colicin Ia, one produced colicin Ib and one was Col$^-$, as were the six human cultures.

The remaining 23 cultures of type 141/1f isolated in 1972 and 1973 were from patients in different parts of England and Wales. Two of them produced colicin Ia and the rest were Col$^-$. Ten earlier isolations of type 141/1f were from patients in London in 1958, the Netherlands in 1960 and Australia in 1962 and 1963. The 141/1f strain continued to cause a few infections in Britain in 1974 and 1975, including an outbreak in cattle on a Scottish farm.

Phage type/biotype 141/31bd

Twelve of the phage type 141 cultures isolated in 1972 and 1973 were biotype 31bd. Nine were from Danish chicken imported into Scotland in 1972, one was from a patient in Aberdeenshire and two were from patients in London. Imported chicken may have been the source of human infections, but a culture of type 141/31bd had been isolated from cattle in Aberdeenshire in 1965, so that an indigenous source may also have been present. One culture of type 141/31b was isolated from imported chicken; it was probably an *l*-tartrate-positive mutant from the 141/31bd strain.

The co-existence in Britain of types 141/1f, 141/9f and 141/31bd could have been the result of a variation in one of these biotypes converting it into the other two, but there was no evidence that any such variation took place. In no case did the isolations from a focal outbreak in a farm or town include more than one of the biotypes, and interconversion of the biotypes was not observed in the laboratory.

Evidence of relationship in widely dispersed strains

In several groups of cultures isolated from sources widely separated in time and place, the association of a particular biotype with a particular phage type in all members of the group suggested that all belonged to the same, widely dispersed clone.

Phage type/biotype 143/17a

All of 43 cultures of phage type 143 isolated in 1967 from bovine and other sources in 14 different localities in England and Wales and in one in Scotland belonged to biotype 17a. This association of phage type and biotype suggested that the cultures were members of a single epidemic strain, a conclusion supported by the finding that all produced colicin Ib and were resistant to streptomycin and sulphonamides.

Phage type/biotype 3/17j

Failure to form gas from glucose (j) is a rare character in *S. typhimurium*, yet 11 of the 20 anaerogenic cultures in our series belonged to phage type 3 and primary biotype 17; nine were full biotype 17j, one was 17bj and one 17fj. Anaerogenic cultures of phage type 3 have been received by the Enteric Reference Laboratory from many countries over a period of about 30 years and it is thought that they represent a single line. Phage type 3 also includes many aerogenic cultures of biotype 17a, so that this phage type is not necessarily anaerogenic. The association of the phage type and biotype characters in the anaerogenic cultures seems to be explicable only if these cultures are clonal in origin. The single aberrant cultures of biotypes 17bj and 17fj were probably mutants of the 17j clone.

Phage type/biotype 4/1f

All 27 of our cultures of phage type 4 and primary biotype 1 were trehalose negative (f); 24 were biotype 1f, two were 1cf and one was 1df. They had been isolated between 1923 and 1969 in England, Scotland, Australia, Guyana, Mexico, Poland, Sweden and the USA, but they may represent a clone of biotype 1f, of which the 1cf and 1df cultures were mutants.

Phage type/biotypes 99/25hi and 99/27hi

Cultures of phage type 99 are characteristically found in pigeons and other birds. All but one of our 21 cultures of this phage type showed the combination of two infrequent characters: that of weak fermentation of rhamnose (h) and that of not fermenting inositol at 25 °C (i). The former of these characters was present in only 54 and the latter in 101 of the 2092 cultures. Their association in almost all isolates of phage type 99 suggests that these cultures, which originated in England, Scotland, Belgium, Canada, Mexico and the USA between 1958 and 1969, may be members of a single strain. If this is so, the strain has diversified in several of its biotyping characters, for eleven of the cultures were *d*-tartrate-positive (primary biotype 25), nine were *d*-tartrate-negative (primary biotype 27) and a few showed

other variations, e.g. non-fimbriate or Nic$^-$. The cultures lacked 0-antigen 5 and may therefore be identical with the Bitter's-xylose-negative, Bitter's-rhamnose-negative cultures from pigeons described by Edwards & Bruner (1939).

Evidence of relationship after variation in phage type

The phage type is relatively stable in *S. typhimurium* and usually remains the same in all cultures from different sources in an outbreak. Occasionally, however, a sub-line of an epidemic strain may be altered in phage type by a change in lysogeny, by gain or loss of a plasmid (Anderson, 1966, 1968*a*, 1969*a*) or by chromosomal mutation. When this happens, cultures of different phage types may be isolated from different sources in the same outbreak, and the demonstration that they belong to the same biotype suggests that they are derived from the same clone.

Phage types 14 and RDNC

Such an observation was made on a group of strains examined shortly after they had been isolated from patients in a localized outbreak of food-poisoning. Some of the cultures were phage type 14 and others had a typing pattern not corresponding with that of any recognized type (RDNC). The cultures of both phage types were biotype 31b, suggesting that the outbreak was due to a clone of phage type/biotype 14/31b which had given rise to an RDNC variant during growth in the infected food. This conclusion was supported by the finding that lysogenization of a type 14 culture with a temperate phage isolated from one of the RDNC cultures converted the type 14 culture to the typing pattern of the RDNC culture. The reverse variation, presumably due to spontaneous loss of lysogeny with this phage, was demonstrated when some colonies picked from aged cultures of the RDNC lines were found to be phage type 14.

Phage types 8, 9 and 64

Our 13 cultures of phage type 8, 36 of phage type 9 and 20 of phage type 64 were isolated in many different countries during a period of 23 years, yet all belonged to primary biotype 17. Groups of cultures of the same full biotype isolated from localized outbreaks included representatives of more than one of the three phage types. Thus, a group of eight cultures of biotype 17h isolated in Melbourne, Australia, in 1963–4 included five of phage type 9 and three of phage type 64. A group of 15 cultures of biotype 17g isolated in Britain between 1958 and 1964 included seven of phage type 8, two of phage type 9 and six of phage type 64. The suggested phylogenetic relationship of these three phage types was confirmed by the demonstration that cultures of each type could be converted into either of the other two types by changes in their state of lysogeny.

Phage types 29 and 44

Most cultures of phage types 29 and 44 belonged to biotypes 26a, 26d, 26f and 26i, which are potentially interconvertible by mutation in their *l*-tartrate, trehalose and 25 °C-inositol characters. It seemed, therefore, that these phage types might

be interrelated , and we obtained both epidemiological and experimental evidence that this was so.

Phage type 44, formerly designated 1 var.5/U9, is characteristically a bovine type and was common in British herds before 1962. Phage type 29 became the predominant type in British cattle from 1964 to 1969. Our series included 75 cultures of type 29 and 41 of type 44 isolated in Britain between 1956 and 1975. All but five of these cultures (one type 29/31b, two 29/1f and two 29/1bf) belonged to primary biotype 26, and those of each phage type were distributed mainly in the full biotypes 26a, 26d and 26i.

Strains of phage type 29 carry the Δ transfer factor (Anderson & Lewis, 1965) and when a strain of phage type 44 was infected with Δ, it was converted into phage type 29. Conversely, when Δ was displaced by the colicinogenic factor, *colIb*, with which it is incompatible, a culture of phage type 29 changed into type 44 (E. S. Anderson & H. R. Smith, unpublished observations: see Anderson, Threlfall, Carr & Savoy, 1973). Probably, therefore, the strain of phage type 29 that became predominant after 1962 was derived from a strain of phage type 44, already prevalent in British cattle, by infection with Δ.

The cultures of phage type/biotype 29/26a isolated between 1956 and 1962 were sensitive to antibiotics, but in the years 1963–5 the clone of this phage type/biotype that had arisen by infection of a phage type 44 line with Δ successively acquired resistance to streptomycin and sulphonamides, tetracyclines, furazolidone and ampicillin (Anderson, 1968*a*, *b*; 1971). In 1965 an *l*-tartrate-negative variant (biotype 26d) emerged from the antibiotic resistant line and became the predominant strain until 1969. The derivation of the 26d variant and some other, rarer variants (26i, 26bd, 26dgi and 26dz) from the 26a clone was recognized because the phage type and antibiotic sensitivities were the same in the parental and variant isolates (Duguid *et al.* 1975).

The five cultures of phage type/biotypes 29/1f, 29/1bf and 29/31b isolated in Britain between 1963 and 1970 had so many differences in biotyping and antibiotic-sensitivity characters from the predominant strain of biotypes 26a and 26d that they appeared to be unrelated to it.

The FIRN clone

Biotyping delineated a large group of cultures that were non-fimbriate, non-inositol-fermenting and non-rhamnose-fermenting. Such cultures were termed 'FIRN' by Morgenroth & Duguid (1968). They comprised 13 % (275) of the 2092 cultures. All were Bitter's-xylose-negative and belonged to the four primary biotypes 29, 30, 31 and 32, of which they constituted all the members (Duguid *et al.* 1975). Whilst all 275 cultures of biotypes 29–32 were non-fimbriate, only 44 of the 1817 cultures in the other biotypes showed this character.

Genetic evidence has suggested that all FIRN strains are descended from a single clone of biotype 25 which successively underwent fimbriae-negative (Fim$^-$) and rhamnose-negative (Rha$^-$) mutations (Morgenroth & Duguid, 1968; Old, 1972). The earliest of our FIRN cultures was isolated in Scotland in 1947, but Rha$^-$ cultures that may well have been FIRN were isolated earlier in Germany from

ducks (Hohn & Herrmann, 1936, 1937) and the group is probably ancient. Since its origin, the FIRN clone has differentiated into at least the 4 primary and 22 full biotypes of which we now have cultures (29b, bc, bd, bf, bcf; 30by, bfy; 31b, bc, bd, bf, bg, bi, by, bz, bgz; 32b, bf, bi, by, bz, bef). This differentiation has involved mutation in the abilities to ferment *d*-, *m*- and *l*-tartrates, trehalose, glycerol, inositol (at 25 °C) and xylose, to form flagella and to synthesize growth factors.

The clone has also diversified in phage type. Our FIRN cultures belonged to 27 different phage types: types 1, 13, 14, 25, 29, 40, 41, 56, 58, 71, 80, 81, 85, 86, 89, 92, 93, 120, 123, 126, 129, 141, 146, 160, 161, 166 and 195, with a few cultures RDNC or U. The phage types with the largest numbers of FIRN cultures were type 13 (43 cultures), type 14 (105), type 40 (16) and type 80 (25).

Except for five of these phage types, of which we had only single cultures, all the types that contained FIRN lines also contained some non-FIRN lines. However, the common types 13 and 14 were almost exclusively FIRN. In phage type 13, only 1 of 44 cultures was non-FIRN. This exceptional culture, which was biotype 23by, resembled FIRN strains in all its properties except that it fermented inositol at 37 °C; it was probably an Inl^+ back-mutant from a FIRN strain of biotype 31by. In phage type 14, only 2 of 105 cultures were non-FIRN; their biotypes, 2a and 18a, were unrelated to the FIRN biotypes.

Sources of FIRN strains

FIRN strains are $Fim^- Inl^- Rha^-$, whereas most other strains of *S. typhimurium* are $Fim^+ Rha^+$. Few cultures show an intermediate combination of characters such as $Fim^+ Rha^-$ (21 cultures in primary biotype 7), $Fim^- Rha^+$ (41 cultures in biotypes 1, 3, 9, 12, 17, 25 and 26) and $Fim^- Inl^+ Rha^-$ (3 cultures in biotypes 7, 21 and 23). The largely bimodal distribution of characters between the FIRN and non-FIRN cultures suggests either that the FIRN combination of characters is specially adapted for colonization of a particular kind of niche within the range of habitats occupied by *S. typhimurium* or, more probably, that the FIRN strains have fortuitously achieved a wide dissemination in a special niche during recent years.

FIRN strains are particularly common in avian hosts. Phage types 13 and 14 are the main types containing FIRN lines and for many years most cultures of these types received by the Enteric Reference Laboratory from non-human sources have been from birds, type 13 characteristically from ducks and type 14 from fowls (Anderson, 1969*b*). Table 2 shows the relative importance of avian and non-avian sources for our FIRN and non-FIRN cultures. For the comparison, the 206 cultures from unspecified sources and the 1292 from human sources, which were mostly food-poisoning cases of unknown animal origin, are disregarded. Of the remaining 124 FIRN cultures, 78 % were from birds and 22 % from mammals, whilst of the 470 non-FIRN cultures 23 % were from birds, 76 % from mammals and 1 % from cold-blooded animals.

Dissemination of clones

The choice of a wide range of independent cultures of *S. typhimurium* for this study was deliberate, since we were attempting to determine the maximum degree of subdivision of the serotype that could be achieved by the combination of phage typing and biotyping. Observations on the groups of strains known to be epidemiologically related showed that the preferred single method of discrimination is phage-typing. The consistency of the biotype in multiple strains of a single phage type of which the origins were either unknown or widely distributed in time and space suggests that particular clones of *S. typhimurium* have become dispersed on almost a world-wide scale. Similar widespread dissemination of particular antibiotic-resistant lines of this serotype has recently been described by Anderson, Threlfall *et al.* (1977).

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