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Phage type/biotype groups of *Salmonella typhimurium* in Scotland 1974–6: variation during spread of epidemic clones

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SUMMARY

Biotyping by the scheme of Duguid *et al.* (1975) of 2010 cultures of *Salmonella typhimurium* received by the Scottish Salmonella Reference Laboratory in 1974–6, the definitive phage types of which were known, revealed 137 different phage type/biotype groups. Four major epidemic clones, comprising 52% of the cultures, were recognized: 1/2a, 49/26a, 56/17g and 141/9f. The sources of each of these four groups of strains were primarily bovine (587 cultures) and human (361), suggesting a close association between infections in the two hosts.

Epidemiological evidence showed that most of the outbreaks were caused by cultures of a single phage type/biotype, suggesting that both phage typing and biotyping characters were usually stable in the course of spread of epidemic strains.

Thirty-two of the 63 phage types contained strains of more than one biotype. Cultures from 11 of the phage types were of two or more closely related biotypes and those from 21 others were of unrelated or distantly related biotypes. The combined use of phage typing and biotyping made it possible to detect occasional variations in the phage type or biotype in epidemic clones during their spread, e.g. phage type 49 to 204, 56 to 193, 141 to 193 and biotype 2a to 10a, 9f to 9bf, or 9cf, 26a to 26f.

INTRODUCTION

Variation of character in an epidemic strain during its spread through a host community is of great interest, because such variation may affect the infectivity, carriage or virulence of the strain. Furthermore, variation in the typing characters used to monitor the spread and distribution of strains may invalidate the epidemiological conclusions drawn from the typing information. If a few isolates in an outbreak differ from the majority, it is always difficult to know whether the minority variants are closely related to the majority type, derived from it by variation in typing characters, or are of a distinct type causing aberrant secondary infections in the same or related hosts. Because such questions may be resolved if many typing characters are observed in each culture, combined typing studies are important.

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Anderson *et al.* (1978) showed that biotyping considerably extended the sensitivity of phage typing and that their combined use sharpened the precision of identification of strains of *Salmonella typhimurium*. They differentiated their 2092 cultures into 204 phage types by the scheme of Anderson *et al.* (1977) and into 147 full biotypes by the scheme of Duguid *et al.* (1975). Used together, these typing systems allowed the recognition of 574 different 'phage type/biotypes' and the demonstration of variation in phage type and/or biotype in epidemic clones (Anderson *et al.* 1978). Since most strains had been derived from different epidemic episodes in many countries over many years that series was of limited value in testing the claim that relatively few lines would be responsible for the majority of infections in any country in which conditions are favourable for the establishment of salmonellas in man and animals (Anderson, 1971).

The serotype of Salmonella most commonly causing infections in man and cattle in Scotland continues to be *S. typhimurium*, and between January 1974 and December 1976, medical and veterinary laboratories reported to the Communicable Diseases (Scotland) Unit 3182 isolations of *S. typhimurium*, of which 2103 cultures had been sent for confirmation of identification to the Scottish Salmonella Reference Laboratory (SSRL). In addition to cultures from sporadic cases they included many groups of cultures isolated from different sources within an outbreak. As strains representative of *S. typhimurium* infection in Scotland over a limited period of time, they were suitable for detailed examination to identify the major epidemic clones present and to observe variations in typing character in the epidemic clones.

MATERIALS AND METHODS

Bacteria

The 2010 cultures of *S. typhimurium* were nearly all of the 2103 cultures sent to SSRL, Stobhill Hospital, Glasgow, from medical and veterinary laboratories throughout Scotland in 1974 (416 cultures), 1975 (693) and 1976 (901). They were obtained from man (883), cattle (915), other animals (99), birds (39) and the environment (74). We received from SSRL stock cultures stored on Dorset's egg medium. Each was plated on DCA agar (Oxoid, deoxycholate citrate agar), incubated overnight at 37 °C and single colonies picked for biotyping.

Phage typing

Cultures had been sent to the Enteric Reference Laboratory, Colindale Avenue, London, for phage typing, either directly from the laboratory of isolation or from SSRL. The phage type designations are those of Anderson *et al.* (1977), except types 46a, 49a and 104b, which are related, respectively, to the definitive types 46, 49 and 104.

Biotyping

The primary biotype of each culture was allocated after assessment of its reactions in biotyping media with the test substrates D-xylose, *meso*-inositol, L-rhamnose, *d*-tartrate and *meso*-tartrate according to the criteria of Duguid *et al.*

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(1975). Within the primary biotypes, subtypes (designated by the letters a–j, and x, y, z) were defined by the reactions of the cultures in ten secondary tests (see Duguid *et al.* 1975).

Colicin typing

Examination for colicin production was by the overlay method of Ozeki, Stocker & Smith (1962). Cultures found colicinogenic with indicator *Escherichia coli* strain CL104 were tested for identification of colicins Ia, Ib, B and E_2 with indicator strains listed by Barker & Old (1979) and of colicins E_1, I with E_1 and I with E_2 with strain CL136 (carries ColE_1), strain CL152 (colicin I- and colicin E_2-resistant mutant of CL104) and strain CL184 (colicin I-resistant mutant of CL136).

RESULTS

Phage types, biotypes and phage type/biotype groups

The phage types of the 2010 cultures of *S. typhimurium*, and their correlation with biotypes, are shown in Table 1. Phage typing divided 1784 cultures into 55 definitive types and three others (46a, 49a and 104b) closely related to definitive types. Of the remainder, 211 reacted with the typing phages but gave patterns not conforming with those of any definitive type (RDNC) and 15 cultures were resistant to all phages and classified as untypable (U). The commonest phage types, each containing between 12·5 % and 15·2 % of the cultures examined, were 1, 49, 56 and 141, followed by phage types 12a, 44, 104, 195 and 204, each containing between 2·5 % and 3·9 % of the cultures. On the other hand, 13 phage types (20, 39, 46a, 66, 72, 82, 85, 88, 104b, 147, 155, 166 and 208) were represented by single cultures.

The biotypes of the cultures, and their correlation with phage types, are shown in Table 2. Biotyping divided the 2010 cultures into 14 primary biotypes. The commonest primary biotypes, each of which included more than 5 % of the cultures examined, were 1, 2, 3, 9, 17 and 26 which, together, accounted for 93 % of the cultures. The remainder (7 %) belonged to one of the eight less common types, 10, 18, 19, 21, 25, 28, 29 and 31. Cultures in which all secondary characters were positive (subtype a) were represented in many of the primary biotypes and 57 % of all cultures were subtype a. Some major epidemic strains, however, belonged to subtypes other than a, e.g. 289 cultures of the trehalose non-fermenting type 9f and 293 cultures of the glycerol non-utilizing type 17g. In all, 45 different full biotypes were represented.

Strains in 32 of the phage types were subdivided by biotyping and strains in 20 of the full biotypes were subdivided by phage typing. Thus, there were 116 different phage type/biotype combinations among the 1784 cultures of definitive (or related) phage types and a further 21 combinations among the 226 cultures of types RDNC and U. The commonest phage type/biotype combinations were: 1/2a (244 isolates), 141/9f (288), 56/17g (272) and 49/26a (247) accounting for 52 % of the cultures. Another 15 phage type/biotypes (6/26a, 12a/3a, 32/1a, 44/26i, 74/2h, 104/2a, 110/2a, 170/3a, 193/1a, 195/3a, 195/31b, 204/26a, RDNC(1)/

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2a, RDNC(2)/17a and RDNC(3)/2a) each of which included at least about 1% of the cultures, accounted for a further 31% of the 2010 cultures examined. Sixty of the combinations were represented, however, by single cultures.

Table 1. *The phage types of* 2010 *cultures of* Salmonella typhimurium *and their correlation with biotypes*

Phage type	Biotype (and number of cultures*)	Phage type	Biotype (and number of cultures*)
1	1a (8), 2a (244), 2b, 26h	92	2a, 26a (3)
2	17a (2), 17g (4)	93	1a, 31b (3)
4	1f (5), 1fz	95	3a (10)
6	2a, 17a (3), 26a (19)	96	25i (4)
8	17g (4)	99	19dx (3), 21f, 21j, 25hi (2), 31bd
9	17b, 17g (5), 17eh, 19eh	104	2a (55)
10	3a (2), 25be (2)	104b	10a
11	1a (3)	108	3a, 18a
12	2a, 17a, 19a, 26a,	110	2a (21)
12a	3a (59), 3f (7)	123	31bd (13), 31bdy
13	31bd (2)	124	1a (2)
14	29b (2)	135	25a (7), 25h (6), 25x (15)
17	1a (7)	141	1f (16), 9f (288), 9bf, 9cf
18	1a (4)	145	1a(3), 2a (3), 17a, 26a
20	1a	146	1f (3), 2a, 19a (6), 29b, 31b
24	3a (2), 25i	147	3a
32	1a (28)	155	9a
39	3a	166	25a
40	29b, 29bi (2), 31b (5)	170	3a (26)
42	3a (8)	180	1a (6)
44	26a (2), 26i (76), 28ij	193	1a (24), 1c (2), 1f (4), 2a(2),
46	17a (8), 17y, 25x		3a (3), 9f, 17g (8), 26a (2)
46a	19a	194	1f (7), 9fi
49	26a (247), 26f, 26i (7)	195	1f (3), 3a (19), 31b (28), 31bj
49a	26a (13)	203	1a (3), 1bc
56	2a (2), 17g (272)	204	1a, 26a (55)
66	9a	208	26i
72	1a		
74	2h (25)	RDNC	1a (2), 1f, 2a (7), 3a (2), 25a,
82	25a		25hi, 31bd (2)
85	25a	RDNC (1)	2a (103), 2y, 10a (5), 17a, 31bd
88	25a	RDNC (2)	17a (54), 17y
		RDNC (3)	2a (29)
		Untypable	1a, 2a(4), 3a, 10a, 17a (3), 26i (5)

* Where more than one, the number of cultures in a phage type/biotype is given in parentheses.

Major epidemic types

Strains from four principal phage type/biotype groups accounted for 1051 of the cultures examined.

Phage type/biotype 1/2*a*

In 1974, this epidemic strain was associated primarily with bovine infections in Ayrshire. It was associated with a small meat-borne outbreak in Wigtownshire in 1975 and caused two milk-borne outbreaks in man. The more extensive, in the

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Eyemouth district of Berwickshire in late 1975, involved more than 200 persons who had consumed raw milk from a producer-retailer farm on which cattle were excreting this type of Salmonella. The other was at Barrhead and nearby Paisley

Table 2. *The biotypes of* 2010 *cultures of* Salmonella typhimurium *and their correlation with phage types*

Biotype	Phage type (and number of cultures*)	Biotype	Phage type (and number of cultures*)
1a	1 (8), 11 (3), 17 (7), 18 (4), 20, 32 (28), 72, 93, 124 (2), 145 (3), 180 (6), 193 (24), 203 (3), 204, RDNC (2), U	18a	108
		19a	12, 46a, 146 (6)
		19eh	9
1c	193 (2)	19dx	99 (3)
1bc	203		
1f	4 (5), 141 (16), 146 (3), 193 (4), 194 (7), 195 (3), RDNC	21f	99
		21j	99
1fz	4		
		25a	82, 85, 88, 135 (7), 166, RDNC
2a	1 (244), 6, 12, 56 (2), 92, 104 (55), 110 (21), 145 (3), 146, 193 (2), RDNC (7), RDNC/1 (103), RDNC/3 (29), U (4)	25h	135 (6)
		25i	24, 96 (4)
		25x	46, 135 (15)
2b	1	25be	10 (2)
2h	74 (25)	25hi	99 (2), RDNC
2y	RDNC/1		
		26a	6 (19), 12, 44 (2), 49 (247), 49a (13), 92 (3), 145, 193 (2), 204 (55)
3a	10 (2), 12a (59), 24 (2), 39, 42 (8), 95 (10), 108, 147, 170 (26), 193 (3), 195 (19), RDNC (2), U	26f	49
		26 h	1
		26i	44 (76), 49 (7), 208, U (5)
3f	12a (7)	28ij	44
9a	66, 155		
9f	141 (288), 193		
9bf	141	29b	14 (2), 40, 146
9cf	141	29bi	40 (2)
9fi	194		
		31b	13 (2), 40 (5), 93 (3), 146, 195 (28)
10a	104b, RDNC/1 (5), U	31bj	195
		31bd	99, 123 (13), RDNC (2), RDNC/1
17a	2 (2), 6 (3), 12, 46 (8), 145, RDNC/1, RDNC/2 (54), U (3)	31bdy	123
17b	9		
17g	2 (4), 8 (4), 9 (5), 56 (272), 193 (8)		
17y	46, RDNC/2		
17eh	9		

* Where more than one, the number of cultures is given in parentheses.

in the early months of 1976 (Mills, Gibson & Millar, 1976). The 244 cultures we examined had been isolated in 1974 (25 cultures), 1975 (157) and 1976 (62), from 164 patients in Ayr, Edinburgh, Eyemouth and the Glasgow area, from 72 cattle particularly in Ayrshire and from 8 other sources.

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Phage type/biotype 141/9*f*

This type caused an extensive outbreak of food poisoning in Edinburgh in 1972 and milk-borne outbreaks in Penicuik and other communities in Midlothian in late 1972 and early 1973, followed by incidents on many farms in counties south and west of Edinburgh (Barker & Old, 1979). Between 1974 and 1976, 288 isolations were made, mainly from cattle on farms in Wigtownshire and Midlothian (191 cultures), and also from man associated either with the farm infections or two separate outbreaks in Glasgow in 1975 and 1976 (56 cultures).

Phage type/biotype 56/17*g*

The 272 cultures examined included 195 from cattle, 61 from patients and 16 from other sources and represented all available cultures of this type present in Scotland in 1974 (90), 1975 (47) and 1976 (135). This type was isolated in considerable numbers in each of the years and was responsible for at least 77 separate episodes among cattle on farms mainly in the southern counties of Scotland. Three recognizable human outbreaks that occurred were associated with infected cattle and restricted to farming communities in Ayrshire, Berwickshire and Lanarkshire.

Phage type/biotype 49/26*a*

Strains of this type were associated with many separate incidents, including two human outbreaks linked with poultry and at least 36 separate farm episodes involving cattle. Their distribution over the years was uniform; and the 247 cultures we examined from cattle (127), man (89) and other sources (31) were all the cultures of this type received at SSRL in 1974 (75), 1975 (89) and 1976 (83).

The finding that epidemiologically related strains in an outbreak were generally of the same phage type and biotype confirmed earlier results of Anderson *et al.* (1978) and demonstrated that both phage type and biotype characters were stable during *in vivo* spread from host to host. There were, however, a few instances in which variation in typing characters was observed.

Strains of one phage type subdivided by biotype

Of the 58 phage types that were definitive or related, 28 contained cultures of more than one biotype. Twenty-one phage types (1, 6, 9, 10, 12, 24, 40, 44, 46, 56, 92, 93, 99, 108, 141, 145, 146, 193, 194, 195 and 204) contained strains that differed in one or more primary biotype characters and the epidemiological evidence indicated that each phage type/biotype group of such strains that differed in a primary biotype character represented a distinct clone. For example, strains of primary biotypes 1 and 9, distinguishable by the ability of the former to utilize inositol at 37 °C, were found in phage type 141. However, the date of the first appearance and distribution of strains of type 141/1f compared with those of type 141/9f were such as to indicate that biotype interconversion had not occurred. Furthermore, each outbreak was caused exclusively by strains of only one primary biotype.

Nine of these 21 phage types (1, 9, 40, 44, 46, 99, 141, 193 and 195) and seven

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other phage types (2, 4, 12a 49, 123, 135 and 203) contained strains of one primary biotype that differed in secondary biotype characters. In some outbreaks cultures of both secondary types were isolated. For instance, along with 244 cultures of the epidemic strain 1/2a, a single non-fimbriate culture (1/2b) was isolated from one of two persons in a family; with the 288 cultures of the epidemic strain 141/9f, two variant cultures were detected, one non-fimbriate (141/9bf) from one of eleven infected cows in one herd and the other non-flagellate (141/9cf) from one of 20 cows in another herd. The majority (24) of the cultures of type 193 were full biotype 1a but two cultures from a family of four infected persons were non-flagellate (1c) and one culture from a family of two persons was trehalose non-fermenting (1f). The majority (247) of the cultures of phage type 49 were of full biotype 26a but one variant in a family of two persons was trehalose non-fermenting (26f). Finally, the origin of auxotrophic variant lines was observed from the following prototrophic epidemic strains: 4/1f, 123/31bd, RDNC(1)/2a, RDNC(2)/17a.

Epidemiological information suggested the occurrence of separate outbreaks each caused by strains of a distinct biotype in the following: biotypes 3a and 3f of phage type 12a, biotypes 26a and 26i of phage type 49 and biotypes 25a, 25h and 25x of phage type 135; but was insufficient to explain the origin of the variant strains in the remaining phage types (2, 9, 40, 44, 46, 99, 195 and 203, see Table 1).

Strains of one biotype subdivided by phage type

Twenty of the full biotypes (1a, 1f, 2a, 3a, 9a, 9f, 10a, 17a, 17g, 17y, 19a, 25a, 25i, 25x, 25hi, 26a, 26i, 29b, 31b and 31bd) contained cultures of different phage types. Some instances in which cultures of different phage types were isolated from the same or related sources in the same outbreak suggested that a different subline of a new phage type had arisen *in vivo* from the epidemic strain. Such examples where phage-type interconversion seemed to have occurred were: (i) cultures of types 42/3a and 147/3a from the same patient, (ii) cultures of types 82/25a and RDNC/25a from another patient, (iii) one culture of type 49/26a and two of type 204/26a from three calves in an Aberdeenshire herd.

Table 1 shows that the 46 cultures of phage type 193 belonged to eight different full and six different primary biotypes. Epidemiological information about cultures that were host-related suggested possible interconversion between phage type 193 and two of the commonest phage types: 141 and 56. Thus, the only culture of biotype 9f among 289 cultures isolated that belonged to a phage type other than 141 was a single culture of type 193/9f isolated from a child in a family the other members of which were infected at the same time with type 141/9f. Cultures of type 193/17g were associated with those of type 56/17g in three outbreaks: (i) in two members of one family, (ii) in one cow among five in a herd, (iii) in one cow among 14 from which all cultures of both types also produced colicin Ib.

Colicin typing

One hundred and eighty-four (9·2%) of the 2010 cultures produced colicins, namely, Ia (51 cultures), Ib (81), E_1 (5), E_2 (41), B (1), Ia with E_1 (1), Ib with E_2 (2) and unidentified (2). The colicinogenic cultures were from man (61 cultures),

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cattle (105), other animals (9) and the environment (9). There was little change in the proportion of colicin producers in the three years: 9·3 % in 1974, 8·9 % in 1975 and 9·1 % in 1976.

Colicin-producing strains were found in 31 of the 137 phage type/biotypes but, apart from those represented by single cultures, type 135/25h was the only one in which all members produced the same colicin (Ib). All three cultures of type 145/2a were also colicinogenic but two produced colicin Ia and one E_1. All other phage type/biotype groups which contained colicinogenic strains included other strains that were non-colicinogenic.

There were 24 episodes, each involving two or more hosts, in which colicinogenic strains were involved. In 13 of the episodes, differences in colicinogeny were found in cultures from related hosts infected with strains of the same phage type and biotype; 24 cultures were colicinogenic and 104 were non-colicinogenic. In the other 11 episodes, all strains were colicinogenic and the types involved were: 1/1a/ColIa (2 cultures), 44/26i/ColIa (12), U/26i/ColIa (4), 56/17g/ColIb (17 cultures from three of the episodes), 135/25h/ColIb (6), 204/26a/ColIb (4), 49/26a/ColE_2 (13) and 204/26a/ColE_2 (23 cultures from two of the episodes), a total of 81 cultures. The remaining 79 colicinogenic strains involved single hosts in apparently unrelated incidents.

DISCUSSION

The majority of the cultures (83 %) in this collection of 2010 phage-typed strains of *Salmonella typhimurium* belonged to one of 19 phage type/biotype groups, among which four especially important groups, represented by strains of types 1/2a, 49/26a, 56/17g and 141/9f, accounted for more than 50 % of the cultures. Thus the majority of infections due to *S. typhimurium* in Scotland in the 3-year period of study were caused by strains of relatively few phage type/biotypes.

A long-term investigation of the ecology of *S. typhimurium* led Anderson (1971) to postulate that in any country the association of strains of a particular phage type with a host probably indicated that that phage type was a well-established clone in that host. For example, strains of *S. typhimurium* of phage types 1, 49 and 56 are among those with long associations with bovine hosts (Anderson, 1971) and strains of phage type 141 are also bovine-associated (Anderson *et al.* 1978; Barker & Old, 1979). The results in this paper confirm that cultures of these four epidemic strains were commonly associated with cattle in Scotland in the years 1974–6. The regular association of strains of these phage types with particular biotypes, namely phage type 1 with biotype 2a, 49 with biotype 26a, 56 with 17g and 141 with 9f, is further evidence that these major phage type/biotype groups are clones. The demonstration that each caused considerable outbreaks in man and other animals showed that these clones are not host-specific but that cattle are the major reservoirs of infection from which other animals become infected, evidence in agreement with the epidemiological findings in most cases.

Biotyping of the 2010 cultures of *S. typhimurium* isolated in Scotland from 1974 to 1976 also revealed the existence of two primary biotypes (10 and 28) and 19 full biotypes (1c, 2b, 2y, 9bf, 9cf, 9fi, 10a, 17y, 17eh, 19eh, 19dx, 21f, 21j, 25be,

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26h, 28ij, 29bi, 31bj and 31bdy) not previously described among collections of strains of more diverse origin (Diguid *et al.* 1975; Anderson *et al.* 1978). The *d*-tartrate-negative, anaerogenic culture of biotype 28ij and phage type 44 probably originated by mutation from a *d*-tartrate-positive, aerogenic strain of the major clone of type 44/26i. The seven cultures of biotype 10 (in phage types 104b, RDNC (1) and U) did not utilize *meso*-tartrate and were interesting in that they were isolated in Glasgow in 1974–5 along with 55 cultures of a closely related phage type, type 104. These latter cultures, however, were of primary biotype 2, i.e. utilized *meso*-inositol but not *meso*-tartrate. The biotype 10 line, therefore, was most probably derived from a strain of biotype 2 after mutation in the ability to utilize *meso*-inositol.

The likeliest explanation of the many strains (often represented by single cultures) that differed in a single, usually secondary, biotype character from the majority of strains of the same phage type – strains such as 44/28ij, 1/2b, 141/9bf, 141/9cf, 193/1c, 193/1f, 49/26f, 49/26i and 123/31bd – is that they are variants derived as a result of mutations to loss of functions from the parent epidemic strains during their *in vivo* spread in hosts. On the other hand, the detection of such mutants in the laboratory is difficult. The continued biotyping of cultures from different hosts in future years should reveal whether any of the mutant sub-lines are sufficiently advantaged in particular ecological niches to become dominant. We have described in this paper several examples of strains of the same phage type and closely related biotypes which were, nevertheless, involved in epidemiologically distinct episodes. The origin of mutant sub-lines such as 135/25h and 135/25x from the parental clone (135/25a) presumably occurred long enough ago to allow the sub-lines to establish themselves as distinct clones. Such sub-lines occasionally replace the parental clone from which they arise, as was demonstrated in 1965 when *l*-tartrate non-utilizing strains of biotype 26 and phage type 29 (29/26d) replaced the parental strain (29/26a) as the dominant clone in bovine hosts (Duguid *et al.* 1975).

Among the present series of strains, 21 of the definitive phage types contained cultures that differed in at least one primary biotype character, and in 15 of these phage types, the biotypes of the cultures were distantly related in phylogenetic terms. If the existence of different, unrelated biotypes among cultures of a single phage type, and, hence, of possible different sources of infection by different phage type/biotype groups of strains, is not recognized, epidemiologically incorrect assumptions may be made. We demonstrated, for example, the existence of no fewer than three distinct clones of biotypes 1f, 9f and 31bd among cultures of phage type 141 responsible for epidemiologically distinct outbreaks in Scotland in the years up to 1978 (Barker & Old, 1979).

Truly double infections with cultures of different phage types may occasionally occur, but when the episodes are short in time and occur in single families or herds, another possible explanation is that they indicate phage-type interconversions. The combined use of biotyping with phage typing is useful for confirming clonal relationships between cultures of an epidemic strain isolated before and after its variation in phage type. Several examples of such interconversions of

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phage type have been reported (Anderson *et al.* 1978) and others suggested in this paper. The indication of possible phage-type interconversions is a further benefit obtained from biotyping.

Although nearly 10% of the cultures produced colicin, typing by colicin production added little to the differentiation of epidemic cultures, mainly because the majority of them produced only one of three colicins: Ia, Ib or E_2. Those that produced colicin Ia or Ib belonged to many different phage type/biotypes. Several outbreaks were caused by strains, all of which produced colicin of the same type. When such strains were recognized by identity of phage type and biotype as belonging to a single clone, the colicin type was additional confirmation of their clonal origin, e.g. strains of type 135/25h/ColIb. In many other outbreaks, however, hosts infected with strains of a single phage type/biotype were excreting *S. typhimurium* strains of different colicin type, and whereas some were Col^-, others were Col^+. These colicinogenic strains probably acquired a Col factor from other enteric organisms present in the host (Anderson *et al.* 1978; Barker & Old, 1979). Most of the cultures that produced colicin E_2 belonged to only two phage types, types 49 and 204. Characterization of the resistance plasmids present in cultures of these phage types (Threlfall, Ward & Rowe, 1978) indicated direct interconversion between them, and this suggestion is endorsed by our finding that the majority of cultures in both lines were of the same biotype (26a). The production of colicin E_2 by all of the cultures from some outbreaks revealed the existence of a colicin sub-type within the larger clone.

Because the biotyping scheme for *S. typhimurium* (Duguid *et al.* 1975) is based on 15 biotyping characters, all of them thought to be determined by chromosomal genes, biotype instability due to chance loss or acquisition of plasmids is not a problem. However, even when variation in a biotyping character is encountered during the spread of a strain the relationship between the variant biotype and the parent is readily recognized because so many characters have been simultaneously assessed. Phage typing has proved invaluable in routine investigations of outbreaks caused by *S. typhimurium*. Nevertheless, because typing characters are subject to variation, relationships between cultures will be most accurately analysed when cultures are screened by more than one typing technique. The general stability of biotype characters and the excellent discrimination achieved by biotyping make this the obvious ancillary typing method for use after completion of the routine investigation of outbreaks by phage typing.

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