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BIOTYPING AND COLICINE TYPING OF *SALMONELLA TYPHIMURIUM* STRAINS OF PHAGE TYPE 141 ISOLATED IN SCOTLAND

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STRAINS of *Salmonella typhimurium* of phage type 141 were relatively uncommon in Scotland before 1972 (Scottish Home and Health Department, 1973). In 1972, however, sporadic incidents in Ayrshire in cattle and man were followed by two major outbreaks, one involving over 100 persons associated with an Edinburgh restaurant (Bone, 1975) and the other with a milk-borne epidemic in Penicuik (MacLachlan, 1974). Thereafter, outbreaks continued to occur in the southern counties of Scotland among cattle, sheep and man throughout the years to 1977.

Elsewhere in Britain, *S. typhimurium* phage type 141 was also uncommon before 1972 (Scottish Home and Health Department, 1973). In England and Wales from 1972 to 1976, however, 856 strains of phage type 141 were isolated, 454 from man and 402 from animals and other sources (Professor E. S. Anderson of the Enteric Reference Laboratory, Central Public Health Laboratory, Colindale, London; personal communication).

The preferred method for identifying subtypes within the serotype *S. typhimurium* is phage typing. The system, introduced by Felix and Callow (1943, 1951) and extended by Anderson *et al.* (1977a), distinguishes 207 definitive phage types. When other methods, such as biotyping, colicine typing and the identification of drug resistances, are combined with phage typing they may give further strain discrimination (Anderson, 1971). The biotyping scheme for *S. typhimurium* introduced by Duguid *et al.* (1975) differentiated 144 full biotypes among 2030 strains from different sources in 54 countries. Included in that study were strains of phage type 141 among which the existence of three biotypes was established (Duguid *et al.*, 1975; Anderson *et*

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al., 1978). This paper is a complete report of our findings on 551 strains of *S. typhimurium* phage type 141 isolated in Scotland to the end of 1977.

MATERIALS AND METHODS

Bacteria. The *S. typhimurium* strains of phage type 141 isolated in Scotland included 241 cultures received from Professor E. S. Anderson, Central Public Health Laboratory, Colindale, London and 310 cultures received from Dr J. S. Stevenson, Scottish Salmonella Reference Laboratory, Stobhill Hospital, Glasgow. This collection of strains included one isolated in 1965, the majority of strains isolated in 1972 and 1973, and all of the strains sent to the Scottish Salmonella Reference Laboratory from medical and veterinary laboratories from 1974 to 1977. Among these 551 cultures were 243 from man, mostly from patients with gastroenteritis, 230 from cattle, 33 from sheep, 11 from chickens, 14 from farm streams and ditches, six from milk, five from barley, four from meat, and one each from a dog, a mouse, pig swill, a refrigerator, and a sewer.

Professor Anderson supplied another 72 strains representative of incidents occurring outside Scotland, including 63 from England and Wales isolated from 1958 to 1975, two from the Netherlands in 1960 and seven from Australia in 1962 and 1963.

Stock cultures were maintained at ambient temperature on Dorset's egg slopes.

Phage typing. All cultures were phage typed at the Central Public Health Laboratory, Colindale, London and belonged to the definitive phage type 141, i.e., U65 of the earlier provisional scheme (see Anderson *et al.*, 1977a).

Biotyping. The full biotype designations of Duguid *et al.* (1975) are used throughout. Particulars of the methods have been described by Alfredsson *et al.* (1972) and Duguid *et al.* (1975).

Colicines. Strains were examined for colicine production by the overlay method of Ozeki, Stocker and Smith (1962). Spot cultures of 12 strains were made on nutrient-agar plates and after incubation for 24 h the bacteria were killed by chloroform vapour. The surface was covered with a thin layer of nutrient agar and flooded with a 24-h culture of indicator strain *Escherichia coli* CL104, a streptomycin-resistant mutant of strain ϕ (Gratia, 1925), which is sensitive to most colicines. After overnight incubation the plates were examined for zones of inhibition of growth of *E. coli* strain CL104.

The type of colicine produced was determined by the use of the following *E. coli* indicator strains with resistances to specific colicines (Ozeki, Stocker and Smith, 1962; Stocker, 1966): strain CL150 (colicine I-resistant mutant of strain CL104), strain CL135 (colicine B-resistant mutant of strain K12-W1177V), strain CL148 (colicine E₂-resistant mutant of strain ϕ), strain CL234 (resistant to colicine Ib) and strain CL235 (resistant to colicine Ia).

RESULTS

Biotyping of Scottish strains

Primary biotypes 1 (30 strains), 9 (509 strains) and 31 (12 strains) were represented among the 551 cultures of *S. typhimurium* phage type 141 isolated in Scotland during 1965–77, the distribution and sources being given in table I. The 30 strains of primary biotype 1 were trehalose non-fermenting (secondary biotype character f, i.e. full biotype 1f). Among the 509 strains of primary biotype 9, all of which were trehalose non-fermenting (f), one strain was non-haemagglutinating (b) and another non-motile (c). The non-fimbriate (9bf) strain and the non-flagellate (9cf) strain were isolated from individual cows in herds on Farms 15 and 26, respectively, all other cows in these herds

yielding cultures of biotype 9f. The 9bf and 9cf variants, each of which mutated spontaneously to the 9f biotype during laboratory culture, never became established clones in cattle or any other host. The 12 strains of primary biotype 31 were non-fimbriate (b) and all but one were unable to utilise *l*-tartrate (d). Thus, the following full biotypes were noted: 1f (30 strains), 9f (507), 9bf (1), 9cf (1), 31b (1) and 31bd (11).

Sequence of outbreaks associated with Scottish strains

Strains of phage type/biotype 141/31bd. The strain isolated from a cow in Aberdeenshire in 1965 was of biotype 31bd. Nine strains of biotype 31bd and a variant strain of biotype 31b were recovered from Danish chicken imported to Scotland in 1972. There was also a culture of phage type/biotype 141/31bd from a human source in Aberdeen in 1972 (table I). Thereafter, strains of phage type 141 belonging to the primary biotype 31 were not isolated.

Strains of phage type/biotype 141/9f. The 509 cultures of biotype 9f (9bf, 9cf) came from a multicentred Scottish epidemic, parts of which have been described (Scottish Home and Health Department, 1973; MacLachlan, 1974; Bone, 1975). The first cultures received for biotyping were from cases of infection in cattle and man which started in May 1972 and extended to October 1972 (Kilbirnie and Farms 1, 2 and 3, table I). The Ayrshire episodes were followed by a major outbreak in August associated with an Edinburgh restaurant. We received four cultures isolated from cooked chicken, cooked beef, kitchen waste and the refrigerator of that restaurant, 49 cultures from patrons of the restaurant and another 57 cultures from Edinburgh patients with food poisoning in late August and September 1972 (Bone, 1975). All of these cultures were biotype 9f (table I). Two months after the Edinburgh restaurant outbreak, from November 1972 to January 1973, an outbreak in Penicuik in which more than 200 persons were infected was traced to the consumption of unpasteurised milk (MacLachlan, 1974). Sixteen cultures obtained from that outbreak from human, bovine, ovine and milk sources (Penicuik and Farms 4 and 5, table I) were biotype 9f, as were cultures from small community outbreaks at Currie, Ratho (Farm 7) and Peebles (Farm 9) and sporadic cases in Midlothian, some of which were traced to a contact with a known source of infection (MacLachlan, 1974).

From January 1973 to December 1977 strains of type 141/9f continued to cause many infections in cattle in Berwickshire (Farms 11, 18 and 23), Midlothian (Farms 10, 16, 22 and 27), Lanarkshire (Farms 6, 19, 21 and 31), Wigtownshire (Farms 12, 14 and 15), Dumfriesshire (Farms 25, 26 and 29) and other areas in central and southern Scotland. There were also incidents in man, mainly in and around Edinburgh and Glasgow. There were 104 isolations in 1974, 76 in 1975 and 110 in 1976 (table I). In 1977, only six of the 612 strains of *S. typhimurium* isolated in Scotland were of phage type 141 (Dr J. S. Stevenson; personal communication).

Strains of phage type/biotype 141/1f. The first three strains of biotype 1f

Table I
Distribution and sources of strains of different biotypes of Salmonella typhimurium phage type 141 in Scotland 1965-1977

Year	If	Number, source, place and month of isolation, of strains of indicated biotype 9f (9bf, 9cf)					31bd (31b)	
							1 Cattle	Aberdeen
1965	None	None	None					
1972	None		1 Man 2 Cattle 49 Man, 4 inanimate 57 Man	Ayrshire Ayrshire Edinburgh	Kilbirnie Farms 1, 2 restaurant	May Jun., Aug. Aug.	10 Chickens*	imported Apr.
			1 Man, 1 cattle 4 Man, 1 milk	Ayrshire Midlothian	Farm 3 Penicuik	Aug.-Sep. Oct.	1 Man	Aberdeen Aug.
1973	3 Meat, imported		8 Man 1 Cattle, 1 milk 1 Sheep 3 Man 1 Cattle 2 Man 5 Man, 2 cattle 3 milk	Midlothian Midlothian Midlothian Midlothian Lanarkshire Lanarkshire	Penicuik Farm 4 Farm 5 Currie Farm 6 Wishaw	Nov.-Dec. Jan. Jan. Jan. Jan. Jan., Jun.	None	
	6 Man, Glasgow, May-Dec.		1 Man, 5 cattle 7 Cattle	Midlothian Kirkcudbright Peebleshire	Farm 7 Farm 8 Farm 9	Jan. Jan. Jan.		
	4 Man, West Lothian, Jul.-Sep.		3 Man, 3 cattle 4 sheep, 1 dog 2 Man, 2 sheep 10 Man 2 Cattle 2 Cattle 4 Man 19 Man, 1 milk	Midlothian Berwickshire Midlothian Wigtownshire Roxburghshire Glasgow Edinburgh	Farm 10 Farm 11 Carlops Farm 12 Farm 13	Apr.-Jun. Apr. Apr.-Jul. May Jul. Jul.-Dec.		
	1 Man, Ross and Cromarty, Aug.							
1974	1 Man, Edinburgh, Jan.		42 Cattle, 1 sheep 4 Cattle, 3 sheep, 8 water 3 Cattle 11 Cattle†	Wigtownshire Midlothian Wigtownshire Wigtownshire	Farm 12 Farm 10 Farm 14 Farm 15	Jan.-Jul. Jan.-Jul. Mar.-Apr. Apr.-Jul.	None	

TABLE I (continued)

Year	1f	Number, source, place and month of isolation, of strains of indicated biotype	
		9f (9bf, 9cf)	31bd (31b)
1975	None	1 Cattle	Midlothian Farm 16
	None	2 Cattle	Ayrshire Farm 17
	None	22 Cattle	Berwickshire Farm 18
	2 Man, Ayrshire, Dec.	2 Man	Edinburgh Farm 19
		2 Cattle	Lanarkshire Farm 20
		1 Cattle	Kirkcudbright Farm 21
		2 Cattle	Lanarkshire Farm 21
	10 Cattle, Dunblane, Mar-Apr	1 Cattle	Berwickshire Farm 18
		15 Cattle	Midlothian Farm 22
		1 Man	Lanarkshire Wishaw
		5 Cattle	Berwickshire Farm 23
		1 Man	Dumfriesshire Dumfries
		1 Cattle	Peeblesshire Farm 24
		2 Cattle	Wigtownshire Farm 12
1976	1 Man, Edinburgh, Feb.	21 Cattle†	Dumfriesshire Farms 25, 26
		22 Man	Glasgow Farm 27
		1 Sheep	Midlothian Farm 10
		5 Barley	Midlothian Farm 10
		1 Mouse	Midlothian Farm 10
	1 Man, Glasgow, Apr.	3 Cattle, 1 water	Dumfriesshire Farm 26
		16 Sheep	Roxburghshire Farm 28
		17 Cattle	Dumfriesshire Farm 29
		3 Cattle	Wigtownshire Farm 12
		14 Cattle	Ayrshire Farm 30
1977	None	21 Cattle, 3 water	Lanarkshire Farm 31
		2 Water	Lanarkshire Motherwell
		30 Man	Glasgow Motherwell
		5 Sheep	Roxburghshire Farm 32
		1 Sewer swab	Glasgow Jan.
			None Jan.

• One of 10 chicken carcasses was infected with a variant 31b line.

† One of 11 cattle on Farm 15 carried a variant 9bf line.

‡ One of 20 cattle on Farm 26 carried a variant 9cf line.

isolated in Scotland were recovered in early 1973 from Australian meat imported into Glasgow. Later strains came from patients with gastroenteritis, six from Glasgow, four from West Lothian and one from Ross and Cromarty in 1973; one each from Edinburgh and Paisley and two from Ayr in 1974; and one each from Glasgow and Edinburgh in 1976 (table I). In 1975 type 141/1f was recovered from 10 cows in a self-contained herd on a farm near Dunblane, Stirlingshire. This was the only known occurrence in Scotland of infection of cattle with *S. typhimurium* type 141/1f. Strains of phage type 141 biotype 1f were not isolated from any source in Scotland in 1977.

Colicines produced by Scottish strains

Seventy-four of the 551 cultures of *S. typhimurium* phage type 141 produced colicine; 67 were colicine Ib, four colicine Ia, one colicine B, one colicine E₂ and one an unidentified colicine. The majority of colicine-producing strains were of biotype 9f and the outbreaks with which they were associated are shown in table II. Cultures of strains isolated in Ayrshire in early 1972 were non-colicinogenic (Col⁻), as were the isolates from five staff members and from the food sources in the Edinburgh restaurant outbreak. Among the patrons of the restaurant, only five harboured strains that produced colicine Ib (ColIb) (table II). From human cases in the Edinburgh area after the restaurant outbreak a high proportion, 32 of 57 cultures, were producers of colicine Ib. Duplicate specimens were received from five patrons and, in two instances, one culture was Col⁻ and the other ColIb.

In subsequent outbreaks cultures were of one of three patterns of colicinogeny: (i) cultures from all hosts Col⁻ (26 outbreaks); (ii) all cultures Col⁺ (two outbreaks, Farms 7 and 16); (iii) Col⁻ and Col⁺ cultures from different individuals within the outbreak (10 outbreaks, Farms 4, 6, 10, 12, 14, 21, Edinburgh human cases 1973 and 1974, and Glasgow human cases 1975 and 1976). Of the 50 cultures received from 33 cattle on Farm 12, 46 were Col⁻, three were ColIb and one was ColB. The three ColIa and the ColE₂ cultures came from four separate outbreaks. From 1975 to 1977, among the 129 *S. typhimurium* type 141/9f cultures from outbreaks associated with 11 farms, none was colicinogenic, although three cultures from patients infected in the Glasgow area in that same period were colicinogenic (table II).

Six of the 30 cultures of biotype 1f were colicinogenic. Cultures of strains isolated from imported Australian beef produced colicine Ia (one culture) and colicine Ib (one culture); the culture isolated from imported mutton was Col⁻. Of the 10 cows in the infected herd in Stirlingshire, six yielded cultures that were Col⁻ and four that were ColIb.

All cultures of biotype 31b or 31bd were Col⁻.

Strains from incidents outside Scotland

The three primary biotypes 1 (36 strains), 9 (21) and 31 (6) were present among the 63 cultures of *S. typhimurium* of phage type 141 examined as

TABLE II

Colicinogeny in Salmonella typhimurium strains of phage type 141 and biotype 9f from 44 outbreaks in Scotland 1972-1977

Year	Number of outbreaks	Place	Number of isolates:		
			total	producing colicine	Colicine produced
1972	4	Kilbirnie, Farms 1, 2, 3	5	0	
	1	Edinburgh restaurant	53	5	Ib
	1	Edinburgh (man)	57	32	Ib
1972-73	1	Penicuik, Farms 4, 5	16	1	Ia
1973	6	Currie, Carlops, Farms 8, 9, 11, 13	32	0	
	1	Wishaw, Farm 6	3	1	Ib
	1	Farm 7	10	10	Ib
	1	Edinburgh (man)	20	4	Ib
	1	Glasgow (man)	4	0	
1973-75	1	Farm 10	31	4	Ib
1973-76	1	Farm 12	50	4	Ib(3) B(1)
1974	1	Farm 14	3	1	Ib
	4	Farms 15, 17, 19, 20	16	0	
	1	Farm 16	1	1	Ib
	1	Farm 21	2	1	Ia
	1	Edinburgh (man)	2	1	E ₂
1974-75	1	Farm 18	23	0	
1975	7	Wishaw, Dumfries, Farms 22-25, 27	26	0	
	1	Glasgow (man)	22	2	Ib(1) ?(1)
1975-76	1	Farm 26	24	0	
1976	5	Motherwell, Farms 28-31	73	0	
	1	Glasgow (man)	30	1	Ia
1977	1	Farm 32, Glasgow	6	0	

? = unidentified colicine produced.

representative of the many strains isolated in England and Wales from 1958 to 1975. The earliest culture examined was of biotype 1f, isolated from a man in London in 1958. The other 35 cultures of primary biotype 1, from representative incidents from 1972 to 1975, came from sources that were primarily human (32 strains) but there were two strains from cattle and one from a lion. Of the 32 strains from man, 30 were isolated in 1973, from 18 different towns in England and Wales, suggestive of widespread dissemination of the clone at that time. All 36 strains of primary biotype 1 were of full biotype 1f.

The 21 cultures of primary biotype 9 were from human (16 strains), bovine (4) and equine (1) sources, representative of strains isolated in 15 different parts of England from 1969 to 1975. Ten of them were cultures from patients with gastroenteritis in eight towns in England in 1973. All but one of these 21 strains

were of full biotype 9f. The single variant strain from a human source in 1970 was a cysteine-requiring auxotroph, i.e., full biotype 9fy.

The six strains of primary biotype 31 were from human (5 strains) and bovine (1) sources representing incidents in England in the years 1969, 1971 and 1973. Four of them were of full biotype 31bd. There were two cysteine-requiring variants, i.e., full biotype 31by, which were isolated in 1971 from man and cattle.

Among the nine cultures representative of strains isolated in the Netherlands and Australia in 1960, and 1962-63 respectively, all but one were of full biotype 1f. The single variant strain from Australia required folic acid for growth, i.e., full biotype 1fz.

Thus, among the 72 cultures examined, representative of incidents outside Scotland, the following full biotypes were noted: 1f (44 strains), 1fz (1), 9f (20), 9fy (1), 31bd (4) and 31by (2).

DISCUSSION

Biotyping revealed three different biotypes of *S. typhimurium* of phage type 141 in Scotland from 1965 to 1977. Strains of biotype 31bd, accounting for only 2.2% of the Scottish cultures examined, contributed little to human infection in Scotland at any time from 1965 onwards. The main isolations of this biotype were from chicken carcasses imported into Scotland some months before the Edinburgh restaurant outbreak due to strains of biotype 9f. Although chicken was originally considered as the possible vehicle of infection in that outbreak, its involvement was discounted by epidemiological evidence (MacLachlan, 1974; Bone, 1975), and our results confirmed that the biotype of the chicken strains was different from that of the infecting organism in the restaurant outbreak, despite the fact that both were of the same phage type.

It is clear that the majority of the outbreaks in Scotland due to phage type 141 strains were caused by strains of biotype 9f, which accounted for 92.4% of the 551 cultures examined. They were associated with infections in man and cattle firstly in Ayrshire, and then in the major epidemics in Edinburgh from 1972, when phage type 141 strains first became prevalent. The source of the Edinburgh restaurant outbreak was never traced, but speculation implicating bovine sources received support from our biotyping studies which showed that this outbreak was caused by type 141/9f which had also been recovered from bovine sources in Scotland at that time. Raw milk was established as the vehicle of human infection in the large outbreak caused by type 141/9f at Penicuik and Farm 7 (MacLachlan, 1974; Bone 1975). Subsequent dissemination of the strain to new dairy herds was probably due in many instances to the movement of animals between farms.

Strains of type 141/1f accounted for only 5.4% of the Scottish strains examined. They first caused human infections in Glasgow in May 1973, about three months after cultures of the same phage type/biotype had been recovered from consignments of imported boneless beef and mutton and a long time after the explosive outbreaks due to 9f strains in Edinburgh, Penicuik and associated

communities. Thereafter, biotype 1f strains were isolated sporadically from man in the Glasgow area and later in Edinburgh and Ayrshire. Also it was recovered for the only time from cattle in the self-contained herd at Dunblane, Stirlingshire in March 1975. Its introduction to that herd poses an interesting epidemiological challenge. Thus in Scotland infections by type 141/1f were associated usually with human sources.

Strains of biotype 31bd differ from those of biotype 1f in four primary and three secondary biotype characters and from those of biotype 9f in three primary and three secondary characters. Wild-type *S. typhimurium* of biotype 31 are quite different phylogenetically from strains of biotypes 1 and 9 (Duguid *et al.*, 1975) and conversion from biotype 31bd to either biotypes 1f or 9f is unlikely. Strains of biotypes 1f and 9f are more closely related to each other than to 31bd, and differ only in that the former ferment inositol in 24 h at 37°C (Inl⁺) whereas the latter do not (Inl⁻) (Old, 1972; Duguid *et al.*, 1975). Two findings suggest that interconversion of biotypes 1f and 9f was rare. Firstly, all cultures isolated from related sources in any one of the many outbreaks described in table I were always of the same biotype, and no incident was discovered that yielded strains of biotypes 1f and 9f. Secondly, although some biotype 9f strains from our larger collection of *S. typhimurium* cultures yielded Inl⁺ mutants, i.e., became biotype 1f, (Old, 1972), repeated experiments with the Scottish series of 9f cultures indicated stability in their Inl⁻ character. Thus, the strains of biotypes 1f and 9f probably represented stable, distinct clones.

Strains of *S. typhimurium* with a new combination of phage type and biotype can arise either by alteration of phage type in a clone of a stable biotype or by mutational change of a biotype character in a clone of stable phage type (Anderson *et al.*, 1978). Strains of biotype 31bd are so distinct from those of biotypes 1f and 9f, however, that they probably arose by the former method—i.e., by a change in lysogeny, by gain or loss of a plasmid or by chromosomal mutation—in a strain of stable biotype 31bd and of a phage type not yet appreciated as being capable of conversion to phage type 141. The biotype of our earliest isolate of phage type 141, in London in 1958, was 1f and strains of biotype 1f were also isolated in the Netherlands and Australia from 1960 onwards. In a survey of 4167 *S. typhimurium* cultures, 57 of the 185 cultures of biotype 1f were of phage type 141; the other 128 cultures were found in combination with 42 other phage types (Duguid *et al.*, 1975; Ruth Barker, unpublished observations). On the other hand, there was no evidence that strains of biotype 9f other than of phage type 141 have become established. For example, among the 4167 cultures examined, there were only four cultures of biotype 9f not associated with phage type 141; there were two cultures of phage type 49, one of phage type 68, and one of phage type 193. This latter culture was isolated in Scotland in 1976 from a girl whose sister was infected with *S. typhimurium* type 141/9f which suggests the possibility of a direct conversion between phage types 141 and 193.

Strains of type 141/9f have been found in Britain only since 1969. It seems probable, therefore, that a 1f bacterium which mutated in the inositol character at some time before 1969 was the ancestor of the 9f clone. Inability to

ferment trehalose, the f secondary biotype character, was stable throughout the epidemic spread of the 9f and of the 1f lines of *S. typhimurium*. Cultures from primary isolations were always trehalose-negative but mutants able to ferment trehalose in 24 h were found occasionally in subcultures stored on Dorset's egg (Duguid *et al.*, 1975). The finding that 1f and 9f lines of phage type 141 were trehalose negative further indicates their relatedness. Chance circumstances or perhaps the acquisition of a plasmid-borne character contributing to communicability (Anderson *et al.*, 1977b) may have led to their later dissemination throughout England and southern Scotland.

The incidence of colicinogeny in this series of Scottish strains (13.4%) was similar to that found in other series (Hamon and Péron, 1966; Ruth Barker, unpublished observations). Our results suggested that the original 141/1f line was non-colicinogenic. Again, because the early isolates of type 141/9f, including those from the staff members and food sources associated with the Edinburgh restaurant outbreak, were non-colicinogenic, it seems likely that the index strain of type 141/9f was non-colicinogenic. Cultures from five patients in the restaurant outbreak that produced colicines probably acquired their Col factors from the patients' resident intestinal bacteria. Although epidemiological evidence did not implicate these five persons with any further spread of infection, the high incidence of colicinogeny in subsequent isolates in the Edinburgh area suggested either that a colicinogenic line became successfully established or that most patients were infected with Col⁻ bacteria which acquired Col factors at a high rate from resident ColIb-carrying intestinal organisms.

Regardless of whether the Col factors transfer themselves or are transferred by an independent transfer factor, the plasmid status of the infecting *S. typhimurium* strain must be such as to allow entry and establishment of the new plasmid. The genetic elements determining the phage type 141 did not appear to restrict the entry of Col factors and when circumstances were favourable for transfer, it occurred at a rate of approximately 1 in 4 infected hosts. In any one outbreak, the Col factors acquired by *S. typhimurium* strains were usually of the same colicine type. Thus, within most circumscribed communities such as a family or a herd, the different hosts probably carried intestinal organisms producing the same colicines. One exceptional outbreak (Farm 12, table II) yielded cultures that were Col⁻ (46 cultures), ColIb (3) and ColB (1).

Of the outbreaks studied, 37 involved infection of two or more hosts and one of these (Farm 7, table II) yielded cultures from cattle and man all of which were Col⁺. Although it is possible that a Col⁻ line of *S. typhimurium* type 141/9f acquired a Col factor in one host before diversification, it is more likely that the outbreak was initiated by a ColIb line and that the Col character remained stable during spread to the several hosts. Because no other episode involving more than one case of infection was caused exclusively by a ColIb line it is unlikely that Farm 7 was the source of any subsequent outbreak. Knowledge of the pattern of colicinogeny within individual outbreaks of *S. typhimurium* strains of known phage type and biotype can sometimes be of

value in tracing the source of a fresh outbreak, but was of relatively little importance over the long period of the present study.

Our biotyping observations clearly established the presence of three distinct clones of *S. typhimurium* of phage type 141 in Scotland in the years of our study and assisted in unravelling the epidemiology of the different outbreaks described. The demonstration of so many full biotypes of *S. typhimurium* by the biotyping system of Duguid *et al.* (1975) makes it a strong contender as the best additional differential typing technique for use in conjunction with phage typing.

SUMMARY

Cultures of *Salmonella typhimurium* (551 strains) of phage type 141 isolated in Scotland from 1965 to 1977 were examined for biotype and colicine type. Three main biotype clones were recognised: 1f (30 strains), 9f (507 strains) and 31bd (11 strains) with subtype variants 9bf (1 strain), 9cf (1 strain) and 31b (1 strain). The contribution made by each biotype clone to outbreaks in Scotland was analysed. The findings confirmed the distinctness of the three biotype clones within the single phage type and indicated possible origins of strains of biotypes of 9f and 31bd.

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