Genet. Res., Camb. (1967), **9**, *pp.* 283–297
With 1 *text-figure*
Printed in Great Britain

Behavior of three colicine factors and an R (drug-resistance) factor in Hfr crosses in *Salmonella typhimurium*

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(*Received* 22 *July* 1966)

1. INTRODUCTION

The term 'plasmid' was proposed by Lederberg (1952) for extrachromosomal genetic units of bacteria and higher organisms. Jacob & Wollman (1958) proposed the term 'episome' to describe bacterial plasmids which at times existed independently of the chromosome, replicating autonomously, and at times became integrated in the bacterial chromosome. There is unequivocal evidence for the acquisition of a chromosomal location by plasmids of two classes, the F factor and the prophage forms of certain temperate phages. No such proof is available in respect of various other plasmids; the prophage form of phage P1, the colicine factors and the R (transmissible drug-resistance) factors. Alföldi and his colleagues (Alföldi *et al.*, 1957, 1958) reported observations on Hfr crosses between colicinogenic and non-colicinogenic strains which led them to infer that an E1 colicine factor had a characteristic chromosomal location in *Escherichia coli* K12—but other workers have questioned their interpretation of the data and in similar crosses have found no evidence for chromosomal location of several different colicine factors in this species (Nagel de Zwaig, Anton & Puig, 1962; Clowes, 1963; Fredericq, 1963*b*; Monk & Clowes, 1964; Puig & Nagel de Zwaig, 1964; Nagel de Zwaig & Puig, 1964). We here report the behavior of three different colicine factors and an R factor during crosses of an Hfr derivative of *Salmonella typhimurium* strain LT2 carrying all four plasmids to an acceptor LT2 line carrying none of them. The transmission of the three colicine factors *colI*, *colE1* and *colE2*, between F^- strains of *S. typhimurium* has previously been investigated in detail (Ozeki, Stocker & Smith, 1962; Stocker, Smith & Ozeki, 1963; Smith, Ozeki & Stocker, 1963). In the present investigation, of which a preliminary report has appeared (Dubnau & Stocker, 1964), we found no evidence of a chromosomal location for any of these three colicine factors, or for the R factor, in the *S. typhimurium* Hfr strain used.

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2. MATERIALS AND METHODS

(i) *Media*

Bacterial strains were maintained on Dorset egg slopes in small screw-cap bottles kept at room temperature. Nutrient broth was prepared from a tryptic digest of beef. Peptone agar was used as a solid complete medium. The defined medium was that of Davis & Mingioli (1950); amino acids and adenine were added at 20 μg./ml., and sugars at 0·2%. Citrate was omitted from this medium when the ability to utilize a sugar as sole carbon source was to be tested. Ability to ferment sugars was tested on either deoxycholate neutral-red or tetrazolium peptone agar containing 0·5% of the sugar. Motility was tested in a semi-solid nutrient medium (Stocker, Zinder & Lederberg, 1953). Sodium azide was added to peptone agar at M/300 or M/400. Streptomycin sulphate was added to peptone or minimal agar at 1 mg./ml., to select for bacteria with high-level streptomycin resistance due to chromosomal mutation, or at 25 μg./ml., to select for bacteria with low-level resistance due to possesssion of the R factor. Tetracycline hydrochloride was added to peptone or minimal agar at 25 μg./ml. Sulphanilamide was added to minimal medium at 1 mg./ml.

(ii) *Bacterial strains and plasmids*

The strain used as donor was an LT2 Hfr line, SR305, described by Zinder (1960). It carries the markers *hisD-23 metC-30 gal-50* and transfers its chromosome 'clockwise', with *ile* as the earliest marker, of those here considered (Zinder, personal communication; Sanderson & Demerec, 1965). (Genotype abbreviations. Nutritional requirements: isoleucine, *ile*; adenine (or purine), *ade*; proline, *pro*; methionine, *met*; histidine, *his*. Failure to ferment: galactose, *gal*; rhamnose, *rha*; arabinose, *ara*; inositol, *inl*; maltose, *mal*. Drug resistance; to azide, *azi-r*; to streptomycin, *str-r*; to tetracycline, *tet-r*; to sulphanilamide, *sul-r*. Absence of flagella, *fla*. Phase 1 flagellar antigen, *H1*.)

The female strains used in most experiments were SL680, that is LT2 *adeC-7 proA-46 H1-iM10 fla-56 str ile* (Smith & Stocker, 1962; Joys & Stocker, 1963) and various sub-lines obtained from it by repeated exposure to the mutagen ethyl methane sulphonate (Loveless & Howarth, 1959)—in particular SL809, which carries the additional markers *ara rha inl azi*.

The three colicine factors used were: *colE1*, derived from strain K12-30 of Fredericq, and *colE2* and *colI*, both derived from the *Shigella sonnei* strain P9 of Fredericq. In the usage of Lewis & Stocker (1965), they would be described as *colE1-30*, *colE2-P9* and *colI-P9*. The three colicine factors were simultaneously transferred to the Hfr strain by contact with a high-frequency colicinogeny transfer system obtained by the mixed culture of appropriate colicinogenic strains (Stocker *et al.*, 1963; Smith *et al.*, 1963). Colicinogeny was determined by standard methods (see Ozeki *et al.*, 1962).

The R factor used came from a multiply resistant strain of *Salmonella typhimurium* of phage type 27, isolated from a hospital outbreak in London (Datta, 1962).

It confers resistance to tetracycline and sulphonamides and low-level resistance to streptomycin. It is presumably identical with the R factor from the same source now called *R2* by Meynell & Datta (1966) and we shall therefore use this designation. The LT2 Hfr strain, which ferments arabinose, was infected with *R2* by 2 hours' incubation with an LT2 *ara*$^-$ line carrying *R2*, and selection of an arabinose-positive colony on tetracycline-supplemented indicator agar.

(iii) *Mating methods*

Overnight (unshaken) broth cultures of the Hfr and F$^-$ strains were diluted ten-fold in fresh broth and incubated at 37°C. for 2 hours, then mixed in equal parts. Control F$^-$ and Hfr cultures, diluted to one-half with broth, were run in parallel with the experimental mixture. After incubation for 90 min. or more the cultures were centrifuged, the cells washed twice and resuspended to the original volume in saline; 0·1 ml. volumes of suitable dilutions were spread on media selective for various classes of recombinants. Selection for growth-factor independence was made on minimal plates supplemented with all the requirements of the recipient except one, and usually with all the requirements of the Hfr, streptomycin at 1 mg./ml. serving to eliminate the male parent. In some experiments the Hfr was instead contra-selected by omission of histidine. Selection for fermentation loci was made on minimal medium lacking both glucose and citrate, and containing the sugar concerned. Selection for *fla*$^+$ *str* recombinants was made in streptomycin semi-solid medium. In the following description the locus selected from the donor parent will be indicated by a subscript 1, and that selected from the recipient by a subscript 0. For example, $ile_1\ str_0$ indicates recombinants selected for possession of the *ile*$^+$ allele of the donor and of the *str-r* allele of the recipient. Selection plates were incubated at 37°C. for 48 hours; $fla_1\ str_0$ recombinants were, however, picked both after overnight and 48-hour incubation. Recombinants were purified by streaking, generally on the same medium as used for their selection; one discrete colony was picked from each recombinant clone to a peptone-agar master plate, which after incubation was replicated by a multi-prong replicator to test plates, for characterization of unselected markers. Resistance to streptomycin or tetracycline conferred by the R factor could be satisfactorily scored by replication to drug-supplemented peptone agar, but sulphanilamide resistance had to be scored by streaking on sulphanilamide minimal agar, since the heavy inoculum transferred by the replicator gave growth even of sensitive strains.

Interrupted mating experiments were made difficult by the low fertility of the Hfr crosses—10^{-4}–10^{-5} recombinants per donor cell even for early loci. Centrifugal washing of the mating mixture after blendor treatment would have caused cell contacts and possible new matings. Young broth cultures of the Hfr and F$^-$ strains to be crossed were therefore washed and suspended in minimal medium with glucose and the common amino acids (except any corresponding to a selected locus), and these suspensions were mixed. The yield of recombinants was about the same as from matings in broth. At intervals samples were treated in a blendor

and pour plates were immediately made, from 0·1 ml. volumes of treated suspension added to 20 ml. of molten selective medium. A few recombinant colonies (< 10/plate) developed even from samples treated in the blendor immediately after mixing. They presumably arose from matings occurring in the selective medium plate, despite the 200-fold dilution of the mating mixture.

(iv) *Transduction of drug-resistance characters*

Phage P22 was propagated by the soft-agar layer method on the strain to be used as transductional donor, generally the Hfr strain carrying the three colicine factors and the R factor. The lysates were sterilized by filtration or by heating at 60°C. for 1 hour. A young culture of the recipient, usually the *ade pro str-s* parent of the female strain used in the crosses, was mixed with the phage, at a multiplicity of 5 phage particles/bacterium. After 30 min. incubation, the bacteria were washed in saline, resuspended in $\frac{1}{10}$ volume saline and plated on agar containing either tetracycline or sulphanilamide.

3. RESULTS

(i) *Linkage map inferred from Hfr × F⁻ crosses*

The yield of recombinants varied considerably between experiments, but the relative abundance of each class was always about the same. In a typical experiment in which the selected recipient locus was *str-r* the yield per input Hfr (Hfr : F⁻ ratio about 1 : 1) was $1{\cdot}3 \times 10^{-4}$ for ile_1, $5{\cdot}8 \times 10^{-6}$ for pro_1, $7{\cdot}7 \times 10^{-7}$ for fla_1 and 9×10^{-9} for $adeC_1$ recombinants. These yields are much lower than those obtained in comparable Hfr crosses in *Escherichia coli* K12. We think that the low fertility results from a low rate of pair formation. The *pro*⁻ locus of the recipient used produced one inconvenient complication. When *rha*⁺ or *ara*⁺ were selected from the donor, all or nearly all the recombinants obtained were found to have also the donor *pro*⁺ locus. Observations on crosses involving this and other *pro*⁻ recipients (Smith & Stocker, 1962, Mäkelä, personal communication) indicate that the apparent absence of pro_0^- recombinants in certain selections results from the failure of such recombinants to form colonies on the selection plates, despite the presence of proline, and not from any anomaly of recombination. We do not know why there was 'inadvertent selection' of pro_1^+ when ara_1^+ or rha_1^+ were selected, but not when ile_1^+ or ade_1^+ were selected. It was thus impossible to obtain a true estimate of the total yield of rha_1 and ara_1 recombinants. The yield of ile_1 his_0 recombinants was about the same as that of ile_1 str_0, and the yield of pro_1 his_0 about the same as that of pro_1 str_0 recombinants. Presumably, *str* and *his*, which with this Hfr donor enter later than *ile* and *pro* (Sanderson & Demerec, 1965), are incorporated into a negligibly low proportion of ile_1 and pro_1 recombinants.

In many crosses recombinants selected for various donor loci were purified and scored for unselected characters. No indication of persistent heterozygosis was observed. Tables 1 and 2 record the results of typical experiments. The decreasing

representation of the donor alleles amongst recombinants selected as ile_1 str_0 (Table 1, lines 1 and 5) suggest that the loci enter in the order:

ile—rha—mal—inl—(ara, azi, pro)

with the loci *gal, fla, his* and *adeC*, whose donor alleles were unrepresented in the samples tested, presumably entering even later than the (*ara, azi, pro*) group. When a late locus, *pro, fla* or *adeC*, was selected from the donor, the representation of unselected late donor loci was such as to suggest the order:

(ara, azi, pro)—gal—(fla, his)—adeC

The representation of the donor alleles at loci earlier than the selected donor marker was much less than the 50% expected to result from free crossing-over between

Table 1. *Representation of unselected donor markers*

			Recombinants										
			% *with indicated donor marker**										
Cross	Selection	Number tested	*ile*	*rha*	*mal*	*inl*	*ara*	*azi*	*pro*	*gal*	*fla*	*his*	*adeC*
XIII	ile_1 str_0	100	(100)	52	—	—	9	8	5	0	0	0	0
XIII	pro_1 str_0	100	25	40	—	—	64	63	(100)	15	0	0	0
XIII	fla_1 str_0	37	16	16	—	—	14	16	14	11	(100)	27	3
XIII	$adeC_1$ str_0	74	4	8	—	—	10	8	11	15	15	24	(100)
XXX	ile_1 str_0	48	(100)	41	20	12	8	6	4	0	—	—	—

In both crosses the donor was the Hfr strain. The recipient in cross XIII was SL809 and in cross XXX was a *mal* mutant of SL809.

— indicates no data, because the locus concerned was not segregating (*mal* in cross XIII) or was not scored.

* Parentheses indicate selected donor marker.

† Subscripts 1 and 0 indicate donor and recipient markers selected.

Table 2. *Frequency of recombinant classes obtained by selection for* ile_1 str_0

Recombinant type*	No.	%
ile$^+$	26	54
ile$^+$ *rha*$^+$	11	22
ile$^+$ *rha*$^+$ *mal*$^+$	2	4
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$	3	6
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$ *ara*$^+$ *azi-s*	1	2
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$ *ara*$^+$ *azi-s pro*$^+$	2	4
ile$^+$ *mal*$^+$	2	4
ile$^+$ *rha*$^+$ *ara*$^+$ *azi-s pro*$^+$	1	2
Total tested	48	

Data from cross XXX, in which the donor was the Hfr line and the recipient was SL809.

* Donor alleles only shown. The donor allele of *gal*, which maps to the 'right' of *pro*, was not present in any of the 48 recombinants tested.

https://doi.org/10.1017/S0016672300010582 Published online by Cambridge University Press

paired chromosomal segments. For instance, amongst recombinants selected for the late loci pro_1, fla_1 and $adeC_1$, the representation of the early marker ile_1 was, respectively, 25%, 16% and 4%. The order:

ile—rha—(ara, azi)—pro—gal—fla—his—adeC

was also supported by calculation of the total number of cross-overs required, on various hypothetical orders, to account for the observed distribution of recombinant classes, e.g. in the ile_1, pro_1, fla_1 and $adeC_1$ selections of cross XIII (Table 1).

The Hfr strain carrying four plasmids was used in interrupted mating experiments; their presence did not appear to affect chromosomal recombination. Because of the low yields when late markers were selected, and because of the inadvertent selection of pro_1^+ in certain selections, only ile_1^+ and pro_1^+ selections were used. In the interpretation of the results the 'background' of zero-time recombinants (< 10/plate) was disregarded. The plots of recombinant yield against

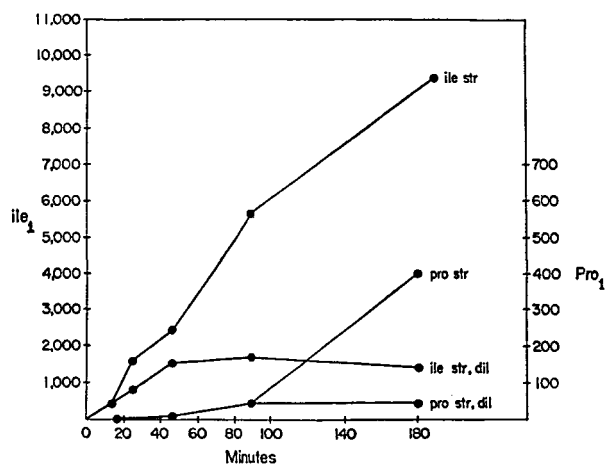

Fig. 1. Kinetics of appearance of ile_1 str_0 and pro_1 str_0 recombinants in a cross in defined medium of the Hfr line, carrying three colicine factors and *R*2, to SL809. After 15 min. part of the mating mixture was diluted ten-fold in the defined medium; at the times indicated samples from the diluted and undiluted mixtures were treated in the blendor and inoculated in pour plates of selective medium. Note that vertical scale (number of recombinant colonies per ml. mating mixture) differs for the two classes selected.

time of interruption (Fig. 1) indicated that ile_1 entered at about 15 min. and pro_1 at about 80 min. after mixing. In the experiment shown in Fig. 1 a portion of the mating mixture was diluted tenfold 15 min. after mixing, so as to reduce the rate of collisions. The early leveling off of the curves for recombinant yields for the diluted mixture, compared with the long-continued rise for the undiluted mixture, suggests that in this system the formation of effective pairs is the rate-limiting process, and that when the mating mixture is not diluted only a minority of pairs form during the first 15 min. The earliest time at which various unselected donor

loci began to appear amongst ile_1 str_0 recombinants was as follows: *rha*, 15 min.; *mal*, 25 min.; *inl*, 40 min.; *ara*, *azi* and *pro*, 80 min.

Combination of all the data indicate that the Hfr used injects in the sequence:

$$\leftarrow ile—rha—mal—inl—(ara, azi)—pro—gal—(fla, his)—adeC,$$

with *ile* entering at about 15 min. and *pro* at about 80 min.

(ii) *Plasmids in Hfr crosses*

Some colicine factors and some R factors when introduced into Hfr or F^+ lines of *E. coli* K12 greatly reduce or abolish their fertility. The introduction into our LT2 Hfr line of *colI*, *colE2* and *R2* did not affect its fertility; in some crosses the presence of *colE1* seemed to have resulted in a slight (about twofold) reduction in fertility. The pattern of segregation of unselected markers was not altered by the presence of any or all the plasmids in the male line.

(*a*) *Transfer of plasmids to the F^- population*

In experiments in which the Hfr strain carried *colI* or *colE1*, or both, and in which the mating mixture (Hfr:F^- ratio about 1:1) was incubated for 90 min. before plating on a defined medium selective for the recipient strain, the proportion of the recipient cells which had acquired a colicine factor was 10^{-2}–10^{-3}, compared to a frequency for ile_1 str_0 recombinants of about 10^{-4}. When the Hfr strain carried *R2* the proportion of the recipient population which acquired the R factor was likewise in the range 10^{-2}–10^{-3}. The transfer of plasmids to the recipient population at a frequency 10–100 times higher than the frequency of recombination for the early locus *ile* argues against the plasmids being transferred only on the chromosome.

(*b*) *Plasmid transfer to recombinants*

In crosses in which the Hfr parent carried plasmids, the proportion of recombinants with a given colicine factor was much higher than the proportion in the recipient population at large. The frequencies for each plasmid varied considerably between experiments, but the relative frequencies for the different plasmids were approximately constant. For ile_1 recombinants, the proportion with *colI* ranged from 6% to 32%, with $colE_2$ from 28% to 40%, with *colE1* from 45% to 82%, and with *R2* from 0% to 2%. When recombinants selected as ile_1 were scored for other segregating loci, there was no obvious correlation between presence of any donor marker and of any plasmid, i.e., no close linkage. However, some colicine factors were commoner in certain recombinant classes than in others. The number and percent of recombinants carrying each plasmid are recorded in Table 3 for each of the common classes (10 or more recombinants) amongst 744 recombinants from two crosses. The proportion carrying the R factor is too low to permit any inference and the differences in representation of *colE1* are perhaps not significant. The representation of *colI* and of *colE2* varied between classes, being lower (8% and 5%) in recombinants with only ile_1, the leading locus of the Hfr, than in those with a

Table 3. *Frequency of plasmids in common types of recombinant*

Recombinant type*	Number tested	*colI* No.	*colI* %	*colE1* No.	*colE1* %	*colE2* No.	*colE2* %	*R2* No.	*R2* %
ile$^+$	319	24	8	135	42	17	5	2	0·6
ile$^+$ *rha*$^+$	88	15	17	48	55	13	15	2	2
ile$^+$ *rha*$^+$ *mal*$^+$	28	3	11	19	68	3	11	0	
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$	25	8	32	17	68	6	24	3	12
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$ *ara*$^+$ *azi-s pro*$^+$	54	17	32	18	33	16	30	1	2
ile$^+$ *rha*$^+$ *mal*$^+$ *inl*$^+$ *ara*$^+$ *azi-s pro*$^+$ *gal*$^+$	11	10	91	10	91	10	91	1	9
pro$^+$	30	2	7	13	43	3	10	0	
ara$^+$ *azi-s pro*$^+$	30	2	7	13	43	3	10	0	
mal$^+$ *inl*$^+$ *ara*$^+$ *azi-s pro*$^+$	12	2	17	4	33	1	8	0	
Pooled types with 'early' (*ile*$^+$, *rha*$^+$ and 'late' (*azi-s*, *pro*$^+$) donor alleles, separated by recipient alleles	116	21	18	45	29	2	18	3	3
Total	744	121	12	343	50	102	14	14	2

Pooled data from crosses XXXI and XXXII, in which the Hfr donor carried the three colicine factors and the R factor, and the recipient was SL809 *mal*. Classes not comprising at least 10 recombinants were not recorded.

* Donor alleles only shown. The late loci *fla*, *his* and *adeC* were not scored.

longer stretch of donor chromosome, especially the class of 11 recombinants with all the segregating donor loci from *ile* through *gal*, 10 of which (91%) had both *colI* and *colE2*. The correlation of acquisition of *colI* and of *colE2* with the presence of a long stretch of donor chromosome (which was observed also in other crosses) did not result from close linkage of either of these plasmids to any single late-entering locus. Amongst recombinants with both an early donor marker (*ile* or *rha*) and a late marker (*azi* or *pro*) but with intervening recipient markers, the representation of *colI* (18%) and of *colE2* (18%) was not obviously greater than in other recombinant classes (Table 3). It thus appears that the acquisition of these two factors was correlated with incorporation of a long stretch of donor chromosome, rather than with incorporation of any particular locus, or with the long period of uninterrupted mating presumably needed for transfer of a late locus. To test this correlation, three selections were made in an additional cross: for ile_1 str_0, expected to yield a very low proportion of recombinants with a long donor region; for ile_1 pro_1 str_0, expected to yield a high proportion of such recombinants, and for pro_1 str_0, which would give an intermediate proportion. The representation of *colI* was 32% (35/108) when ile_1 was selected, 64% (52/81) when both ile_1 and pro_1 were selected, and 52% (55/105) when pro_1 only was selected. We do not know why *colI* was more frequently acquired by recombinants in this cross than in those recorded in Table 3, but the relative frequencies in the three selections were as predicted by the correlation inferred above.

(c) *Plasmid associations*

When the Hfr donor carried several plasmids it was found for some pairs of plasmids, say *a* and *b*, that amongst recombinants with plasmid *a* the proportion which had also plasmid *b* was consistently greater than the proportional representation of plasmid *b* amongst all recombinants. Data from a typical cross are given in Table 4*a*. Amongst recombinants with *colI* the proportions with *colE1* (84%) and with *colE2* (86%) are much higher than the corresponding proportions (38% and 14%) amongst all recombinants. Similarly most recombinants with *colE2* have also *colI* and *colE1*, though each of these latter factors is present in only a minority of the whole population of recombinants examined. Recombinants with one or more colicine factors did not have *R2* more often than did other recombinants, but most recombinants with the R factor had also one or more colicine factors.

Table 4. *Association of plasmids amongst clones from Hfr cross:* (*a*) *chromosomal recombinants;* (b) *recipients acquiring* R2

	Plasmid	Representation*	Representation* in sub-class Amongst recomb. which acquired:			
(*a*)		Amongst 458 chrom. recomb.	*colE1*	*colE2*	*colI*	*R2*
	colE1	174 (*38*)		58 (*66*)	61 (*84*)	5 (*72*)
	colE2	66 (*14*)	58 (*33*)	—	63 (*86*)	3 (*43*)
	colI	73 (*16*)	61 (*35*)	63 (*95*)	—	4 (*57*)
	R2	7 (*2*)	5 (*3*)	3 (*5*)	4 (*6*)	—
			Amongst recip. which acquired *R2* and:			
(*b*)		Amongst 147** recip. with *R2*	*colE1*	*colE2*	*colI*	
	colE1	86 (*59*)	—	31 (*72*)	30 (*71*)	
	colE2	43 (*29*)	31 (*36*)	—	38 (*90*)	
	colI	42 (*29*)	30 (*35*)	38 (*88*)	—	

Data from cross XXXI, of Hfr carrying three colicine factors and *R2* to SL809 *mal*. Recombinants from several selections pooled.

* Clones with plasmid indicated, stated as number and (italicized, in parentheses) as percent of all clones in class or sub-class.

** Clones obtained by plating samples of mating mixture on sulphanilamide-supplemented defined medium selective for recipient. All 147 purified *sul-r* clones were also *tet-r* and none had any donor chromosomal marker. Pooled data from samples taken at various intervals from mating mixture.

These plasmid associations were also found amongst non-recombinant F^- clones which had acquired a plasmid from the Hfr. All of 147 clones picked as resistant to sulphanilamide were resistant also to tetracycline, as would be expected if they had acquired the entire R factor (their acquisition of the low-level streptomycin-resistance conferred by *R2* could not be tested because the recipient strain was already fully resistant). None of these clones had any donor chromosomal locus, but all three colicine factors were frequent in them (*colI* 29%, *colE1* 59%, *colE2*

29%). The plasmids *colI* and *colE2* were nearly always associated (Table 4*b*). Though *colE1* was often present without the other two factors it was more frequent amongst the clones which had *colI* and/or *colE2* than amongst the drug-resistant clones at large. Clones of recipient genotype which had acquired *colE2* were also picked from platings from Hfr crosses; *colI* and *colE1* were present at high frequency (over 90% in one experiment, in which 3 hours had been allowed for mating), but the *R* factor and donor chromosomal loci were unrepresented.

(*d*) *Kinetics of transfer of plasmids to recombinants*

In some interrupted mating experiments in which the donor carried the three colicine factors and the R factor, we attempted to determine the minimum time of entry of the plasmids by testing for their presence in ile_1 str_0 recombinants from samples treated in the blendor at various times. In the earliest available ile_1 str_0 recombinants, from the sample interrupted at 20 min., the representation of *colE1* was 20%—so that no minimum time of entry could be inferred. By 180 min. its representation had risen to 80%. Factors *colI* and *colE2*, which were usually transferred together to recombinants, were not present in significant proportion amongst ile_1 str_0 recombinants until 180 min., at which time they were each found in less than 10% of recombinants; at 300 min. their representation was 30%. Thus, *colI* and *colE2* seem to have a minimum time of entry between 100 and 180 min. The R factor was transferred to ile_1 str_0 recombinants at frequencies too low to permit an estimate of minimum time of entry.

(iii) *Co-transduction of colicine factors with drug-resistance (R) traits*

Phage P22 grown on the Hfr strain carrying the three colicine factors and the R factor was used in transduction to SL489, the *ade pro str-s* parent of the *str-r* F^- line used in the mating experiments. From two to five colonies per plate, corresponding to 10^{-9} transductants/plaque-forming unit, were obtained on tetracycline peptone agar. On defined medium with sulphanilamide an average of about twenty-seven colonies per plate, corresponding to 10^{-8} transductants/plaque-forming unit, were obtained. No colonies appeared on either the sulphanilamide or the tetracycline plates when the recipient was plated without phage treatment, or after treatment with phage grown on a strain not carrying the R factor. The large number of mutants made it impossible to test for transduction of the low-level streptomycin-resistance conferred on LT2 by the *R2* factor.

Testing of the unselected markers of the drug-resistance transductants revealed the same pattern of association and segregation of resistance traits derived from *R2* as has been observed in transduction experiments with other R factors, in *S. typhimurium* by phage P22 (Watanabe & Fukasawa, 1961) and in *Salmonella* of O group E by phages ϵ 15 and ϵ 34 (Harada *et al.*, 1963). None of the *tet-r* transductant clones had the *sul-r* or *str-r* trait of the donor; nearly all the *sul-r* transductants had the *str-r*, but none had the *tet-r* trait of the donor. As was to be expected, none of the *tet-r* or *sul-r str-r* transductants had the ade^+ or pro^+ character of the donor.

Unexpectedly, however, some of the drug-resistant transductants were colicinogenic, producing either colicine E1 or colicine E2. We attribute this to co-transduction of the *colE1* or of the *colE2* factor with the *tet-r* or with the *sul-r str-r* determinant(s) forming part of the *R2* factor in the donor strain. None of the 317 *sul-r*

Table 5. *Co-transduction by phage P22 of* colE1 *and* colE2 *with drug-resistance traits derived from* E2

		Drug-resistant transductants		
Lysate*	Selection	Number tested	Carrying *colE1***	Carrying *colE2***
P22-A	*sul-r*	4	1 (*25*)	3 (*75*)
P22-A	*sul-r*	84	0	20 (*23*)
P22-B	*sul-r*	50	3 (*6*)	10 (*20*)
P22-B	*sul-r*	40	3 (*8*)	8 (*20*)
P22-B	*tet-r*	6	0	1 (*15*)
P22-B	*tet-r*	1	0	1
P22-C	*sul-r*	92	0	0
P22-C	*sul-r*	47	4 (*9*)	5 (*10*)
P22-C	*tet-r*	43	0	0

* The three lysates were made on separate occasions by growth of phage P22 on the Hfr line carrying the three colicine factors and the R factor. The recipient was the *ade pro str-s* parent of SL809.

** Number and (italicized, in parentheses) percent of transductants found to be colicinogenic.

and the 50 *tet-r* transductants tested carried both *colE1* and *colE2*, and none carried *colI*. The P22 lysates used transduced the *ile* and *proA* characters at about the expected rates; none of 42 *ile*$^+$ and 47 *pro*$^+$ transductants tested carried any colicine factor or drug-resistance trait of the donor strain. The frequency of colicinogeny amongst the drug-resistance transductants varied greatly from one experiment to another (0–25% for *colE1* and 0–75% for *colE2*), but the phenomenon was observed in several experiments and with several independently prepared phage lysates (Table 5).

4. DISCUSSION

The chromosomal recombination observed in our crosses of the *S. typhimurium* LT2 Hfr line to LT2 F$^-$ resembled that seen in similar *E. coli* crosses, with minor differences, some of which at least seem to be characteristic of Hfr crosses in *Salmonella* (Mäkelä, 1963; Johnson, Falkow & Baron, 1964; Sanderson & Demerec, 1965). Thus the yield of recombinants was low even when an early-entering donor locus was selected. The effect on recombinant yield of early dilution of the mating mixture in interrupted mating experiments suggests that this low fertility may result from a low probability of pair formation per collision. The yield of recombinants fell off sharply when selection was made for later-entering donor loci—but

loci corresponding to about half the linkage map appeared at detectable frequencies. In recombinants selected for a late-entering locus the representation of unselected earlier loci was much less than 50%. This may indicate incomplete synapsis of the entering chromosome fragment with the chromosome of the recipient—even though in our crosses both parents derive from a common ancestor, strain LT2.

The yields in different selections, linkage data in respect of unselected donor loci and minimal time of entry results were all consistent with the entry of the donor loci in the sequence

$$\leftarrow ile—rha—mal—inl—(ara, azi, pro)—gal—(fla, his)—adeC$$

This order agrees well with the map inferred from crosses mediated by colicine factors in *S. typhimurium* LT2 (Smith & Stocker, 1962) and from Hfr crosses in this species and in *S. abony* (Sanderson & Demerec, 1965; Mäkelä, 1963). The locus for fermentation of inositol, for which we propose the symbol *inl*, was not previously mapped. The inferred order also agrees well with that in *E. coli* K12 (Taylor & Thoman, 1964), if the *mal* locus we mapped corresponds to their *malB*.

The introduction of the three colicine factors and the R factor into the Hfr did not much affect its fertility, though *colE1* perhaps caused a slight reduction. In this respect these colicine factors differ from those, such as a *colB* factor studied by Puig & Nagel de Zwaig (1964), which when introduced into Hfr or F^+ lines (of *E. coli* K12) profoundly depress their fertility. The fertility of the LT2 Hfr was also unaffected by introduction of the *R2* factor—which is surprising considering that this factor when tested in *E. coli* K12 is of the *fi*$^+$ (fertility-suppressing) kind (Meynell & Datta, 1966).

The three colicine factors and the R factor were transferred to a small proportion of the total F^- population. We did not observe the transfer of *colE1* from the Hfr to a high proportion of the F^- population which occurs in *E. coli* (Alföldi *et al.*, 1958; Clowes, 1963; Nagel de Zwaig & Puig, 1964)—perhaps because of the relatively low rate of pairing in the *Salmonella* Hfr mating. All the plasmids were transferred to recombinants at much higher frequencies than to the total F^- population. We infer that F conjugation facilitates the transfer of plasmids from the Hfr to the F^- partner, though it does not ensure it. Factor *colE1* was transmitted early (< 20 min.) and with high frequency to all classes of recombinants—as happens also in *E. coli* Hfr or F^+ crosses. Factors *colI* and *colE2* entered later (transfer first detected between 100 and 180 min.), and were acquired by recombinants incorporating only the leading locus of the Hfr less often than by other classes. In *E. coli* Hfr crosses some colicine factors, including the *colI* factor we used (Monk & Clowes, 1964) and another one (Nagel de Zwaig *et al.*, 1962), are more frequent in recombinants selected for a late, rather than an early marker—as though the probability of their transfer increased the longer the partners remained paired. In our experiments, however, the representation of *colI* and *colE2* was higher in recombinants which had incorporated a long stretch of donor chromosome than in those with only an early, or with only a late marker, or with both early and late markers separated by recipient alleles. It is difficult to interpret this observation; one possible explanation would

be that these two factors are transferred in consequence of unstable attachment to the Hfr chromosome at any of a large number of sites, and become established in a recombinant clone only if the locus concerned is integrated into the chromosome. The R factor was transferred to few recombinants, and no inference could be drawn about its time of entry. Our linkage data do not indicate any constant chromosomal site for *colI*, *colE1*, *colE2* or *R2*, and in this respect agree with the conclusions drawn by others about various colicine factors in *E. coli*. As the evidence available indicates that colicine factors, R factors and the defective prophage P1*d1* (Boice & Luria, 1963) lack characteristic chromosomal sites they are perhaps better termed 'plasmids' than 'episomes'. However, a colicine factor may sometimes be associated with an F factor, and in consequence become located at the chromosome 'tail' in an Hfr (Fredericq, 1963*a*); and R factor genes transduced by P22 sometimes become associated with a defective P22 prophage and in consequence become located at the P22 prophage site (Dubnau & Stocker, 1964).

Some interesting correlations of transfer of different plasmids were observed, both amongst chromosomal recombinants and non-recombinants (Table 4). Nearly all cells which acquired *colI* or *colE2* acquired both, and *colE1* also. Most cells which acquired *R2* acquired also *colE1*, and many also acquired *colI* and/or *colE2*. If the plasmids are transferred by a process distinct from chromosome transfer these correlations, and the earlier entry of *colE1* than of *colI* and *colE2*, might reflect the sequential transfer of several plasmids joined in a linear linkage group:

$$\leftarrow colE1—(colI,\ colE2)—R2$$

or it might indicate that in those pairs in which some event necessary for plasmid transfer occurs the probability of transfer per time unit is highest for *colE1* and lowest for *R2*. The unexpected co-transduction of *colE1* or *colE2* with resistance determinants from *R2* suggests the physical proximity of each of these colicine factors to the R factor in the phage-infected Hfr cell. This presumably indicates some kind of linkage. An alternative explanation, that co-transduction reflects the existence of many copies of each plasmid in the phage-infected cell, seems unlikely in view of the absence of co-transduction of any plasmid or drug-resistance trait with chromosomal loci. The absence of co-transduction of *colI* with resistance traits was to be expected, since phage P22 is apparently unable to transduce this factor (Ozeki & Stocker, 1958).

SUMMARY

An LT2 Hfr strain, *his metC gal*, was crossed to a multiply marked LT2 F^- line. Analysis of recombinant yields, segregation of unselected markers and interrupted matings indicated injection of the Hfr chromosome in the sequence

$$\leftarrow ile—rha—mal—inl—(ara,—azi,—pro)—gal—(fla,\ his)—adeC$$

The introduction into the Hfr of the colicine factors *colI*, *colE1* and *colE2* and the R factor *R2* had little or no effect on its fertility. All four factors were transmitted

at low frequency to the F^- population, and to recombinants at higher frequencies (*colI* 5–30%, *colE1* 30–80%, *colE2* 5–30%, *R2* 0–9%). Transfer of *colE1* occurred before 20 min., that of *colE2* and *colI* later than 100 min. Segregation data did not reveal close linkage of any factor to any chromosomal locus, but recombinants with a long stretch of donor chromosome were more likely than others to have acquired *colE2* and *colI*. Nearly all recombinants and F^- cells which acquired *colI* or *colE2* acquired both, and *colE1* also. Most cells which acquired *R2* acquired one or more colicine factors. These plasmid associations can be formally represented by transfer of plasmids, independently of the chromosome, in the sequence *colE1*—(*colI*, *colE2*) —*R2*. Phage P22 grown on the Hfr carrying the four plasmids transduced the *tet-r* trait of *R2* at very low frequency, and the *sul-r str-r* characters, together, at low frequency. Some of each sort of drug-resistance transductant, but no transductants in respect of chromosomal characters, acquired *colE1 or colE2* by co-transduction.

This account is based on the University of London Ph.D. thesis of E. D.

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