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Conjugation, Colicinogeny and Transmissible Drug-Resistance

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Preface

Bacterial plasmids take many forms and are found in nature as sex factors, colicin factors, phages, and most recently of all, as the R factors responsible for the transmissible drug-resistance that has become so widespread. Their similarities have been recognised as more significant than their differences since the earliest days of bacterial genetics, and, moreover, many of their properties are now known to be shared with the extra-chromosomal DNA of higher cells. It is these general biological properties that form the subject of this book. This is not to disregard the practical significance of plasmids, many of which are of the greatest importance as chapters 1 and 7 will show, but this is a field that needs separate treatment. Still less has it been possible to include the whole literature, and my aim has been to relate past and present observation, and observations from different disciplines, in an attempt to show how they bear upon each other.

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Contents

1. Context and definitions

To begin by defining the scope of this book: many species of bacteria possess, in addition to their chromosome proper, other genetic elements which lead an independent existence within the cell: these are *plasmids*. Some can unite with the chromosome by crossing-over: these are *episomes*. In practice, the existence of a plasmid is usually recognised by the functions it determines. This may be resistance to sulphonamides or antibiotics, as with drug-resistance (R) factors; the ability to transfer genes by conjugation, the operational characteristic of a sex factor; or it may be the synthesis of a protein coat which enables the plasmid to be transmitted in extracellular particles like those of bacterial viruses.

Even these few examples are enough to show that that part of bacterial genetics which is specifically 'bacterial', as distinct from that which is broadly 'genetical' like replication, coding, transcription, and so on, is largely taken up with the study of plasmids. Without them, many of the classical experiments in molecular biology would have been impossible. Some twenty-five years of intensive research have produced a large body of data on the structure and function of the plasmids which form the subject of this book. The data have, in fact, become so numerous and so detailed that a much larger book could have been written on phage λ or the F sex factor alone, and the most that a fairly short account can now hope to achieve is to indicate some of the problems and the nature of their solutions, particularly by including something on comparable non-bacterial autonomous genomes like the DNA of polyoma virus[103,575] and of organelles like mitochondria[94,299,391,451] the study of which is in many ways more advanced than that of the bacterial plasmids. The fundamental properties of all these genomes are largely identical and it is now almost pointless to consider one group without reference to the others. What has been omitted here are details of technique. The bacteriological aspects have already been described elsewhere,[389] but the index to this book gathers the appropriate entries under the heading, *Methods*.

Of course, a completely different book on this subject would have been equally feasible. Bacteriology is of far reaching practical significance and, not surprisingly, plasmids have already become of central importance in many problems of human and veterinary medicine.[155,551,553,612] At present, the most celebrated instance is probably the ubiquitous transmissible drug-resistance of gram-negative enterobacteria determined by R factors which are now found in strains isolated from animals, patients and, even more

alarming, polluted rivers.[12,122,393,394,498,525,583,584] Its national implications and the possibilities of control by allocating individual antibacterial drugs to specific uses were discussed by the Swann Committee, many of whose recommendations have been adopted in the United Kingdom.[458] Once the selective pressure favouring drug-resistant bacteria is lifted by ending the use of a drug, there are good grounds for hoping that the incidence of the corresponding resistance genes will decline. R factors probably existed before the discovery of the agents to which they confer resistance[194,522] but they were not as widespread as they are today. One hopes that the limitation of chemotherapy will gradually bring about a return to the original position. Indeed, a natural process limiting the spread of R factors in enteric pathogens is already known: their preferential transfer to 'rough' strains of low virulence.[298,559,585]

It is an open question whether R factors have become widespread because they are transmissible or because their resistance is so often multiple. The potential dangers of transmissibility are easy to demonstrate in laboratory experiments, particularly by transfer from symbionts like *E.coli* to highly dangerous pathogens like *Shigella flexneri*, *Vibrio cholerae* or *Pasteurella pestis*.[301,362,447] No less alarming than the spread of drug-resistance from symbionts to pathogens is the possibility that pathogens may transfer the genetic determinants of parasitism to otherwise harmless species of bacteria. Transmission of drug-resistance between enterobacteria certainly occurs after feeding R^+ bacteria to experimental animals,[209,454,483,524,534] although it is not quite so clear how transfer occurs in nature. This is hardly surprising, remembering that the normal caecal contents are not only unfavourable to conjugation but severely limit the total number of enterobacteria that are present.[381] Equally, it is not surprising that when the source of these unfavourable factors, the normal anaerobic bacteria of the caecum, is removed or is absent, the number of enterobacteria increases dramatically,[381] as does the spread of R factors.[208] In fact, chemotherapy in the presence of R factors may very well encourage their spread. Nevertheless, it seems at least arguable that the prevalence of R factors in an environment dominated by chemotherapy is largely due to their enormous selective advantage in conferring simultaneous resistance to as many as eight of the anti-bacterial drugs in current use.

An analogous class of plasmids of outstanding practical importance is that determining drug-resistance in *Staphylococcus*, a widespread cause of sepsis in man.[417,418,461,553,612] Conjugation is unknown in this genus and transmission is by transduction. At a genetic level, however, this is a minor distinction and fundamentally the plasmids of staphylococci do not seem to behave differently from those of enterobacteria, despite the differences in terminology used to describe them.

There are, of course, many other examples of practical importance. Plasmids intrude into the working of phage-typing schemes (section 7.4). Some

determine the synthesis of toxins (section 7.5). Others may interfere with clinical diagnosis, notably in enteric disease where the common bacterial pathogens like *Salmonella typhi* are conventionally regarded as non-lactose fermenting—which could be calamitous when they present as fermenters due to carriage of a plasmid.[33,141,156,455]

The terminology used here is as follows. *Plasmid* has its usual meaning of a genetic element able to exist stably in the autonomous, that is extrachromosomal, state.[332] An *episome* is a plasmid capable of uniting with the chromosome.[293] Some plasmids may obviously be episomes but have not yet been shown to be so for technical reasons, notably the lack of a suitable method of selection. The act of uniting with the chromosome is *integration* and the reverse, *excision*.[511] Integration occurs by a reciprocal cross-over between plasmid and chromosome (section 3.1) and, to this extent at least, it resembles recombination between homologous chromosomes. Although the use of 'recombination' to describe crossing-over between non-homologous DNAs, like those of the F sex factor and the bacterial chromosome, can be criticised, its use and derived terms like 'recombination enzyme' or 'site-specific recombination' have become so frequent that they will also be found here. A *transmissible* plasmid can ensure its own transmission by conjugation; a *non-transmissible* plasmid cannot, though it may be *co-transferred* with one that is transmissible. Transmissibility has its own genetic determinants, only now beginning to be distinguished individually (section 4.5). These are often referred to by various portmanteau terms: *sex factor* used originally for the F factor as a whole, *fertility factor*, *conjugal fertility factor*, *resistance transfer factor* (RTF), *transfer factor* and so on. All these will eventually be superseded by the names of the specific gene loci carried by a particular plasmid but, in the meantime, 'sex factor' has proved convenient. 'Resistance transfer factor' is too specific because these functional groups are known to transfer genes other than resistance determinants: while 'transfer factor' has other connotations in biochemistry and immunology. A *sex factor* is, therefore, the complex of genes responsible for conjugation and gene transfer. Some of its genes specify filamentous appendages which are not flagella and have therefore been classed with the filaments named *fimbriae* or *pili* which are formed by many enterobacteria.[60,138,253] In the field of conjugation, 'pili' and 'pilus' are usually used. The pili involved in gene transfer are conveniently called *sex pili*,[385] with *pilin* for their protein, giving sex factor, sex pili, pilin. Sex pili serve to classify naturally occurring sex factors into major groups, at present two in number which are exemplified by F, the sex factor of *E. coli* K12, and by I, the sex factor of the plasmid colicin factor I (section 4.2). Within each group, many minor differences are apparent and so they are best termed 'F-like' and 'I-like' to convey that each group is not homogeneous. The pili not directly involved in transfer also differ markedly amongst themselves[138] and, in view of their widespread occurrence, can be grouped together as *common pili*. A sex factor exerts two other named effects. It largely pre-

vents its host accepting the same sex factor by conjugation, the phenomenon of *exclusion*, also called *surface exclusion* or *entry exclusion*, suggesting that its mechanism is known which is not the case (section 4.4). In the minority of cells that do accept, the resident sex factor prevents the replication of the donated plasmid (section 6.2). Whatever the latter mechanism may prove to be, there is an exact parallel in the behaviour of lysogenic bacteria superinfected by their phage to which they are necessarily immune. This second effect on replication is therefore best called *superinfection immunity*. The two terms are sometimes used in the reverse sense.[584] Genetic symbols generally follow the usual convention[127] and the properties of the various chromosomal loci of *E. coli* K12 (figure 3.1) and *Salmonella typhimurium* LT2 can be traced from reviews.[389,557] Plasmid-borne resistance loci follow a different convention used widely in the literature[583] which serves to distinguish them from the chromosomal loci producing the same phenotype. Thus, the plasmid allele determining streptomycin-resistance is written (Sm) while the chromosomal allele is *str-r*. Other plasmid genes determine resistance to sulphonamides (Su), tetracycline (Tc), chloramphenicol (Cm), kanamycin (Km), and so on. Colicin factors are designated by their type and their original host:[175] thus ColIb-P9 is the plasmid determining colicin Ib which was discovered in *Shigella sonnei* strain P9.

Two trends are apparent at the time of writing. The first is a shift in interest at the fundamental level from phage and bacteria to more complex subjects like animal cells. However, the problems that remain unsolved in this field of bacteriology are amongst the most central of the subject, extending as they do from the mechanisms co-ordinating replication of plasmid and chromosome to the phylogeny of plasmid-determined characters. One question which is at present almost completely obscure is the biological significance of the antibiotic-inactivating enzymes determined by R factors. It is almost certainly wrong to think of the antibiotics in current use as rare and unusual. They belong to a large class of related compounds synthesised during microbial growth[670] and may conceivably be formed in reactions by which the antibiotic-producing organism detoxifies its own metabolic products.[636] The well-known antibiotics are merely representatives selected for their high toxicity for pathogenic micro-organisms and low toxicity for their hosts. Antibiotic-inactivating enzymes may represent the next step in natural habitats, in which antibiotic molecules are in turn detoxified by organisms other than those which produce them. Reasonable though this explanation may be, it remains speculation until the ecology of these enzymes is understood. If they were originally specified by chromosomally-located genes in species so far not identified, there are two well-recognised mechanisms by which a chromosomal gene could become autonomous in a plasmid; namely, chromosomal pick-up (section 2.1) and duplication (section 2.4). Once autonomous, the spread of a gene is considerably simplified because its survival in a foreign species becomes much more likely when it is part of a self-

replicating autonomous plasmid than when it can survive only by integrating in the recipient's chromosome, a far more complex process which is likely to fail when the gene and its new host are unrelated (section 2.4).

The second trend is the application of existing knowledge to new types of problem. To take but two examples. The only methods available for determining the true division rate of bacteria within infected animals depend entirely on the discoveries of phage genetics.[368,378] Their success is due to the fact that a phage provides a far more sensitive method of labelling than an isotope since it can be induced to replicate autonomously at will, so increasing the unit label—the individual phage genome— by many orders of magnitude. The other obvious application is to the study of bacterial toxins. Diphtheria toxin is a prime cause of the disease and its synthesis has been known to be determined by phage for some 20 years, yet the details are still far from agreed.[31,32,251] Despite this and other suggestive observations on the synthesis of toxins by streptococci[622] and staphylococci,[132] the whole field of toxin synthesis is still open for analysis. It would, however, be completely misleading to expect these problems to be solved instantly by applying what we know of λ and of F. Bacterial genetics as a whole will no doubt continue to throw up fresh surprises, while each new system has its own peculiarities. What is certain is that the analysis of these applied problems will only be possible if we understand the behaviour of the classical systems, systems which became classical precisely because they were in a sense simple to analyse.

2. The discovery of plasmids

The identification of the first bacterial sex factor, the F factor of *Escherichia coli*, is now part of the history of modern bacteriology. The immediate result was a great advance in our understanding of gene transfer by conjugation but, beyond this, F provided a model of a new kind of autonomous genetic element which later could be invoked as an example, whether rightly or wrongly, in the analysis of other genetic systems. There was a time, about 1958, when the main problems of bacterial conjugation appeared to have been resolved but, as time passed and F and other plasmids were examined in greater detail, it became clear that the earlier explanations were far from universally valid and that numerous difficulties remained (section 4.3). To take only two points, it is still uncertain if chromosome transfer due to F, not to mention other sex factors, is invariably preceded by its integration in the chromosome, let alone how the plasmid or the chromosome comes to be transferred from cell to cell.

2.1. The F factor

The existence of F came to light during the analysis of genetic recombination in *E.coli*. In the original experiments, two distinct lines of multiply-marked mutants were derived from strain K12 which have dominated the literature ever since.[329,338,339] The first line was *thr leu thi* and is exemplified by strains W10 and W677; the second was *met bio*, of which a well known member is strain 58–161, now *bio*$^+$.[89] The mutations of one line of mutants were thus complementary to those of the other. When cultures of the two lines were mixed and plated on unsupplemented glucose-salts agar, a small proportion of the mixed culture (ca. 10^{-7}) formed colonies which proved to be prototrophic recombinants. It might be noted in passing, that these experiments succeeded where earlier attempts had failed not only because strain K12 was F$^+$ but also on account of their design. The nutritional markers could be used to prevent growth of the parents and to select rare recombinants. Moreover, knowing that a singly-marked mutant could revert to prototrophy at a frequency up to 10^{-7}, revertants were avoided by choosing multiply-marked parents. Thanks to this powerful selection, which was missing in the experiments of earlier workers, only recombinant colonies appeared on the plates.

The early K12 crosses established, amongst other important findings, the haploid nature of bacteria,[329] the exceptional occurrence of partial diploids

after mating,[330,337] the presence of linkage and the ordering of different markers in genetic maps, and the demonstration of dominance and recessiveness.[330,331] At the same time, as more and more data accumulated, the bacterial genetic map began to appear more and more complicated and, to that extent, somewhat contrived. It became necessary to consider whether, for example, the map was branched[337] or whether the bacterium contained several chromosomes.[598] Throughout this period, the presumption was that the donor and recipient cells contributed genes equally to the progeny of the mating and fused to form a complete zygote from which recombinants segregated during subsequent growth. The presence of a sex factor determining a difference between the roles of the two parents was unsuspected, and mating ability was assumed to be an inherent property of all mutants of K12. The first sign that this view of conjugation was in error came from experiments designed to measure the kinetics of recombinant formation using the K12 strains, 58–161 and W677. One of the two parental strains was streptomycin-sensitive (*str-s*) and the other, streptomycin-resistant (*str-r*). After mixing broth cultures of the two strains to allow mating, colony counts were made on unsupplemented glucose-salts agar selective for recombinants and which also contained streptomycin to kill the *str-s* strain. The then surprising finding was that recombinants were only obtained when W677 was *str-r*. In fact, 58–161 could be treated with streptomycin for as long as 18 h before its addition to W677, without abolishing recombinant formation. This immediately indicated that complete cell fusion could not be occurring as it would then have been immaterial which strain was streptomycin-resistant. Thus, the two strains were evidently not equivalent, and W677 was therefore suggested to be the recipient of genes transferred from the donor, 58–161, which then became redundant.[221] A dramatic difference between 58–161 and W677 was also revealed by exposing them separately to u.v. radiation before mixing: irradiated 58–161 gave many more recombinants whereas irradiated W677 gave many fewer.[222]

What, then, distinguished the donor from the recipient, both being descended as they were from the same ancestor, K12? At the time, the relevant genes might have been in either the donor or the recipient, or in both. Fortunately, however, a number of spontaneous variants of the putative donor strain, 58–161, chanced to be isolated independently in the U.S.A.[75,335] and in the U.K.[223,224] which were infertile when mated with W677, whereas all the clones of W677 remained fertile with the original 58–161. Tests of the various strains in all possible permutations led at once to a definition of fertility as a function determined by a gene named F.[335] Retrospectively, it became clear that the ancestral strain, K12, was F^+, as were its *met* mutants like 58–161; while the *thr leu thi* line including W677 had become F^- very early in its history on mutating from leu^+, probably as a result of repeated exposure to X-rays during mutagenesis.[335] It was, of course, a fortunate chance that a strain like K12 which carried a sex factor was chosen initially

for this work which otherwise could not have succeeded. At the time, the chance of a given strain of *E.coli* possessing a sex factor capable of bringing about chromosomal recombination was about 1/50[335] which would account for failures with other naturally-occurring strains like B and L-15.[128,463]

Many of the fundamental properties of F were noted almost as soon as it was identified. F was clearly far more readily transmitted than the chromosome by F^+ cells, e.g. 10–100% of F^- cells became F^+ in 60 min. Evidently, it underwent 'extra-nuclear' transmission[335] and had a 'free cytoplasmic existence',[224] and was even at that time recognised as a 'plasmid',[75] already distinguished as a class of autonomous extra-chromosomal genetic elements.[332] In view of the great increase in chromosomal recombination produced by u.v.-radiation and the contemporary interest in u.v.-induction of prophage, F was inevitably discussed as an infectious agent akin to a 'latent bacterial virus' [335] or a 'non-lytic infective agent',[223] although it was also clear from experiments with culture filtrates that F could only be transferred by cell-to-cell contact.[75,223,224,335]

Another important property of F came to light when a clone, HfrC, derived from a mutagenised F^+ culture was found to behave quite differently from the usual F^+ donor.[76] A second clone, HfrH, with similar properties was subsequently found in an F^+ culture stored at 4°.[224] Although these unusual clones transferred many of their chromosomal markers far more frequently than did F^+ strains (and so were called 'Hfr' for 'high frequency of recombination'), the recombinants were never F^+. Neither were they Hfr, unless certain chromosomal genes were selected.[224] Also, these Hfr strains reverted to F^+.[75,335] The explanation is now well known: in an F^+ strain, F is autonomous whereas in an Hfr strain, F has integrated in the chromosome at one of many possible points (section 3.1, figure 3.1). Both F^+ and Hfr strains conjugate and transfer DNA equally readily and, with either, the first DNA to be transferred is probably part of F (section 4.5). When this forms part of an autonomous F factor, the remainder of the F genome must follow almost immediately so that F is transferred as a whole. The chromosome is usually unlinked to F in an F^+ strain and is transferred far less frequently. When F is integrated in an Hfr strain (i.e. linked to the chromosome), its leading part and the adjoining chromosome are presumably transferred as frequently by an Hfr donor as is F by an F^+ donor. That part of F in the leading DNA is, however, separated from the remainder of the F factor by the whole length of the bacterial chromosome and, as transfer is usually interrupted spontaneously by separation of the mating cells, the distal part of F rarely reaches the recipient, which correspondingly rarely becomes Hfr. The exception is when the experiment selects one of the last chromosomal markers due to be transferred, because this adjoins the distal part of F. Many recombinants then appear Hfr, as expected.[224,291,292]

In an Hfr strain, the chromosome is transferred sequentially in a given order. Independently isolated Hfr strains usually differ, however, in the order

in which their chromosomes are transferred. One may transfer *thr* first while another may transfer *pro*, and so on. In every case, however, F appears as the last chromosomal marker and consequently is rarely wholly transferred to recipients. In contrast, one Hfr strain gave rise to clones with curious properties. These transferred F very frequently, and so appeared F^+, but at the same time they reverted to Hfr far more often than a conventional F^+ strain. Furthermore, Hfr strains derived from this F were always of the same sort.[2] The explanation appeared to be that the F of the original Hfr had reverted to the autonomous state, taking with it a piece of the adjoining chromosome which subsequently provided a region homologous with the original chromosomal site of integration (figure 2.1, section 3.3). Because of this, the F factor always re-integrated very readily at this site in the chromosome.[245,286] Remembering that the usual Hfr donates F as the last chromosomal marker about 120 min after mixing the two parental cultures, whereas autonomous F is donated immediately, these variant F factors could be isolated from an Hfr culture by selecting for *early* transfer of the *last* chromosomal gene located next to F. This gave, amongst many examples, F linked to lac^+ or to gal^+ in plasmids now referred to as F*lac* or F*gal*, or more generally, as F-prime (F′) factors.

An autonomous F′ factor behaves much like autonomous F. It is a single linkage group, is transferred rapidly, may become integrated in the chromosome to give an Hfr strain, and is lost spontaneously during growth of the culture. Moreover, an F*lac* from *E. coli* can be transferred to bacteria like *Shigella*, *Salmonella* or *Pasteurella*.[286,362] From the point of view of plasmids in general, the formation of F′ factors showed that chromosomal genes could come to form part of an autonomous plasmid and be subsequently transferred by conjugation, even to different genera.

The last notable function of F to be discovered in the fifties was its ability to bring about the transfer of plasmids which alone were not transmissible. Thus, the colicin factor ColE2–P9 was not transferred between two F^- strains of *E.coli* although transfer occurred if either strain was F^+. In the cross, $Col^+F^- \times Col^-F^+$, the transfer presumably occurred in two stages: F first passed to the Col^+ strain and then returned with the Col factor to the Col^- strain.[174] This phenomenon of *co-transfer* is not a unique property of F and is now known to be shared by many other sex factors (section 4.3). But, once again, the behaviour of F provided a valuable model for the interpretation of later experiments with Col factors and R factors.

2.2 Colicin factors

Although the colicins of *E.coli* and other antibiotics formed by bacteria have been known for decades,[175,309,416] the autonomous nature of colicin factors, the plasmids determining the colicins, was revealed more recently, following the discovery of F. It first became apparent in $F^+ \times F^-$ crosses

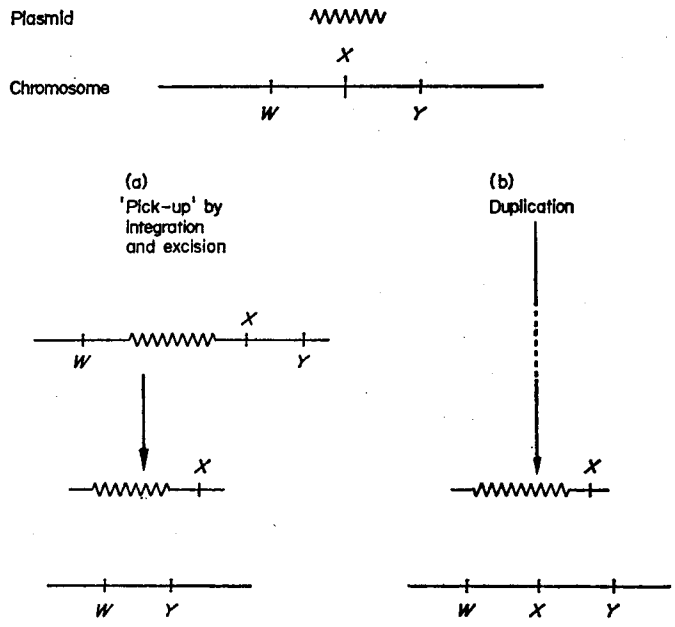

Figure 2.1. 'Pick-up' and duplication. An autonomous plasmid in a host with the chromosomal genes, *W X Y*. *X* could become linked to the plasmid either (a) by 'pick-up' entailing a preliminary integration of the plasmid near *X* followed by excision of the plasmid and *X*, leaving the corresponding deletion in the chromosome (figure 3.4c); or (b) by a duplication in which a second copy of *X* appears in the plasmid, leaving the chromosome unaltered.

where the recipient carried ColE–K30[178] and selection was made for chromosomal recombinants. The striking result was that, regardless of which recombinants were examined, all were Col^+ like the recipient. In other $F^+ \times F^-$ crosses, one or other parent carried ColE–K30. When the F^- recipient was Col^+, all classes of recombinants were once again Col^+: but when the F^+ donor was Col^+, some 60% of recombinants were again Col^+, not Col^- like the original recipient, as would be expected if the *col* gene was chromosomal.[179] The *col* gene therefore behaved unlike any of the fourteen chromosomal genes tested in these crosses; that is, it behaved as non-chromosomal or autonomous. This conclusion has been repeatedly confirmed for many Col factors, as in Hfr $Col^+ \times F^-Col^-$ crosses using a variety of Hfr donors, each differing in the sequence of chromosome first transferred. In every cross, regardless of which donor is used, the *col* gene is equally rapidly transferred, again demonstrating its independence of the chromosome.[88,404,405]

In these experiments with ColE–K30, it is implicit that the Col factor is

non-transmissible alone and that its transfer is due entirely to F. Many other Col factors are similarly non-transmissible although certain ColB, ColV, ColE, ColI and ColK factors are able to bring about their own transfer, in every case, by conjugation.[173] These transmissible *col* genes must therefore be associated with sex factors and, although transmissibility does not in itself show that the sex factor and *col* gene are linked (section 4.3), they do appear to be so in ColB, ColV, ColI and ColK.[377] These Col factors are therefore analogous to F′ factors in so far as they consist of a sex factor linked to unrelated genes (unlike the genes of an F′ factor, however, the origin of *col* genes is unknown), although they have very often been discussed as homogeneous entities. Many strains carry two unlinked Col factors, a notable example being *Shigella sonnei* P9, the source of the well known factors, ColIb–P9 and ColE2–P9.[176]

The study of ColI transfer yielded a result of great general importance for the analysis of sex factors. This was the discovery in *Salmonella typhimurium* LT2 that, although only 0.1% of cells in a culture which had carried ColI for many generations could transfer their factor in the usual period of 20 min allowed for mating ('low frequency transfer', or LFT), as many as 100% could transfer the factor if they had acquired it within the preceding few generations ('high frequency transfer', or HFT:[546]). A long-established $ColI^+$ 'donor' strain was grown overnight with a Col^- 'intermediate' strain in the ratio of 1/20. The Col factor passed from the donor strain to a few cells of the intermediate strain which, being newly made Col^+, could transfer efficiently to other Col^- cells, and so on, to produce an 'epidemic spread' of ColI throughout the intermediate strain. With properly chosen conditions, 50–100% of intermediate cells in an overnight HFT mixture acquire ColI sufficiently recently for them to transfer it at high frequency to a third Col^- recipient strain. At the time of this discovery, the episome model was much under discussion, and the alternation between the LFT and HFT states was tentatively attributed to the Col factor alternating between integration and autonomy. Since then, however, it has become clear that LFT and HFT reflect the effects of repression on sex factor function (sections 4.2: 7.1.2).

2.3 R factors

Drug-resistance factors were first detected in 1957 in Japan when patients with bacillary dysentery yielded strains of *Shigella* resistant to many of the antibacterial drugs then in use. The striking findings were, first, that, in a single epidemic, both fully-sensitive as well as multiply-resistant strains of the same pathogen could be isolated, even from a single patient; and, second, that multiply-resistant strains of *E.coli* were also often obtained. It was therefore suggested that the sensitive organisms were acquiring drug-resistance not by successive chromosomal mutations, but by simultaneous acqui-