Origin of Transferable Drug-resistance Factors in the Enterobacteriaceae

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The observation of a rise in multiple resistance to antibiotics in shigellae by Japanese workers, and their demonstration that this resistance could be transferred to other Enterobacteriaceae by conjugation (Ochiai *et al.*, 1959; Akiba *et al.*, 1960; Watanabe, 1963), opened a new phase both in bacterial genetics and in the study of drug resistance in this group of organisms. Briefly, this type of resistance is transferred from cell to cell by resistance factors (R-factors), which have been postulated to consist of the genetic determinants for drug resistance (R-determinants), and resistance transfer factors (R.T.F.s). It has been assumed that the R-factors are cytoplasmic in location and that the communicability of the complex is dependent on the R.T.F.s, which are episomal in nature.

The drug resistance is usually multiple and is directed against the antibacterial drugs commonly used in human and veterinary medicine—for example, ampicillin (A), chloramphenicol (C), neomycin (N), kanamycin (K), streptomycin (S), sulphonamides (Su), and tetracycline (T). Work in the Enteric Reference Laboratory has been concerned particularly with transferable resistance in *Salmonella typhimurium*. Common resistance patterns currently found in this organism are: S T Su; A S T Su; and S T N K Su. To these combinations furazolidone resistance is now usually added in cultures of both animal and human origin. Anderson and Lewis (1965a) recently drew attention to the disturbing rise of multiple drug resistance in *S. typhimurium*. Of 450 cultures of this serotype examined in the Enteric Reference Laboratory between December 1964 and February 1965 273 (61%) showed drug resistance, mostly multiple, and one particular phage-type, 29, predominated with 168 cultures—61.5% of the total number of resistant strains. At the time of the survey almost all cultures of type 29 examined were drug-resistant. We have continued monitoring this situation and will publish a detailed analysis of our results later. However, it can be stated that of approximately 4,700 cultures of *S. typhimurium* received between December 1964 and October 1965 1,700 (36%) belonged to phage-type 29; that only 40 (2.4%) of the type 29 cultures were completely sensitive to drugs; that 500 of the 1,700 cultures were of human and 1,200 of animal origin; that the overwhelming majority of animal cultures were isolated from calves; and that many of the human infections were directly related to bovine disease. The principal reservoir of type 29 is thus bovine. The prevalence of furazolidone resistance in cultures of human origin was similar to that in bovine cultures, an indication that most human infections of undetermined source were bovine in origin, since furazolidone is most widely used in efforts to prevent and treat calf scours. Only a small minority of the drug-resistant cultures of phage-type 29 have so far been tested, but all those examined could transfer at least part of their drug resistance to suitable recipient cultures, and there is no reason to doubt that the great majority will behave similarly.

We have already pointed out (Anderson, 1965a; Anderson and Lewis, 1965a, 1965b, 1965c) that the high incidence of drug-resistant phage-type 29 in calves is probably due to a combination of poor conditions of hygiene in animal husbandry, especially in the intensive farming, transport, and marketing of calves, and the distribution of infected stock by dealers; and by the too free use of antibacterial drugs in efforts to control the resultant salmonellosis.

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It was recently demonstrated (Anderson, 1965b; Anderson and Lewis, 1965c) that the R.T.F.s and the R-determinants behave as basically independent elements which become associated with each other to form R-factors when they are present in the same cell. Without the R.T.F.s the R-determinants cannot be transferred by conjugation; without the R-determinants the R.T.F.s have no drug resistance to transfer. As the R.T.F.s will mediate the transfer of genetic determinants not only for drug resistance but also for colicinogeny and other characters, the term "R.T.F." will be abandoned hereafter in favour of "transfer factors." These factors, among which the "sex" factors of *Escherichia coli* should be included, mediate the exchange of genetic information by conjugation in the Enterobacteriaceae, although this exchange may be of little practical importance outside the field of drug resistance (Hayes, 1964).

Experimental Work

A test has been devised for detecting transfer factors in strains of Enterobacteriaceae fully sensitive to drugs (Anderson, 1965b; Anderson and Lewis, 1965c). This consists in mixing the wild (donor) strains with a strain carrying an R-determinant but no transfer factor (intermediate recipient), and adding to the mixture a fully drug-sensitive culture (final recipient) which carries neither a transfer factor nor an R-determinant. If the donor strain contains a transfer factor this migrates into the intermediate recipient and combines with the R-determinant to form an R-factor, which is then transferred into the final recipient. A given R-determinant may be mobilized in this way by different transfer factors, and a given transfer factor will mobilize different R-determinants.

The intermediate recipient usually used in these tests is a strain of *S. typhimurium* into which an R-determinant for streptomycin and sulphonamide resistance has been introduced; it is unable to transfer its drug resistance until it is "infected" by a transfer factor. The final recipient is *E. coli* K12F–. Of 90 wild drug-sensitive strains of phage-type 29 of *S. typhimurium* examined, 57 (63%) carried a transfer factor. About half of these sensitive type 29 strains were isolated before drug resistance became a problem in this type and before the type itself became a problem in calf infection.

A proportion of wild drug-resistant strains of *S. typhimurium* will not transfer their resistance. On the assumption that these strains carry R-determinants only, the test described above was modified to demonstrate that the introduction of a transfer factor into such strains would mobilize their R-determinants, which would then be transferred to cultures fully sensitive to drugs. For this test the donor was a strain of *S. typhimurium* into which a transfer factor without drug resistance had been introduced in the laboratory. Wild strains of *S. typhimurium* phage-type 44 resistant to streptomycin and sulphonamides, but unable to transfer this resistance, were used as intermediate recipients. The final recipient was, as before, *E. coli* K12F–. Only three strains of phage-type 44 have so far been tested in this way, but all behaved as predicted—that is, their streptomycin-sulphonamide resistance was transferred to the final recipient. This verifies the hypothesis that they originally contained only the R-determinant for streptomycin-sulphonamide resistance, and required the introduction of a transfer factor before this resistance could be mobilized.

This type of experiment may be varied by using the drug-sensitive donor strains as both donors of transfer factors

and final recipients of the R-factors formed by combination between non-transferring R-determinants and the transfer factors. Although it was expected to encounter an exclusion effect, because the presence of a transfer factor in a strain obstructs superinfection with an R-factor which owes its mobility to the same transfer factor, it was found experimentally that the exclusion effect was not serious. The mating mixtures in these crosses consisted of drug-sensitive strains containing transfer factors (=donor-final recipient) and drug-resistant strains containing non-transferring R-determinants (=intermediate recipient). When the donor-final recipient was *S. typhimurium,* the intermediate recipient was a suitably selected strain of *E. coli*; when the donor-final recipient was *E. coli,* the intermediate recipient was a strain of *S. typhimurium.*

As an example of this technique, in a mixture containing a wild drug-sensitive strain of phage-type 29 of *S. typhimurium* carrying a transfer factor as the donor-final recipient, and *E. coli* K12F− carrying a non-transferring R-determinant for ampicillin resistance as the intermediate recipient, the *S. typhimurium* strain became resistant to ampicillin. The R-factor formed by the entry of the transfer factor of type 29 into the ampicillin-resistant strain of *E. coli* K12 had infected the type 29 donor strain to make it resistant to ampicillin.

Discussion

The experiments reported above strongly suggest that the transfer factors and the R-determinants are initially independent, and also suggest a mechanism for the formation of R-factors in the wild state. It can be assumed that transfer factors are present in drug-sensitive *E. coli* and other Enterobacteriaceae, as well as in salmonellae in the animal and human intestine, and it has already been pointed out that strains of Enterobacteriaceae may carry non-transferring R-determinants. Contact between strains carrying these two components separately in the intestine of an animal host will lead to migration of the transfer factor into the strain carrying the non-transferring R-determinant, converting this into a transferable R-factor. The frequent presence in an animal of a drug to which a strain carrying a non-transferring R-determinant is resistant will confer a survival advantage on that strain, and will therefore result in its "enrichment" in relation to non-resistant organisms. The intrusion of a strain carrying a transfer factor into such a population will be followed by spread of the transfer factor throughout the resistant strain, with resultant conversion of the non-transferring resistance into transferable resistance by the formation of the respective R-factor. Thus, the free use of antibacterial drugs probably hastens the initiation and spread of transferable drug resistance.

The demonstration that strains donating transfer factors can also act as final recipients of the R-determinants mobilized by their transfer factors shows that transferable drug resistance can spread under natural conditions into the drug-sensitive donor strain responsible for its initial mobilization. Transfer factors were common in phage-type 29 of *S. typhimurium* before drug resistance became a serious problem in *S. typhimurium.* Although their presence indicates that type 29 was an efficient donor of transfer factors and mobilizer of R-determinants, the fact that drug resistance became a major problem in this same type establishes that the transfer factors operated to the benefit of their original donors by returning to them carrying the R-determinants.

The origin of the R-determinants is unknown. Because of the ease with which they are mobilized by transfer factors, it seems probable that they are cytoplasmic rather than chromosal in location at the time of their mobilization. On the other hand, Anderson and Lewis (1965c) have suggested that the tetracycline resistance of one of their strains of phage-type 29 of *S. typhimurium* was originally chromosal, and that the initial low-frequency transfer of this resistance to *E. coli* K12, which they observed, was a rare instance of the pick-up of the tetracycline-resistance gene from the chromosome by the transfer factor. Once mobilized, this resistance was transferred to recipient cells with a frequency approaching unity—a frequency similar to that of the transfer factor alone. There are two other possible origins of R-determinants: the spontaneous release of drug-resistance genes from the bacterial chromosome into the cytoplasm; or the existence of intrinsically cytoplasmic determinants for drug resistance. If such cytoplasmic determinants exist, and there is a good deal of evidence to suggest that they do, a much wider range of bacterial characters than drug resistance may prove to have cytoplasmic as an alternative to chromosomal inheritance.

Whatever the origin of the R-determinants, they need not be initially common. The selective conditions provided by the widespread use of the antibacterial drugs to which they correspond are sufficient to guarantee their ultimate ascendancy. And the same conditions will ensure their eventual mobilization and spread by the transfer factors which seem to be widely distributed in the Enterobacteriaceae. The advantages offered by the antibiotics extend not only to the R-factors formed in this way, but also to the transfer factors, which often spread from strain to strain at a much higher frequency than the complete R-factors.

The nature of the transfer factors is a matter of conjecture. They are "infective," and we have shown that some of them produce specific changes in the phage-sensitivity patterns (phage-types) of host cells of *S. typhimurium* (Anderson and Lewis, 1965c). These properties suggest that they are related to temperate bacteriophages. But their obligatory transmission between intact cells indicates that their structure is devoid of an independent invasion mechanism. There is evidence that the bacteria they inhabit may provide the structures necessary for their transmission, in the form of fimbriae or pili (Brinton *et al.,* 1964; Meynell and Datta, 1965). The transfer factors of the Enterobacteriaceae may thus represent a race of "viruses" with a degree of host-dependence higher than that of the temperate phages, in that they can operate only from the intact cell. Although they may carry potentially lethal characters such as colicinogeny, there is hitherto no evidence that the transfer factors themselves are potentially lethal to their host cells. It would be interesting to know whether analogous agents exist in the higher forms of life. If they do, and if they can carry genetic characters, we should be faced with an intriguing field of speculation.

Summary

Transferable drug-resistance factors (R-factors) in the Enterobacteriaceae are formed by combination between two initially independent elements—transfer factors and resistance determinants. R-factors will spread not only in the drug-resistant strains initially receiving the transfer factors but also into the strains from which the transfer factors originally came. Drug-sensitive strains of phage-type 29 of *S. typhimurium* frequently carried transfer factors before drug resistance became a problem in *S. typhimurium.* The fact that transferable drug resistance now predominates in this phage-type indicates that the R-factors owing their formation to the transfer factors of type 29 have been returned, by these transfer factors, to type 29. The formation and spread of R-factors are probably hastened by the use of antibacterial drugs corresponding to the resistances concerned.

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Medical Memoranda

Hypertension Due to Renal Artery Obstruction in Eskimo Girl: Case of Primary Arteritis of Aorta

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A recent leading article (*B.M.J.*, 1963) emphasized the importance of arteritis involving the renal arteries in the pathogenesis of arterial hypertension in young people, with special reference to cases of primary arteritis of the aorta described by Danaraj and Wong (1959) and Danaraj *et al.* (1963). The case reported here illustrates the diagnostic problems encountered in these patients.

Case Report

An Eskimo girl aged 17 was admitted to University Hospital, Copenhagen, on 22 February 1962 with a diagnosis of chronic glomerulonephritis and hypertension.

At the age of 11 she had been admitted to a hospital in Greenland with haematuria, hypertension (blood-pressure 150/120 mm. Hg), and mild proteinuria, and a diagnosis of acute glomerulonephritis was made.

She was readmitted at the age of 14 because of recurrent attacks of fever, headache, and vague abdominal pain for several months. On admission she was febrile and complained of right-sided chest pain, and radiology showed some opacification in the costophrenic angle. The blood-pressure was 160/110 mm. Hg, the E.S.R. 33 mm./hour, and the urine contained a little protein, numerous red cells, and a few granular casts.

In August 1961, when she was 16, she was readmitted with a left facial paresis which never resolved. The blood-pressure was recorded only once and found to be 125/80 mm. Hg, the E.S.R. was 24 mm./hour, and the urine contained a little protein with a few red and white cells and granular casts.

In December 1961 she was readmitted with severe haematuria. She had been complaining for a few weeks of pain in both flanks, dysuria, frequency, weakness, and nausea. She was afebrile, the blood-pressure was 165/125 mm. Hg, and the E.S.R. 20 mm./hour.

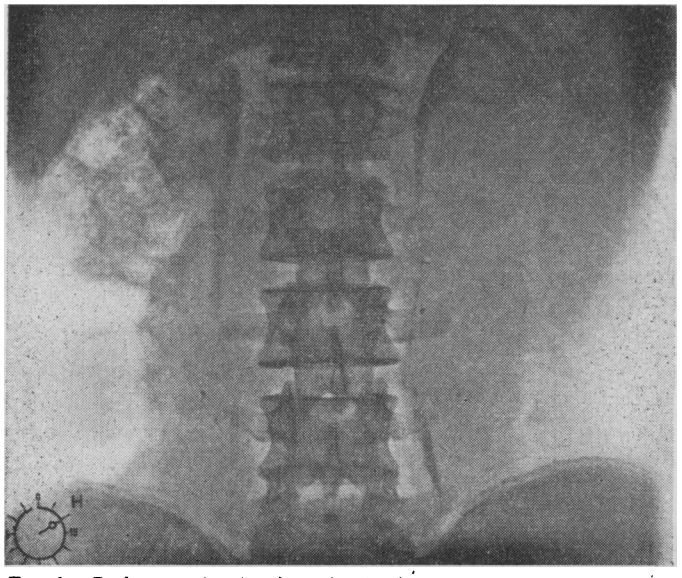

FIG. 1.—Pyelogram showing irregularities in upper and middle thirds of left ureter.

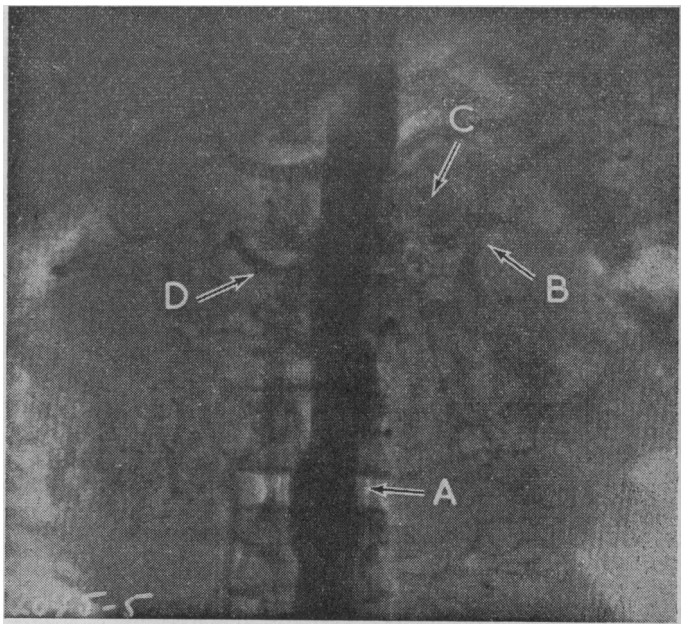

FIG. 2.—Abdominal aortogram showing irregular contours of aorta (A). Both kidneys are visualized and on the left side an excretory pyelogram (B) is seen as well as a plexus of small tortuous vessels (C). The right renal artery (D) is normal.

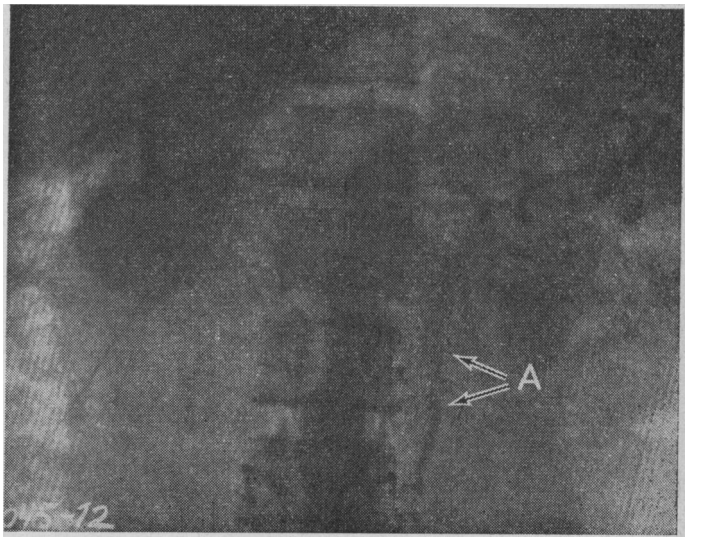

FIG. 3.—Later film demonstrating well-developed plexus of ureteric collateral vessels on left side (A).