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INTRODUCTION

The discovery of infectious (transferrable) drug resistance resulted from careful epidemiological observations on the changing pattern of drug resistant strains of Shigella in Japan (Watanabe, 1963a). From 1957 onwards a large and increasing percentage of these strains, isolated from cases of bacillary dysentery, were found to be resistant to one or more of the commonly used antibiotics (for convenience, synthetic antibacterial drugs will be included in this term), namely, streptomycin, sulphonamides, chloramphenicol and tetracyclines. In a single outbreak of dysentery, resistant shigellae were isolated from some patients; even the same patient sometimes yielded sensitive and resistant shigellae of the same serotype. Moreover, the administration of a single antibiotic to patients harbouring a sensitive organism could cause them to excrete bacteria that were resistant to all four of the aforementioned drugs. In some cases, patients who harboured drug-resistant shigellae also harboured strains of Esch. coli that were also resistant to these four antibiotics.

About this time, it was widely accepted that the principal mechanism responsible for acquired drug resistance was spontaneous mutation at a low rate to a single drug resistance followed by the preferential growth of the resistant cells in the presence of that particular drug. However, this concept of spontaneous mutation followed by environmental selection could not account for these patterns of multiple drug-resistance in shigellae nor the rapid increase in its incidence, and Japanese workers proposed that the transfer of multiple drug resistance from multiple drug-resistant Esch. coli to shigellae in the intestinal tract of the patients was responsible for the epidemiological findings. Such a transfer was demonstrated independently by
Ochiai et al (1959) and Akiba et al (1960) by cultivation of a mixture of resistant and sensitive strains in vitro and subsequently in human volunteers (Kagiwada et al, 1960) and in the sterile intestinal tract of newly hatched chicks (Walton, 1966a).

THE TRANSFER OF RESISTANCE

The original Japanese workers provided evidence that the transfer of multiple drug resistance involved the process of conjugation - the material transferred from donor to recipient cell being termed an R (resistance) factor (Watanabe, 1963a). First of all, microscopic examination of a mixed culture of resistant and sensitive bacteria revealed pairing between the two kinds of cells. Also, when a mixed liquid culture was agitated in a blender to break off any cell to cell contact, and the culture was then diluted to prevent further pairing, the transfer of resistance ceased. It was thought that neither transformation nor transduction were involved in the transfer of resistance because both free DNA and cell free filtrates, extracted from the resistant organisms, were incapable of converting sensitive recipient cells to resistance. Moreover, it seemed that the bacteria acquired a new mechanism for the promotion of conjugation, since the transfer of resistance did not depend on the presence of F (the classical sex factor of Esch. coli that promotes cell conjugation and the transfer of genetic material from a "male" to a "female" cell) - transfer occurring between F- cells.

However, it is now known that bacteria carrying R factors, like those carrying F, possess specialized pili or "sex fimbriae", and that only cells bearing these structures are able to promote conjugation and the transfer of resistance (Lawn et al, 1967). Bacteria that have recently acquired R factors can transfer it at very high frequency, so called "High Frequency Transfer Systems" (Watanabe, 1963b), and this ability, although being lost after several division cycles, accounts for the rapid spread of the R factors through a bacterial population and so is
largely responsible for the infectious nature of the conjugal transfer of multiple drug resistance. The low frequency of transfer in those cells carrying R factors for many generations has been shown to be due to the relative scarcity of cells with "sex fimbriae" (Watanabe, 1967) and it seems certain that they are involved in the process of conjugation but their precise function has not yet been established beyond doubt. Brinton (1965) suggests that they may serve as the conjugation tubes through which the genetic material passes from donor to recipient, but the possibility remains that they may simply be a means of making the initial cell-to-cell contacts, so bringing the bacteria into apposition.

Early on in the study of infectious drug resistance it was recognized that known chromosomal traits of certain bacterial strains, such as the inability to synthesize particular substances, were not usually transferred along with the resistance traits. Also, the resistant cells became resistant almost immediately after the transfer occurred whereas chromosomal drug resistance is ordinarily expressed only after the drug-sensitive genes have been lost in the course of cell division by the process known as segregation. From these observations, it has been concluded that R factors are extra-chromosomal elements (Watanabe, 1963 a). A number of additional observations have since confirmed the cytoplasmic nature of these factors (Watanabe, 1966); these may be summarised as follows:

a) R factors are acquired by bacteria only by infection from other R factor containing cells, never by spontaneous mutation in which case the genes responsible for resistance are chromosomal.

b) the R factors can be eliminated from the bacterial cell by acridine dyes, which are known to eliminate various cytoplasmic factors, like the sex factor F when it is in the cytoplasm (F+ state) but not when it is chromosomally integrated (as in the Hfr state) - suggesting by analogy that the R factors are in a cytoplasmic state.

c) the rapid spread of R factors through a bacterial population as in the "High Frequency Transfer Systems" occurs
at a much faster rate than the overall growth of the culture. Since chromosome replication is synchronized with cell division, the R factor in such systems is replicating faster than the chromosome, and must therefore replicate outside the chromosome, that is, in the cytoplasm of the cell.

Although it was originally reported that transduction was not involved in the transfer of drug-resistance, later workers succeeded in transducing multiple drug-resistance in vitro between lines of Salmonella typhimurium LT-2 by phage P22 (Watanabe & Fukasawa, 1961b), and between salmonellae of group E by phage epsilon (Harada et al, 1963). However, the relative importance of transduction in the in vivo transfer of multiple drug-resistance between gram-negative bacteria is largely unknown. It seems that even if such a mechanism did take place in vivo, the mass of genetic material (and hence the spectrum of drug resistance) transferred could be limited by the size of the transducing phage. Furthermore, owing to the limited host range of phages, transduction is for the most part intra-species, whereas conjugal transfer of multiple drug resistance can take place, in addition, between organisms of various gram-negative species, such as Escherichia, Salmonella, Shigella, Citrobacter, Klebsiella, Proteus, Pseudomonas, Serratia, Pasteurella and even Vibrio cholerae (Datta, 1965).

But the transduction of drug resistance has attracted attention ever since Ritz & Baldwin (1958) showed that typing phage 52 could carry penicillinase determinants from penicillin-resistant to penicillin-sensitive strains of Staph. aureus in vitro. Since then a similar mechanism has been shown capable of transmitting resistance to streptomycin (Morse, 1959) tetracycline (Collins & MacDonald, 1962), the macrolide group of antibiotics (Pattee & Baldwin, 1962) and chloramphenicol (Collins & Ray, 1963). Transduction of drug resistance between strains of Staph. aureus has also been achieved in vivo (Novick & Morse, 1967) but it is not definitely known to what extent transduction is responsible for drug resistance in clinical isolates of staphylococci. A property of many
staphylococci responsible for epidemics of hospital infection is resistance to mercuric chloride (Moore, 1960) and co-transduction of this character with the penicillinase determinant has been demonstrated by Richmond & John (1964); more recently, MacDonald (1966) has shown that staphylococci of the phage types associated with outbreaks of infection in hospital (types 52/52A/80/81 complex of Group I or Group III) are also those which readily acquire the ability to produce penicillinase and resistance to antibiotics by transduction in vitro. The available evidence thus suggests that the rapid emergence of penicillin-resistant staphylococci during the 1950's was brought about by the dissemination of penicillinase determinants by transduction.

Also of interest is the recent recognition of transduction of drug-resistance in staphylococci as a phenomenon somewhat analogous to the conjugal transfer of multiple drug resistance in gram-negative bacteria. Most of the work has been done on the penicillinase determinant: this character is lost at a high rate (c. about 1 per $10^3$ generations) from the cell (Barber, 1949); also, when the transducing phage preparations are irradiated with ultra-violet light, there is an exponential fall in transducing frequency with irradiating dose (Novick, 1963). These two features are shared by known extrachromosomal elements in bacteria (Richmond, 1968), suggesting by analogy that the penicillinase determinants, like the R factors, are plasmids (extrachromosomal elements of genetic material). But the available evidence is not conclusive, for a number of strains of Staph. aureus have been reported in which the penicillinase determinant behaves like a chromosomal gene in transduction (Ashehov, 1966): the frequency of transduction being initially low, but is markedly stimulated by ultra-violet irradiation. The fact that a determinant for mercury-resistance has recently been located on a number of R factors of coliform bacilli has further stimulated speculation as to the ecological similarity between these factors and the staphylococcal plasmids (Novick, 1967). But as far as it is known, staphylococcal plasmids, unlike R factors, are unable to promote conjugation: conjugation having never been observed to occur in staphylococci, nor, for that matter in any gram-positive bacteria.
COMPONENTS OF R FACTORS

There is considerable evidence that R factors consist of two components -

a) the resistance determinants; extrachromosomal genetic elements that confer drug resistance on the host bacterium, but which in the absence of a "Resistance Transfer Factor" (RTF), are transmitted in a lineal fashion only in the host bacteria.

b) the "Resistance Transfer Factor": the extrachromosomal element, the presence of which determines the ability of their hosts to conjugate and their auto-transmission throughout a bacterial population: the RTF, although transmissible, has no resistance per se to transfer.

The independent viability and potential autonomy of the resistance determinants on the one hand, and of the RTFs on the other, can be demonstrated by various experiments in vitro. These show that dissociation between the RTF and the R-determinants, and between the determinants conferring resistance to individual, unrelated drugs, occurs either spontaneously or during transfer by conjugation or transduction. For example, in the transduction of R factors between lines of Salmonella typhimurium (Watanabe & Fukasawa, 1961 b) the great majority of transductants lose the ability to transfer the acquired resistance by conjugation and there was also segregation of the sulphonamide-streptomycin-chloramphenicol-tetracycline resistance pattern into sulphonamide-streptomycin-tetracycline resistant and tetracycline-resistant types. That the loss of auto-transmissibility is due to lack of the RTF and not to a defect in the bacteria is evident from the fact that conjugal transfer is regained when the same transfer factors are introduced into the transductants by conjugation; nor is the loss of auto-transmissibility due to immobilisation of the resistance determinants because of chromosomal integration, since infection of the transductants with the sex factor F restores the ability of the bacteria to transfer resistance, independently of chromosomal transfer.

Furthermore, Anderson & Lewis (1965 b) have shown that RTFs exist in a majority of drug-sensitive strains of Salmonella typhimurium by a form of experiment involving the mixing of 3 cultures: the drug-sensitive culture of
Salm. typhimurium suspected of carrying the RTF (A), another culture of Salm. typhimurium possessing R-determinants only (B), and a drug-sensitive 'indicator' strain of Esch. coli (C) carrying a specific selective marker. Neither A nor B can transfer resistance to C, so that the acquisition of resistance by C is the result of the two elements in the first two cultures combining and being transferred to C. These investigations indicated that 63% of 90 sensitive strains of Salm. typhimurium carried RTFs and, more recently, Lewis (1968), using the same experimental approach, has found that 3% of a sample of 300 strains of Esch. coli isolated from a general population, carried RTFs not associated with R determinants.

It is now evident that the RTFs are also capable of potentiating the conjugal transfer of colicinogenic factors (Anderson & Lewis, 1965 b), surface antigens (Ørskov & Ørskov, 1966) as well as the genetic determinants for resistance to ultra-violet irradiation (Smith, 1967 a), heavy metal resistance (Smith, 1967 b) and haemolysin and enterotoxin production (Smith & Halls, 1967), and it seems likely that the range of genetic characters associated with RTFs will continue to increase as more refined methods for their recognition are employed. It is thus clear that the term "Resistance Transfer Factor" implies a spurious functional specificity in these agents and they are better termed simply "transfer factors". One potentiality of clinical importance of transfer factors is their possible effect on virulence in potential pathogens. Although the property of virulence is controlled by a multiplicity of genes, it can be imagined that an organism may reach the state in which the addition of a single specific character will endow it with virulence for a given host. This is pure speculation at present, but that it may occur is suggested by Formal et al (1965) who crossed a Hfr strain Esch. coli K12 (which is non-pathogenic) with an avirulent mutant of Shig. flexneri, thereby restoring virulence to the latter.

STRUCTURE AND ORIGIN OF R FACTORS

Based on experiments on the transduction of multiple drug resistance in Salm. typhimurium (see previous section)
Watanabe (1963 a) proposed that the resistance determinants were distributed on a linear linkage group, at one point of which was inserted the transfer factor. However, attempts to separate the R-determinants by interrupting the conjugal transfer of multiple drug resistance failed: either no resistance at all was transferred, or all the determinants were transferred en bloc; in other words, a gene order could not be demonstrated by conjugation. A different view was put forward by Anderson & Lewis (1965 a) on the basis of conjugation experiments carried out with multiple drug-resistant Salmonella typhimurium phage type 29. These workers noted that the determinants of three resistances - ampicillin (A), streptomycin (Sm), tetracyclines (T) - were transferred independently and at different rates during conjugation with a sensitive strain of Escherichia coli K12; thus, while the transfer frequencies of A and Sm alone were about $10^{-2}$, that of T about $10^{-6}$ and that of the joint transfer of A and Sm were about $10^{-3}$. The fact that the joint transfer of A and Sm was ten-fold higher than would be expected by chance ($10^{-2} \times 10^{-2} = 10^{-4}$) suggested that these two independent structures frequently became associated on the same structure. Anderson & Lewis thus proposed that the only linkage between the R-determinants was their independent association with the transfer factor during joint transfer.

The method of formation of the complete R factor has stimulated much discussion. Anderson (1965 b) has suggested a possible method of formation of R factors by demonstrating that transfer factors devoid of R-determinants can, upon passage through an organism carrying R-determinants, pick up these determinants and together form an infectious R factor. However, in the situation where this has been demonstrated, the R-determinants involved were actually R-determinants whose original transfer factor had been lost. This begs the question as to the location of R determinants when they first emerge in their bacterial hosts.

The transfer factor and the sex factor F show many similarities (vide infra), and Watanabe (1963 a) has postulated that the relationship between the transfer factors
and R factors (transfer factors + R determinants) is comparable to that between the F+ and F' states of F. This implies that the R factors develop as a result of chromosomal 'gene pick-up' by transfer factors: the R determinants would then be of chromosomal origin, and the R determinants and transfer factor together would form a single episomal unit. There is, however, little support for this hypothesis apart from the successful incorporation of supposedly chromosomal resistance elements into a preformed R factor. Thus, Mitsuhashi et al (1962) derived a tetracycline-resistant strain of Esch. coli 0-26 by exposing a large number of cells to the antibiotic in vitro. Subsequent passage of a 3 drug-R factor through the organism led to the transfer of tetracycline resistance as well as that of the other drugs. Although the authors provided no evidence for a chromosomal origin of the tetracycline-resistance determinant, the kinetics of the transfer suggested such a possibility: when the original three drug R factor received by Esch. coli 0-26 was transferred from that organism to a third host, the transfer rate was high, whereas the new four drug R factor was transferred at a much lower frequency. A similar low frequency transfer of tetracycline resistance has been observed by Anderson & Lewis (1965 b) in a strain of Salm. typhimurium and these workers suggested that this was an instance of the 'pick-up' of a tetracycline-resistance determinant from the chromosome by the transfer factor. Moreover, in the case of tetracycline resistance, there is some evidence to suggest that both transferable resistance, as well as that acquired by spontaneous chromosomal mutation in vitro are due to specific alterations in the permeability of the cell wall (Izaki et al, 1966; Franklin, 1967).

On the other hand, the mass of present day knowledge is against a chromosomal origin for the majority of R determinants. First of all, the R determinants do not exhibit homology with the chromosome; chromosome integration of R determinants having never been unequivocally demonstrated except where they were associated with the genome of transducing phage P22 (Dubnau & Stocker, 1964) and E 15 (Harada et al, 1967) and were integrated at the phage attachment region. Even so, there is
no clear evidence that such chromosomally integrated R determinants can pass reversibly from the chromosomal to the extrachromosomal state like episomes. Also, apart from tetracycline resistance, differences in the biochemical mechanisms and kinetics of drug resistance conferred by extrachromosomal and chromosomal elements makes a chromosomal origin for the R determinants even less feasible. A striking example is the case of streptomycin resistance which, in Esch. coli, usually arises by a single chromosomal mutation to a high level, and is recessive to sensitivity in diploids; the effect of mutation being an alteration in the structure of the ribosomes (so that, unlike sensitive bacteria, they are resistant to streptomycin in both in vivo and in vitro protein synthesis) (Brock, 1966). In contrast, the streptomycin resistance conferred by R factors is low level, does not result in altered ribosomes and, as is evident from its rapid expression in infected cells, is dominant to sensitivity (Rosenkranz, 1964).

As far as the transfer factors are concerned, they show operational similarities to F in that they promote cell conjugation and, in at least some cases, chromosomal transfer into an R- cell, where genetic recombination occurs (Sugino & Hirotta, 1962). But there are quantitative differences between the two: conjugation with R factors occurs at a much lower rate than with F and the frequency of chromosomal recombination with R factors is about $10^{-8}$ to $10^{-6}$ compared with about $10^{-4}$ in F mating. Also R+ cells are neither agglutinated by F-specific antiserum, nor lysed by F-specific ("male-specific") phages (Meynell et al, 1968). These differences were apparently underlined by the observation that many R factors, when present in strains of Esch. coli carrying F, suppressed the function of F - a phenomenon termed "fertility inhibition" (Watanabe et al, 1964). However, Meynell et al (1968) have proposed that this apparent difference between the transfer factor and F really reveals their close similarity in that "fertility inhibition" and the suppression of transfer factor function in those cells carrying R factors for several generations is the result of the production of a repressor by the transfer factor which acts not only on F but also on itself. The temporary absence of this repressor in cells newly infected with R
factors would explain the high frequency of conjugation and transfer of resistance in these systems. All this work has somewhat justified the classification of the transfer factors, the F factor and I (the transfer factor of col I factors) as a group of "sex factors", and the possible viral nature of these factors has been raised. Thus Anderson (1968 c) has emphasized that, like viruses, these factors are infective (although they cannot be obtained in cell-free suspension like virions), possess only one type of nucleic acid (namely DNA), and depend on the metabolic equipment of the host cells to provide energy for their replication and duplication. At least one of the sex factors, namely F, can insert itself into and mediate transfer of host chromosome, and Anderson & Lewis (1965 b) have observed that some transfer factors exert phage restricting effects in Salmonellae which resembles the interference with the multiplication of invading phages by prophages already in the host cell. The available evidence thus suggests a close resemblance between the "sex factors" and prophages. But the evidence is still far from conclusive since, unlike most viruses, the transfer factors are usually very stably inherited in the cytoplasmic state, and except for the "High Frequency Transfer Systems" in cells newly infected with R - and colicin - factors, their replication and segregation are co-ordinated with that of the bacterial chromosome.

EPIDEMIOLOGICAL ASPECTS

The importance of infectious drug resistance in human and animal medicine, and its interest to the fields of microbial genetics and molecular biology, have stimulated a wide search in recent years for R factors in pathogenic and, less frequently, in non-pathogenic enterobacteria, and they have been found wherever they have been sought (Anderson, 1968d). Apart from their world-wide distribution a more alarming finding is that the number of enterobacteria carrying R factors has dramatically increased in the last few years and together with this there has been a progressive expansion of the resistance spectrum conferred by such R factors. A continuing survey, started in 1961, of strains of Salm. typhimurium
isolated in Britain not only illustrates these points but also sheds light on the ecology of R factors (Datta, 1962; Anderson & Lewis, 1965 b; Anderson, 1968 b).

During the period of the survey, the incidence of resistant strains of Salm. typhimurium rose from about 3% in 1962 to 61% in 1965. This was associated with an increase in the proportion of a particular phage type, type 29, which rose from about 10% of the resistant Salm. typhimurium strains in 1962 to more than 60% in 1965. Relatively few of the thousands of drug-resistant cultures of type 29 were tested for transferability of their resistances, but all those that were tested transferred all the R-determinants (apart from the one conferring furazolidone resistance) in vitro. When the sources of these type 29 resistant strains were explored, the great majority (73%) were found to have been isolated from calves with only 3% of these strains isolated from other animals. Also, the infected calves came almost exclusively from farms specialising in "intensive breeding" - the conclusion being that the salmonellosis emerged and rapidly spread among the calves as the result of crowding of animals under very poor hygienic conditions. Numerous antibiotics - furazolidone, ampicillin, chloramphenicol, framycetin, neomycin, streptomycin, sulphonamides and tetracyclines - singly, and often in combination, were used not only in the treatment of infected animals, but also in the prophylactic treatment of apparently healthy animals in affected herds, in an attempt to prevent or limit the spread of the infection. That there was a concomitant progressive expansion in the resistance conferred by the R factors involved illustrates clearly that strains of gram-negative bacteria carrying R-determinants become predominant in the intestinal tract when drugs to which they are resistant are administered to their hosts. Finding that all strains of type 29 isolated before the advent of "intensive breeding" were drug sensitive and almost all of these carried transfer factors, Anderson (1965 b) suggests that the formation of the complete R factor is a result of the entry of a strain carrying a transfer factor into such a population; the mobilization of the R-determinants following
on the introduction of the transfer factor into the strains carrying the R-determinants. He also proposed that the involvement of phage type 29 in the evolution of resistance in this outbreak reflects that the transfer factors involved defined the phage type/manner analogous to the way prophages determine the specific susceptibility of Salmonella strains to the typing phages.

It also turned out that many of the resistant strains of human origin could be directly related to the epidemic of bovine infection. This occurred in farm workers and their families and in veterinary workers handling infected calves; there was also a spread to the general population by way of unpasteurised milk drawn from infected dairy cows. In the instances where direct connection with cattle could not be demonstrated there was considerable indirect evidence that the source of human infection was the affected calves. Type 29 Salm. typhimurium strains accounted for 23% of the cultures from human sources and this phage type also predominated in calves. Also the resistance pattern found in the strains isolated from both calves and man were the same; in this respect, furazolidone resistance, which was prevalent, was especially important, since it occurred with similar frequency in strains from bovine and human sources. This drug is exclusively used for the treatment of infectious diarrhoea in cattle and very little in man, and so resistance to this drug must have arisen in animals. Furthermore, the initial low transferability of tetracycline resistance in most type 29 strains tested was a characteristic feature of both human and bovine strains. In this outbreak, therefore, all the evidence points to a bovine origin of the multiple drug-resistant Salm. typhimurium causing human salmonellosis.

THE THREAT TO ANTIBACTERIAL THERAPY IN MAN AND ANIMALS

The conjugal transfer of multiple drug resistance occurs, as far as is known, only between organisms of various gram-negative species, including most of the Enterobacteriaceae (Datta, 1965; Mitsuhashi et al, 1967). The threat to antibacterial therapy is that by the widespread, and sometimes indiscriminate use of antibiotics in man and animals, a reservoir
of R factors is being rapidly established in the commensal flora of the intestinal tract. R factors conferring resistance to as many as seven different antibiotics—tetracyclines, streptomycin, chloramphenicol, sulphonamides, kanamycin, neomycin and ampicillin—may be subsequently transferred en bloc from the commensal flora to various gram-negative pathogens, rendering antibacterial therapy of certain serious infections ineffective. Also, it is well known that in vitro "sensitivity" patterns of some bowel pathogens, e.g. Salmonellae, bears little relation to the in vivo efficacy of antibacterial therapy; this problem may become of increasing importance generally if, as suggested by Smith & Stewart (1966), R factors are transferred between resistant and sensitive strains during the in vitro sensitivity determination by the disk diffusion method.

The situation in animals has been receiving much attention in recent times. Here, antibiotics are employed in three situations—continuous, low-level feed supplementation for growth promotion, relatively short courses of higher level dosage for prophylactic purposes, and full dosage treatment of infections. That this extensive use of a wide range of antibiotics has resulted in the rapid build up of a population of bacteria carrying R factors has been well documented in the case of Salm. typhimurium in calves (Anderson, 1968 b), non-pathogenic strains of Esch. coli in pigs and poultry (Smith, 1968), as well as in pathogenic strains of Esch. coli in pigs, poultry and calves (Smith & Halls, 1966; Walton, 1966 b). The importance of these findings for veterinary medicine is clear: multiple drug-resistant strains of pathogenic enterobacteria can be expected to cause an increasing proportion of animal infections hitherto caused by sensitive strains. However, what is not immediately apparent is that the emergence and spread of bacteria carrying R factors in animals may have serious repercussions for antibacterial therapy in man. This has been well established in human salmonellosis of bovine origin. But the possible risks do not stop there. It is highly likely that the R factors of such strains, when communicated to man, may be transferred, directly or indirectly, to both pathogenic and non-pathogenic bacteria in the human
intestinal tract. An even greater danger may be provided by
the much commoner and more widespread communication of
multiple drug-resistant, non-pathogenic strains of Esch. coli
of animal origin to man.

At present, it is impossible to distinguish between
R factors of human and animal origin, so no objective estimate
of the animal contribution to the prevalence of R factors in
human enterobacteria can be made. It has been argued that
the size of the animal contribution is exaggerated and
hospital wards, in which it has long been known that the
extensive use of antibiotics promotes the emergence and spread
of drug-resistant strains of pathogens, are par excellence the
place where infectious drug resistance is nurtured and spread.
This may be so at the present time, but there can be no doubt
that there is an animal contribution of R factors, and because
of the disturbing potentialities of the situation, Anderson
has repeatedly called for "a re-examination of the whole
question of the use of antibiotics and other drugs in the
rearing of livestock". The expanding range of antibiotics
employed in animal husbandry and veterinary medicine, and the
increasing trend towards mass medication on the farm
(Editorial, 1968) makes such a reassessment even more pressing.
It is something of a reassurance that, at least in Britain,
some progress in this matter has been made with the setting up
of the Swann committee - "To obtain information about the
present and prospective use of antibiotics in animal husbandry
and veterinary medicine, with particular reference to the
phenomenon of infectious drug resistance, to consider the
implications for animal husbandry and also for human and animal
health and to make recommendations".

As to the threat to antibacterial therapy in man,
relatively little is known, at present, of the prevalence of
R factors in commensal enterobacteria of healthy people.
Lewis (1968) isolated 300 strains of Esch. coli from samples
of faeces from the general population in Nottingham and found
that 72 (24%) were drug-resistant, and 31 (10%) of the 300
strains carried R factors. If one accepts that the strains
from the population used in the study may not differ greatly from those which would be isolated from a truly random sample of faeces of healthy people, then the prevalence of R factors in the English population at large, is at present of the order of 10%. Moorhouse & McKay (1968) examined the faeces of 22 infants on admission to hospital and found that 15 (68%) of these carried R factors in non-pathogenic strains of Esch. coli. Nine of the 22 infants were admitted from other hospitals, and of these, 7 were excreting Esch. coli carrying R factors: the other 13 came direct from their homes, and of these 8 were excreting Esch. coli carrying R factors on admission to hospital. Moreover, the 7 infants from whom fully sensitive strains of Esch. coli were isolated on admission excreted multiple drug-resistant strains within a week; 4 of these infants had no antibacterial therapy in hospital and the other 3 received only one antibiotic. The suggestion that these 7 infants might have acquired the R factors from other children in the hospital is supported by the observation that faecal swabs of 34 (82%) of 41 children from the wards which housed the 22 infants yielded non-pathogenic strains of Esch. coli carrying R factors. Although the numbers involved in the latter study were small, the available evidence suggests that R factors are present to a significant level outside the hospital environment, and apparently freely exchangeable within it.

The transfer of R factors from the normal bowel flora to previously sensitive pathogens has already been incriminated in two outbreaks of infections with organisms carrying R factors. Lewis (1967) reported an outbreak of bacillary dysentery in a mental hospital which continued for several months; patients and "excreters" were treated at various times with ampicillin, ampicillin plus nalidixic acid, tetracyclines, and neomycin plus nalidixic acid, and, at one time, all persons at risk were treated with ampicillin in an attempt to arrest the spread of the infection. The organism involved, Shig. flexneri, was originally resistant to streptomycin and sulphonamides, but during the outbreak various multiple drug-resistant patterns varying from full sensitivity to resistance to six unrelated antibiotics was noted, and this multiple drug resistance was shown to be of the "infectious" type in vitro. The most likely explanation of these findings
is that the epidemic started with the introduction into the ward of an initially fully sensitive strain of Shig. flexneri and that different lines of this strain acquired by conjugation different R factors already present in the commensal enterobacteria of the patients. This is supported by the results of subsequent investigation by Lewis (1968) who found that 49 (21%) of 232 strains of Esch. coli isolated from patients in the same institution carried R factors conferring multiple drug-resistance. The second instance was an outbreak of infantile gastro-enteritis in which Anderson (1968 a) found that infecting enteropathogenic serotypes of Esch. coli carried R factors conferring resistance to eight different antibiotics in differing combinations. Again, the author raised the possibility that the R factors found were originally present in non-pathogenic strains of Esch. coli. Furthermore, the transfer of multiple drug resistance to other pathogens, such as Salm. typhi remains a disturbing possibility. The reality of this danger is borne out by a recent report from Israel of the isolation of a strain of Salm. typhi carrying an R factor conferring resistance to chloramphenicol from the faeces of a typhoid carrier treated with the drug (Sompolinsky et al, 1967). The organism was originally drug-sensitive, so that chloramphenicol resistance had evidently been acquired in the patients intestinal tract, but the origin of the R factor concerned remains unknown.

But the threat of antibacterial therapy is not confined to the serious infections of the intestinal tract; enterobacteria, given the opportunity, can wander far from their normal habitat. This is borne out by the report of Smith & Armour (1966) who isolated four species of gram-negative bacteria - Escherichia, Proteus, Klebsiella, and Pseudomonas - from the urine of unrelated cases of urinary tract infection. Strains of all the species isolated, including 69% of all multiple drug-resistant strains transferred part or all of their resistance to sensitive Esch. coli in vitro. As one of the most significant changes in the overall pattern of bacterial infections since the introduction of antibiotics has been the increasing prevalence of serious infections with gram-negative organisms (Finland et al, 1959; Watt & Okubadejo, 1967), the future will surely see infectious drug resistance presenting an increasing threat to
to antibacterial therapy in man.

What can be done to reverse this trend and to reduce the prevalence of R factors in gram-negative bacteria? R factors have been eliminated in vitro by acridine dyes and mepacrine (Mitsuhashi et al, 1966; Levy & Watanabe, 1966), but not all R factors are sensitive to acridines and the frequency of reversal to sensitivity in vitro can show considerable variations, for example, from 4.2 to 100% in Shigella strains (Watanabe & Fukasawa, 1961a). But the result of a clinical study by Sharda et al (1966) may offer an indication that an attempt to eradicate R factors in vivo may be justified. These workers reported that combined antibacterial-mepacrine therapy eradicated chronic urinary infection in 8 out of 10 children who had received, singly or in combination, five or more antibiotics with no therapeutic benefit. This study is difficult to interpret, however, since the carriage of R factors by the resistant organisms was not investigated.

Reducing the selective pressure exerted in favour of resistant organisms by limiting the types of use and the range of antibiotics thus employed seems to be the only answer to the problem at present. Antibiotics must be used, but in some bacterial infections there may be little or no justification for antibiotic therapy. For example, in most non-invasive salmonella infections, antibiotic therapy neither shortens the duration of the carrier state nor the duration of the diarrhoea (Dixon, 1965), and there is little, if any, evidence that the present day widespread use of antibiotics has ever stopped the spread of "acute infective diarrhoea" once that spread has gathered impetus. There are many other situations both in man and animals where antibiotic therapy is of similar dubious therapeutic benefit, and in these situations, at least, the selective pressure in favour of resistant organisms can be avoided. All this points to what must be started now and continued into the future: a thorough assessment of the aims and achievements of antibacterial therapy both in man and animals.
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