Papers and Originals

Drug Resistance in Salmonella typhimurium and its Implications*

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Summary: A rise in Salmonella typhimurium infection was observed in calves in Britain during 1964–6, following the adoption of the intensive farming method. A single phage type of S. typhimurium, type 29, was incriminated as the major pathogen. Attempts to treat and control the disease with a range of antibiotics were ineffective, but resulted in the acquisition of transferable multiple drug resistance by type 29. The transmission of drug-resistant type 29, directly or indirectly, from bovines to man resulted in many human infections. Transferable drug resistance reaching man from enterobacteria of animal origin may ultimately enter specifically human pathogens. Infections such as that caused by type 29 can be eliminated, not by the massive use of antibiotics but by improvement in conditions of animal husbandry and reduction in the opportunities for the initiation and spread of the disease. A reappraisal is needed of the methods of using antibiotics to determine how these methods can be improved, in order to conserve the long-term efficacy of the antibiotics.

Introduction

For many years we have studied in the Enteric Reference Laboratory the distribution of *Salmonella typhimurium* in all its hosts, animal and human, in order to discover the sources and expose the channels of animal and human infection. The initial method of examination is that of phage-typing, but to this are added whatever other genetic characters may present themselves as being useful for strain discrimination. This work has shown that *S. typhimurium* phage types which are prevalent in particular animal hosts used as human food are also common in man (Anderson *et al.*, 1961; Anderson, 1962, 1964). It has become apparent that for some years bovines have been a major source of human infection with this serotype.

It should be emphasized that, although certain phage types are most frequently associated with certain animal hosts, there is no present indication that this association indicates host specificity of the types concerned. It may be the result of no more than a coincidence at a particular point in time of the organism and its host under conditions suitable for the initiation of an outbreak. Whether the association between parasite and host will persist will depend on the opportunities of transmission of the parasite outside the confines of the herd or animal group in which it precipitated its first outbreak. In this respect the symptomless excreter or incubating case plays an important part in the establishment of chains of infection which may become widespread. Once such a chain has been established its eradication may present a problem of major difficulty.

The adoption of a new pattern of husbandry, such as intensive farming, which may favour the spread of infection, may facilitate the initiation of outbreaks and, because of the special conditions under which rearing is being carried out, seriously impede elimination of infection. Clearly, the more valuable the individual animals, the longer the gestation period, and the smaller the number of progeny per gestation the more difficult the problem, because, while the ruthless sacrifice of stocks to eliminate infective foci may be relatively easy in prolific animals such as fowls costing a shilling or two each, an analogous procedure with larger animals such as calves, which may cost £15 to £20, is unwelcome and may be regarded as impracticable. Moreover, while the elimination of infected "master breeding" fowls from which salmonellae may have originated is a relatively simple matter, the tracing and elimination of infected cows present much more difficulty, and in any case the structure of the calf-rearing industry makes the prosecution of an effective salmonella eradication programme very difficult indeed.

Intensive Calf Farming and Rise in S. typhimurium Infection

The roots of the problem which has occupied my attention for the last few years are twofold: in the first place, there was an apparent lack of sufficient realization that a fundamental change in the ecology of an animal, especially at a time when it was most vulnerable to infection, could precipitate serious infective episodes; and, secondly, it was not appreciated that the control of such episodes lay in improvement in the methods of husbandry, and would not automatically follow, on a penny-in-the-slot basis, by dosing the affected animals with antibiotics and synthetic antibacterial drugs. In the event of failure of antibiotic therapy the results need not be limited to the infected animals, because the emergence of drug-resistant organisms can produce further unfortunate ecological effects, as will be seen.

In the intensive calf-farming method as it was practised during the period to be covered, the calves concerned came from breeders, frequently dairy farmers, who separated the young animals from their dams a few hours or days after birth. These animals were transported, either to dealers' premises or to markets, in vehicles in which they were mixed with calves of different origin. They were then passed to rearers, where they were held up to three months, after which they were passed via dealers or markets, or both, to further rearers, also intensive, where they were fattened to about 1 year or more, when they were sent for slaughter.

Opportunities for the communication and spread of salmonellae (mainly *S. typhimurium*) occurred during transport, in markets, in dealers' premises, and in the resident animals of a farm when a new batch of animals carrying infection was introduced into a herd.

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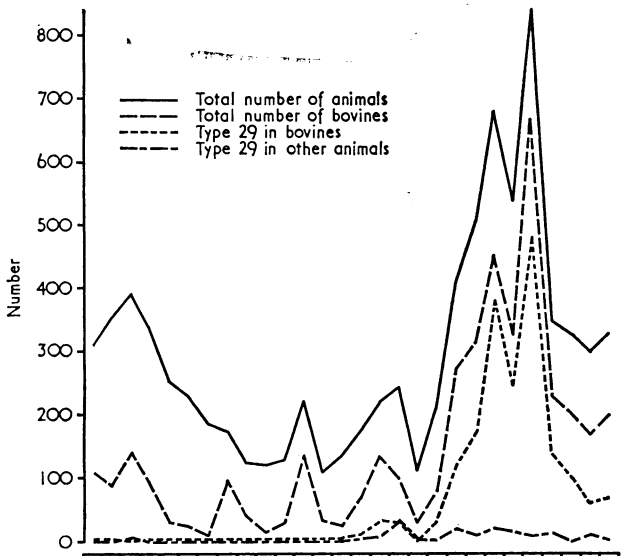
We are accustomed to encountering annual outbreaks of calf infection with *S. typhimurium*. These are associated with the calving season, and are well shown in Fig. 1, which presents the incidence of animal *S. typhimurium* infection in Britain from 1960 to 1966, as represented by cultures examined in the Enteric Reference Laboratory.



	1960	1961	1962	1963	1964	1965	1966	Totals
Number of animals	1386	745	599	637	966	2587	1320	
Number of bovines	430	153	255	259	467	1772	794	
Type 29 in bovines	2	3	2	45	173	1297	347	
Type 29 in other animals	3	3	3	11	52	55	23	

FIG. 1.—Incidence of animal *S. typhimurium* infection, 1960–6.

The top broken line in this graph shows the incidence of *S. typhimurium* in bovines, and the peaks in the autumn and winter quarters indicate the calf outbreaks. Whereas these outbreaks usually subsided during the spring, however, it can be seen that a rise in calf infection which started during the second quarter of 1964 exploded into a protracted outbreak which lasted from the middle of 1964 until the middle of 1966. It should be added that this infection still erupts from time to time to indicate that, although it is no longer so active, it can recrudesce if opportunities of spread present themselves.

Investigations revealed that the majority of the bovines concerned in this outbreak were calves aged a few days to a few months and, moreover, that these were animals being reared by the intensive method. My information is that this method began to be used to an appreciable extent in calves in Britain about 1962–3, and subsequently rapidly increased, so that it was being employed on a large scale by 1964–5.

A pattern emerged in which a high proportion of calves intended for intensive rearing were handled by dealers, who bought them either from breeders or from markets, dispatched them to holding premises, and then distributed them to clients, often crowded in unsuitable transport over long distances. Inevitably, animals of diverse origin were herded together in transport, markets, and holding units, and, equally inevitably, when *S. typhimurium* gained a foothold in animals subjected to this treatment it spread rapidly so that entire herds became infected. The disease usually took the form of diarrhoea with systemic invasion, and the mortality was frequently high. Infected stock were distributed throughout Britain and the disease proved very difficult to control.

Veterinary surgeons were called in to treat sick animals and to control the spread of infection. In accordance with modern practice, it was not surprising that they should prescribe antibiotics for both purposes. Naturally, higher dosage was used for therapy than for prophylaxis. The results in both respects were, however, disappointing. I do not know how many calves died during this period, but the number was large, and the economic loss to individual farmers, both in terms of loss of stock and of expenses of treatment, was frequently heavy.

The Infecting Organisms

As we monitor *S. typhimurium* infection in animals as well as in man, we were aware of the great increase of calf infection from 1964 onwards. For some years we had been studying the occurrence of drug resistance in the organisms received in the Enteric Reference Laboratory. So far as *S. typhimurium* is concerned an impressive picture emerged. Examination of cultures of six common phage types isolated in 1961 and 1962 revealed that, of 1,561 cultures tested, 46 (2.9%) were drug-resistant (Anderson and Datta, unpublished). Tests of cultures isolated during the winter months of 1963–4 and 1964–5 revealed a very different picture (Anderson and Lewis, 1965a), which is shown in the Table.

Antibiotic Resistance in S. typhimurium 1963–4 and 1964–5

	1963–4 November–February	1964–5 December–February
No. of cultures examined	712	450
No. of resistant cultures	150 (21%)	273 (61%)
Commonest resistant phage type	29	29
No. of resistant type 29 cultures	78 (52%)*	168 (61·5%)*

* As percentage of total resistant cultures.

It is apparent from this table that the proportion of drug-resistant cultures had risen to 21% in the 1963–4 group and to 61% in the 1964–5 group. The commonest drug-resistant phage type was 29, which constituted 52% of the 150 resistant cultures in the 1963–4 series and 61.5% of the 273 resistant strains in the 1964–5 series. It was already known from phage-typing examinations that type 29 infections were increasing sharply in calves and man. The high proportion of drug-resistant type 29 strains, and the expansion in range of the resistances, prompted us to examine all cultures of the type back to 1961. The results of this investigation are shown in Fig. 2 and, as these examinations have been continued, are carried to the end of 1966.

In Fig. 2 the incidence of type 29 is given as a percentage of total *S. typhimurium* for the respective years. A number of striking features are evident. In the first place, the proportion of type 29 in the years 1961–2 and during the first three quarters of 1963 was relatively low. Secondly, the dramatic rise in incidence of the type started in 1964 and a high level was

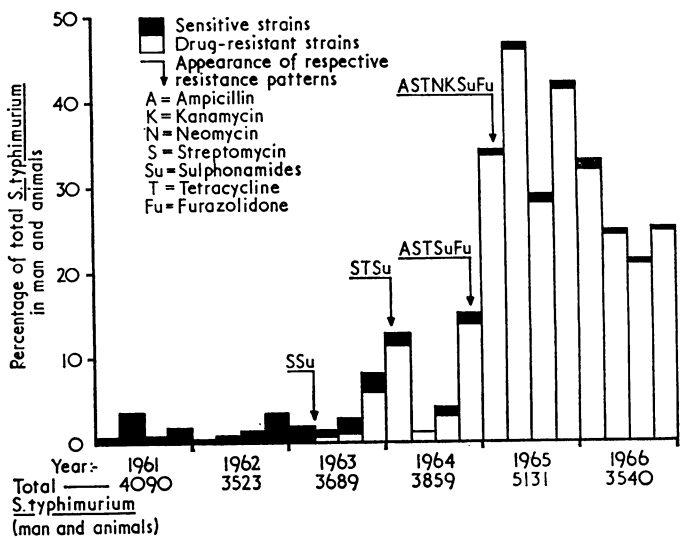


FIG. 2.—Incidence of type 29 as a percentage of total *S. typhimurium*, 1961–6.

subsequently maintained. Thirdly, drug resistance first made its appearance in the type in 1963 and remained a constant feature thereafter, affecting a high proportion of type 29 cultures. Finally, the range of drugs to which type 29 became resistant widened as the type became more frequent. Starting with streptomycin and sulphonamide resistance early in 1963, it acquired resistance to tetracyclines, ampicillin, neomycin, kanamycin, and furazolidone. The latter resistance appeared late in 1964, and chloramphenicol resistance, not shown in Fig. 2, was detected at about the same time.

At an early stage in the studies of the rise in both the incidence and the drug resistance on type 29 we found that the great majority of the animal cultures came from calves intended for rearing by the intensive method. It was evident that the increase in the calf incidence of type 29 had followed the expansion of this industry and resulted from the poor conditions of housing, marketing, and transport already described, which provided the *S. typhimurium* strains with the opportunities for widespread distribution.

In addition to showing the overall incidence of *S. typhimurium* in animals and bovines, Fig. 1 shows the incidence of phage type 29 of *S. typhimurium* in bovines and in other animals. This type was relatively uncommon until the third quarter of 1963, when it increased in bovines, but its frequency fell in the second quarter of 1964. A small peak of type 29 infections in other animals also occurred during this period, though it started after the bovine infections. From the third quarter of 1964 onwards, however, bovine infections with type 29 increased steeply, to reach a high level in 1965, though a diminution was apparent at the end of that year. This diminution was maintained in 1966, but the relative incidence of the type remained high.

In contrast to the frequency of type 29 in bovines during this period, that in other animals, although it was higher than the pre-1963 level, remained relatively low. The high incidence of animal *S. typhimurium* infection from 1964 to 1966 thus reflects the high incidence of bovine infection, largely with type 29, as Fig. 1 shows.

Many patterns of drug resistance were found in type 29, the most extensive being that to ampicillin (A), chloramphenicol (C), kanamycin (K), neomycin (N), streptomycin (S), sulphonamides (Su), tetracyclines (T), and furazolidone (Fu). The commonest resistance patterns in type 29 in 1965 were: SSuTFu; ASSuTFu; and KNSSuTFu. The predominant resistance spectrum at the time of writing is ASSuTFu.

Treatment of Calf Infections

At least one dealer was distributing furazolidone with his calves, with instructions to his clients to administer it to the animals for a few weeks. The inference was that it would act as a prophylactic, presumably against the risk of infectious diarrhoea. As this dealer operated on a large scale the furazolidone probably had extensive use. At what date this practice was started I do not know, but we first found furazolidone resistance in cultures of type 29 isolated in November 1964, and within a short time it was prevalent in the type. Calves distributed by this dealer played a major role in carrying type 29 infection to many parts of the country.

In outbreaks on intensive farms the treatment was predominantly with antibiotics and synthetic antibacterial drugs (these will be referred to collectively as antibiotics hereafter), and fell under two headings: the therapy of sick animals; and the prophylactic treatment of apparently healthy animals in affected herds, in order to prevent or limit the spread of infection. The drugs used for the treatment of sick animals in 1965 were, in order of frequency, furazolidone, ampicillin, chloramphenicol, framycetin, neomycin, streptomycin, sulphonamides, and tetracyclines (Stevens *et al.*, 1967). Combinations of drugs were frequently employed—for example, chloramphenicol was given parenterally and furazolidone orally. Other combinations were used, such as framycetin and neomycin (since these antibiotics are virtually identical, the advantage of the combination is questionable), or streptomycin and sulphonamides (Stevens *et al.*, 1967). Such combined preparations were usually given by mouth.

A study of Fig. 2, which shows the rise in incidence of drug-resistant type 29 and its expanding range of resistance, offers an explanation for the frequent failure of antibiotic treatment.

Frequency of Bovine and Human Infection with Type 29

In 1965 the figures of bovine (largely calf) and of human infection with type 29, as represented by cultures sent for phage-typing, were as follows. Of 1,772 bovine cultures received, 1,297 (73.2%) were type 29, of which 1,294 (99.7%) were drug-resistant. Only 55 cultures of this type, with 43 showing drug resistance, were isolated from other animals (Anderson, 1968a, 1968b).

Human cultures of type 29 examined in 1965 totalled 576, of which 555 (96.5%) were drug-resistant. There were six deaths. As the total number of human *S. typhimurium* cultures received was 2,544, type 29 constituted 23%, and drug-resistant type 29 22%, of all human *S. typhimurium* infection in 1965 (Anderson, 1968a, 1968b). A connexion could often be demonstrated between animal and human infection. This occurred in farm workers and their families and in veterinary workers handling infected calves. Human infection also occurred from raw milk from a dairy farm on which an intensive calf unit had been started. The type 29 infection imported with the calves spread to the dairy cows, the milk of which caused 59 human cases of gastroenteritis (Geoghegan, 1965).

In many instances, however, direct connexion with cattle could not be demonstrated; but we had, nevertheless, convincing evidence of the bovine source of the infection. This can be summarized as follows:

(1) The phage type of the multiresistant human *S. typhimurium* strains, type 29. This type predominated in bovines, but its incidence was low in other animals, as has been shown.

(2) The identity of resistance patterns found in these strains with those found in bovine strains. In this respect furazolidone resistance, which was prevalent, was especially important. It had arisen, not in man, but in calves, in which furazolidone became the most commonly used drug for the prophylaxis and treatment of infectious diarrhoea.

(3) The low transferability of the tetracycline resistance in most type 29 strains tested. This proved to be a feature of human and bovine strains alike, and persists to the present day. This feature also suggested that we were dealing not only with a single phage type of *S. typhimurium* but probably largely with a single strain of that type, which had been presented at a particular time with the opportunity of initiating calf infection, and subsequently with circumstances in which this infection could be widely disseminated. The fact that one calf dealer played a prominent part in this story supports this hypothesis. It is known that he had type 29 infection on his premises, and the first culture of type 29 showing tetracycline resistance of low transferability was isolated in January 1964 from a calf supplied by him (Anderson, 1968b).

There could thus be little doubt that the human infections with drug-resistant type 29 of *S. typhimurium* were almost entirely of bovine origin, and that the drug resistance had arisen in the animal host.

Other Phage Types of S. typhimurium

Many other phage types of *S. typhimurium* have been examined in the Enteric Reference Laboratory. These will not be dealt with in detail, except to say that bovine types predominate in man. In fact, of the 2,544 human cultures we

received in 1965, at least 63% belonged to phage types that are predominant in bovines. Moreover, where drug resistance was prevalent in bovine types it was prevalent in human cultures of the same types. The available evidence again suggested that the drug resistance of the human cultures had arisen almost entirely in the animal host.

This does not exclude the probability that *S. typhimurium* strains sometimes acquire resistance in man, as the result of transfer from non-pathogenic human enterobacteria possessing the relevant resistances. We have encountered a few instances of this, but type 29 almost invariably appeared in man with its resistances fully developed, and this was specially striking when a direct connexion was known to exist between bovine and human infection.

According to a report by Vernon (1966), incidents of human *S. typhimurium* infection fell from 3,176 in 1956 to 1,721 in 1965. It has been claimed (Walton, 1966, 1968) that this fall in the number of human *S. typhimurium* incidents suggests that, although the frequency of calf *S. typhimurium* infection has risen in recent years, the calf is not the main reservoir of human infection. There is a serious flaw in this argument. The figures quoted are crude in the sense that they are not analysed in terms of phage types, and without this information speculation concerning sources of infection is unprofitable, because, as has been pointed out, it is known that certain phage types are characteristically associated with certain animal hosts. The 1956–65 figures of incidents, therefore, give no indication of the sources of human *S. typhimurium* infection.

It is our ambition to reduce *S. typhimurium* in man so far as is possible, both by the control of animal infection and by improvements in hygiene, and the reduction in the number of incidents between 1956 and 1965 is encouraging in this respect. But it should be remembered that man is assailed by this organism from a number of different animal sources, and reduction in infection from some sources may diminish the general incidence of the disease in man sufficiently to disguise the fact that the proportion of infection from other animal sources is static or even increasing. The prevalence in man of predominantly bovine phage types of *S. typhimurium* indicates that bovines are now the main source of human *S. typhimurium* infection in Britain.

It is worth while adding that antibiotic therapy was commonly used in human infections with type 29, but as the drugs employed were often those to which the organisms were resistant there was a notable lack of clinical response. Antibiotic therapy is probably better avoided in this disease in man, unless there is evidence of systemic invasion, in which case chloramphenicol would be the drug of choice, as it is in typhoid fever.

Transferable Drug Resistance

I have concentrated on the practical aspects of the rise of drug resistance in *S. typhimurium*, and though transferable drug resistance played an important part in the acquisition and expansion of the resistances it will only be summarized here.

Because we received some thousands of drug-resistant cultures of type 29 we have been able to test only relatively few for transferability of their resistances. However, all cultures tested would transfer resistance, and it can be assumed that the great majority will do so. Observations in the Enteric Reference Laboratory suggest that the genetic determinants for drug resistance, and the agents responsible for their mobility—the so-called resistance transfer factors, or R.T.F.s, the existence of which was originally postulated by Watanabe (1963)—are basically independent entitities, which become associated with each other to form the transferable resistance factors or R factors (Anderson, 1965a, 1965b ; Anderson and Lewis, 1965b). It was shown that under suitable conditions some R factors would dissociate in transfer to yield recipient strains carrying only the R.T.F. on the one hand, and only the resistance determinants on the other. Without the resistance determinants the R.T.F.s, although transferable, had no resistance to transfer. Without the R.T.F.s the resistance determinants were not transferable. Introduction of an R.T.F. into a strain carrying a resistance determinant resulted in the formation of a transferable R factor.

Surveys of wild drug-sensitive cultures of *S. typhimurium* revealed that many carried R.T.F.s, and examinations of some wild drug-resistant strains of *S. typhimurium* in which the resistances were not transferable showed that they became transferable after the introduction of an R.T.F. (Anderson, 1965a, 1965b). This indicated the probable method of formation of R factors in nature. Strains of enterobacteria carrying resistance determinants become predominant in the intestine when drugs to which they are resistant are administered to their hosts. The entry of a strain carrying an R.T.F. into such a population results in transfer of the R.T.F. into the drug-resistant strains, with consequent mobilization of the resistance determinants, in other words the formation of R factors, which are then transferred to all suitable recipient enterobacteria, including that which supplied the R.T.F. (Anderson, 1965a).

This hypothesis is certainly applicable to type 29 of *S. typhimurium*, because we discovered that all strains of this type isolated before the advent of intensive farming were drug-sensitive, and that almost all of these strains carried R.T.F.s (Anderson, 1965a, 1967, 1968a). The type evidently picked up resistance determinants early in its history of dissemination and, as the antibiotics were brought into extensive use to control the calf outbreaks, it progressively added to its range of resistance both by the acquisition of further determinants and by picking up complete R factors (see Fig. 2). Nevertheless, as I have pointed out, most of the calf outbreaks and their associated human cases may have been caused by a single strain of type 29, because of the special opportunities provided for its spread. The entry and growth of drug resistance in this strain probably took place by the processes already described.

I have referred to the mobilizing agents as R.T.F.s, but they will carry genetic determinants for colicinogeny (Anderson and Lewis, 1965b), and it can be accepted that they carry a wide range of genetic characters. They transfer chromosomal characters, and it has recently been shown that they will transfer (probably extrachromosomal) determinants for haemolysis and enterotoxin production in *Escherichia coli* (Smith and Halls, 1967, and in the press). It is thus clear that the term "resistance transfer factor" implies a spurious functional specificity in these agents, and I have suggested that they should be designated simply "transfer factors," which, in general terms, can be defined as the agents mediating the transfer of genetic characters by conjugation in the enterobacteria (Anderson, 1965a, 1965b, 1967, 1968a, 1968c). The well-known F or "sex factor" of *E. coli* (Hayes, 1953a, 1953b ; Cavalli-Sforza *et al.*, 1953) was the first transfer factor to be discovered.

The transfer factors can infect a wide range of bacterial "genera," including *E. coli, Salmonella, Shigella, Citrobacter, Klebsiella, Proteus, Pseudomonas, Serratia, Pasteurella,* and even *V. cholerae,* which represents a different tribe of bacteria. It should not be forgotten that, in addition to identifiable determinants, transfer factors might carry determinants affecting such characters as virulence. If these were closely linked to the transfer factors concerned, and if the transfer factors also carried resistance determinants, the selective pressure exerted by the presence of an antibiotic could promote the spread of the determinant associated with virulence, perhaps with disastrous effects on the host.

The versatility of the transfer factors in relation to the characters they carry suggests that they may have been important in

bacterial evolution, telescoping its time span into a much shorter compass than that required by the usually accepted mechanisms of mutation and natural selection (Anderson, 1966).

Possible Risks to Man from Transferable Drug Resistance in the Enterobacteria of Animals

We have already dealt with the transmission of a pathogen endowed with drug resistance from animals to man: antibiotics that might be used to treat such human infections are invalidated. But the possible risks to man do not stop there. The transferable resistances of such strains may be transmitted to human enterobacteria, and the use of antibiotics in such conditions will act as a selective influence to ensure that the final enterobacterial population of the host's intestine consists largely of resistant organisms—the original pathogen and the recipients to which it has transmitted its resistance. Ordinarily, these recipients would be non-pathogenic enterobacteria, but the risk also exists of direct transfer of resistance to specifically human pathogens such as *S. typhi* or enteropathogenic *E. coli*. However, a bigger potential risk lies in the creation of a reservoir of commensal enterobacteria carrying stable R factors which may ultimately hand on their resistances to organisms such as those I have mentioned.

A threat to man potentially greater than that resulting from infection with resistant *S. typhimurium* of animal origin is the communication of non-pathogenic animal enterobacteria equipped with transferable drug resistance, which must take place on a large scale. Even if these organisms do not succeed in colonizing the human intestine, there is no reason to doubt that they can transfer their resistance to their human counterparts, and transfer may be such an efficient process that the presence of antibiotics may not be necessary for quantitative conversion of the human enterobacteria to drug resistance. The presence of antibiotics in the human intestine will, of course, make the resistant populations all the more predominant and resistance transfer all the more certain. It is thus possible to establish reservoirs of transferable drug resistance in the non-pathogenic enterobacteria of man by transfer from non-pathogenic enterobacteria of animal origin.

As chloramphenicol remains the drug of choice for the treatment of typhoid fever, a special risk to man lies in the possibility of the transfer of chloramphenicol resistance to the typhoid bacillus. This has rarely occurred hitherto, but it has been reported from Israel (Sompolinsky *et al.*, 1967), though there was no evidence to indicate that the R factor concerned was of animal origin. However, chloramphenicol resistance is easily transferred to *S. typhi* in the laboratory by R factors of either animal or human origin, and the widespread use of chloramphenicol in animal husbandry may add to the risk of eventual communication of chloramphenicol R factors, passed from animals to man, reaching the typhoid bacillus. This possibility is obviously greater in countries where typhoid is highly endemic, so that *S. typhi* is present in the human intestine relatively often.

I have concentrated on transferable drug resistance of animal origin. We know of the dangers it presents to animals, and in a defined sense—that is, in *S. typhimurium* infection—we know of dangers it presents to man. And I have speculated on the possible additional ways in which it may affect man. But this does not imply that I believe that the use of antibiotics in livestock is the major source of transferable resistance in human enterobacteria. These drugs are used very freely in man, often with little discrimination, for diseases of which the infective agents are unidentified. These are uses which should be discouraged. Man has long been able to deal with minor infections, and it would do no harm, and from the point of view of developing immunity it might even do good, to let him resume his fight against these infections, reserving the antibiotics for those cases where their use is justified on clinical or bacteriological grounds, and for specific diseases such as typhoid fever, where they are undoubtedly necessary. Whether it will now ever be possible to achieve such a policy is doubtful, but it would be worth while to try. It would be advisable to abandon the routine treatment of human salmonellosis with antibiotics, reserving them for cases in which there is evidence of systemic invasion. In the latter event chloramphenicol is probably the drug of choice.

Use of Antibiotics as Feeding Additives in Animal Rearing

The most commonly used feeding additives are penicillin and tetracyclines, so that this use now impinges directly on our problem. In Britain, benzylpenicillin, chlortetracycline, and oxytetracycline are the only scheduled antibiotics permitted as additives (Agricultural Research Council, 1962), and up to the present they can legally be used only in fowls and pigs, though to what extent this restriction has been observed it is impossible to say. Though the levels at which these additives are permitted are relatively low they are sufficient to select in favour of drug-resistant strains of *E. coli*, as Smith and Crabb (1957) showed. Moreover, it is now known that tetracycline-resistant organisms emerging after the use of tetracycline feeding additives can transfer their resistance.

It is therefore evident that the use of these antibiotics as feeding additives cannot now be considered separately from their therapeutic and prophylactic uses in animal husbandry.

Tylosin is an unscheduled antibiotic which is on unrestricted sale for use as a feeding additive. This drug is a macrolide, and organisms that become resistant to it also become resistant to other macrolides such as erythromycin and oleandomycin, which are scheduled antibiotics. The use of tylosin as a feeding additive should therefore be subjected to the same critical scrutiny as that of the scheduled antibiotics, and the same principle should be applied to any antibacterial drugs used in animal feeds.

Possible Methods of Improving the Situation

The first and most important of these is to raise the standards of animal husbandry so as to reduce the possibilities of initiation and spread of animal outbreaks of salmonellosis or other types of infectious disease.

It would be an advantage to delay the separation of calves from their dams until the calves are sufficiently robust to withstand the practices associated with intensive farming, and better able to resist infection. Improvement in the conditions of marketing and transport are needed so as to limit the spread of infection. Calves should be bought by rearers from sources known to be reliable, preferably directly from breeders, and the mixing of stock of diverse origin should be avoided. If possible, an all-in all-out stocking policy should be practised by intensive farmers, with disinfection of premises between consecutive batches. If this is not practicable, freshly bought calves should be isolated from stock already held on the farm until the new animals have been shown to be free from infection. In intensive farms the maximum possible space per animal should be allowed, and the general conditions with regard to warmth, lairage, feeding, and attention to the animals should be optimal.

The fact that there has been a fall in the incidence of calf infection with *S. typhimurium* suggests that conditions of calf rearing have improved to some extent. But type 29 still smoulders on in these animals, and a reminder that it retains its sting was provided by a recent outbreak originating in a batch of 72 newly bought calves which arrived at the farm in poor condition. They passed their infection to 15 previously healthy calves already present on the farm. Within 12 days 42 of the 87 calves had died, and most of the remainder were

ill. All 87 were then slaughtered. The organism responsible for this incident was type 29 showing the resistance spectrum ASSuTFu, which is now the commonest pattern found in this type.

It is thus apparent that constant vigilance is necessary to keep infection to a minimum. When salmonellosis does occur vigorous measures to limit its spread should be introduced. These include effective isolation of sick and suspect animals, disinfection of premises, and the comprehensive bacteriological examination of stock, personnel, and premises to detect the full extent of the infection. Organisms isolated should be routinely tested for drug sensitivity and sent for phage typing.

The local veterinary investigation officer, the medical officer of health, and the director of the local laboratory of the Public Health Laboratory Service all have an important part to play in these investigations, and should collaborate freely. This collaboration extends to any reference laboratory to which strains are sent for special examination. In the Enteric Reference Laboratory, our continuous records, and the fact that we are the national centre for this work, enable us to monitor the overall distribution of particular phage types of the organisms we examine at a given time, and also to gain information about their prevalence in perspective. Such information is helpful in the epidemiological study of current incidents of infection.

Energetic efforts should be made to trace the source of the salmonellosis through dealers, markets, and, if possible, to farms in which the original focus of infection may lie. The form of identification of each calf should therefore be adequate for this purpose. If it is discovered that calves from a dealer have spread infection, his stock, premises, and transport should be investigated to determine whether they are still infected. In the event of persistent infection being demonstrated, measures should be instituted to prevent its continued distribution to other farms and to eliminate it from the dealer's premises.

The possibility of making animal salmonellosis a notifiable disease should be studied.

Vaccination.—The development of an effective *S. typhimurium* vaccine for use in livestock might lead to a reduction in the incidence of disease caused by this organism. As most bovine infections occur in very young calves, however, vaccination would have to be carried out as soon after birth as possible.

Use of Antibiotics in Animal Husbandry

As Feeding Additives

A reappraisal of this use is needed in order to assess its present advantages. If it is decided that feeding additives still offer advantages, attempts should be made to find additives which are used neither in animal nor in human medicine, and which present no risk, in terms of tissue residues or of bacterial drug resistance, to animals or man. Regulations would also be needed to prevent their misuse in respect of dosage, or application to animals in which their use is banned.

Antibiotics as Therapeutic Agents

Ideally, antibiotics used in animal therapy should be different from those employed in human medicine, though the range of antibiotics available at present may be inadequate to make this practicable. However, the indiscriminate administration of antibiotics to sick animals suffering from undiagnosed diseases is as undesirable as the similar practice in human medicine.

So far as the treatment of *S. typhimurium* infection of calves is concerned, the information at my disposal suggests that the antibiotics were largely ineffective within a short time of the start of the widespread type 29 outbreak. Whether they were effective in the early stages I do not know. Though it has been suggested that the use of furazolidone is justifiable (Stevens *et al.*, 1967), the predominance of furazolidone resistance in type 29 does not support this view. Moreover, on the basis of the commonest range of resistances now shown by type 29, ampicillin, streptomycin, sulphonamides, and tetracyclines are also unlikely to be effective. Many cultures of type 29 are resistant to neomycin (framycetin) and kanamycin, so that the efficacy of these drugs is also doubtful. Chloramphenicol was apparently occasionally effective, and the proportion of type 29 cultures resistant to it is relatively low, though any incidence of transferable chloramphenicol resistance is disturbing because of the possibility that it may ultimately reach the typhoid bacillus.

With this possible exception, then, there seems to be little evidence that the antibiotics were effective in curing *S. typhimurium* type 29 infection in calves, and the decision to be made is whether it is justifiable to pursue this form of treatment, in view of the potential risks it engenders both for the animal and for the human hosts of these organisms.

Prophylactic Use of Antibiotics

Though antibiotics were used prophylactically on a large scale for the protection of apparently healthy animals in infected herds, this objective does not seem to have been attained in *S. typhimurium* infection. Indeed, it may have done harm when the epidemic organism was resistant to the drugs administered. In this respect it should be remembered that the reduction of competition in the form of the non-pathogenic drug-sensitive bacteria in the animal intestine could actually favour infection with the resistant pathogen, and thereby help it to spread. Moreover, any drug represented in the resistance spectrum of a multiresistant pathogen will act as a selective agent for that pathogen with its multiple resistance.

It should thus be seriously debated whether it would be advisable to abandon the blanket prophylactic treatment of herds known to be infected with salmonellae.

As for "prophylaxis" in herds not known to be infected—and this was certainly in use during 1965—this is indefensible and should be abandoned altogether.

General Investigation of Use of Antibiotics

Provided that reliable information could be obtained, it would be worth while to carry out a survey of the use of antibiotics by veterinary and medical practitioners in order to determine to what extent they are used empirically for conditions caused by unidentified organisms on the one hand or, on the other, for bacterial infections in which the pathogens have been identified and shown to be sensitive to the antibiotics administered.

Reduction of Transmission of S. typhimurium from Livestock to Man

Stockmen and veterinary surgeons handling calves infected with *S. typhimurium* may not have realized the high infectivity of the disease for man, and direct transmission of type 29 to this selected group was relatively frequent. This can probably be reduced by the maintenance of a high standard of hygiene when dealing with infected animals. Nevertheless, it must be accepted that, because of the possibility of heavy environmental contamination by sick animals, the risks of infection of handlers of animals are considerable.

Transmission of *S. typhimurium* to the population at large results occasionally from the consumption of infected raw milk (Geoghegan, 1965). The obvious solution to this type of infection, apart from necessary precautions to avoid infection of

dairy cows and elimination of infected animals, is the pasteurization of all milk, which is long overdue in any case.

Most human infection with *S. typhimurium* probably comes through meat. In this context the animal which is evidently ill is probably not the major risk, because it should not be sent for slaughter for human consumption. The apparently healthy animal which is incubating the disease, or is recovering or has recovered from it, presents a greater risk, because infection may not be apparent. Such animals produce abattoir contamination, so that the organism may be transmitted to animals or carcasses that were originally uninfected.

The build-up of infection at a calf-collecting centre was described by Anderson *et al.* (1961), who observed that the rate of salmonella infection in calves held from two to five days before being sent for slaughter was 35.6%, whereas in calves held for only a few hours it was 0.6%. Naturally, the higher the number of infected animals reaching the abattoir, the greater the risk of contamination of the environment and of healthy carcasses.

It is clear, therefore, that stock should be held for the shortest possible time before slaughter.

Abattoirs should maintain a high standard of hygiene in order to limit the spread of infection. The measures needed to achieve this will not be discussed here.

Carcasses infected with salmonellae will convey the organisms to butchers' shops, where environmental contamination may spread the infection still further.

Finally, infected meat carried into the consumer's home may cause contamination of utensils and working surfaces. Undercooked meat can transmit the infection, but if it is adequately cooked the meat is innocent of direct transmission of salmonellosis. However, the contaminated domestic environment can result in the organisms being carried to a food which is eaten uncooked, or is not heated to a sufficiently high temperature during cooking to kill the salmonellae. These will multiply in such food if conditions are suitable.

Meat from the carcasses of sick animals infected with salmonellae may also reach the public as pet food bought from pet-meat shops which are supplied by knackers' yards (Beasley *et al.*, 1967). Measures should be taken to ensure that all such meat is cooked before being sold to the public.

It is natural to expect housewives to maintain a reasonable standard of kitchen hygiene, but they cannot be expected to handle all fresh meat as if it were infected. Protection of the public at large from infections such as that we have discussed must therefore depend on reduction of the frequency of animal infection at all stages of animal husbandry and slaughter, before carcasses reach the butcher's shop. The events described earlier in this paper indicate that, so far as animal rearing is concerned, such reduction can be achieved, not by the indiscriminate use of antibiotics, but by improvements in the practices of husbandry.

Conclusion

I should like to emphasize that I do not suggest that transferable drug resistance in man is entirely or even largely of animal origin. With the exception of the clearly defined instances of human infection with drug-resistant *S. typhimurium*, I do not know to what extent drug resistance is of animal origin, and unless we can find a method of distinguishing R factors arising in the bacteria of animals from those arising in the bacteria of man we shall have no exact information on this subject.[1] But there can be no doubt that R factors in animal enterobacteria are passed to man, and I believe they represent a danger to be eliminated if possible.

The antibiotics are of great value and should not be squandered by short-sighted uses. Nor should they be used wastefully for conditions in which they are ineffective. I have been pressing since 1965 for a re-examination of their uses in the light of the new information at our disposal, so that policies may be evolved which will protect their long-term efficacy in man and animals. Others have supported the call for such a re-examination. It is gratifying to note that an official study of the subject has now started in Britain. But the terms of reference of the committee carrying out this study suggest that it will operate for a defined period only, after which it will presumably issue a report and be dissolved. A permanent autonomous body is required which, like the United States Food and Drug Administration, has a watching brief that enables it to institute studies of these and related problems as they arise, without the necessity for committees that can be set up only by ministerial decision.

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[1] This could be done if a different range of antibiotics were used in animals from those used in man, and if the two groups of antibiotics gave no cross-resistance to each other. The occurrence of enterobacteria in man which showed resistance to antibiotics of the animal range would then indicate that the resistance had originated in animals