INSTITUTE OF MEDICINE

REPORT OF A STUDY

Human Health Risks with the Subtherapeutic Use of Penicillin or Tetracyclines in Animal Feed

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HUMAN HEALTH RISKS WITH THE SUBTHERAPEUTIC USE OF PENICILLIN OR TETRACYCLINES IN ANIMAL FEED

Committee on Human Health
Risk Assessment of Using Subtherapeutic
Antibiotics in Animal Feeds

INSTITUTE OF MEDICINE
Division of Health Promotion
and Disease Prevention

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PREFACE

In 1980, at the request of the Food and Drug Administration (FDA), a committee of the Assembly of Life Sciences of the National Research Council (NRC) prepared a report evaluating the effects on human health of the use of penicillin and two tetracyclines (chlortetracycline and oxytetracycline) at subtherapeutic concentrations[1] in animal feed. That committee concluded that the postulated hazards to human health from such use of antimicrobials had been neither proved nor disproved. It drew the conclusion largely because a direct detailed epidemiologic investigation of the hazards had not been feasible and in part because it was impossible to ascertain prior antimicrobial exposures of individual animal sources of meat products for human consumption. The committee recommended various epidemiologic studies (especially of human illness due to salmonellae and pathogenic Escherichia coli) and monitoring and surveillance of the occurrence of antimicrobial resistance of enteric bacteria in humans, animals, and foodstuffs.

Several years later, in 1987, FDA asked the Institute of Medicine to conduct an independent review of the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. The Institute established a committee and gave it a tightly drawn charge: specifically, to perform "a quantitative risk assessment" of those consequences--to "assess the adequacy of existing human health data and use such data to arrive at an estimate of

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[1] The Center for Veterinary Medicine considers any extended use of antibiotics in feed at 200 g/ton or less beyond 2 weeks as "subtherapeutic use," whether it is for growth enhancement or disease prevention. "Use levels are generally 200 g or less of penicillin or tetracycline per ton of feed, but dosage units will vary by species. Levels approved for growth claims and disease prophylaxis are usually lower than those approved for disease treatment; however, there is some overlap in the claims for dose levels of 200 g per ton or less." There is more concern in the agency "with the length of time the antibiotic is used in feed than in the level of drug." (FDA personal correspondence, April 26, 1988)

risk, the basis of which will be justified." If complete quantification of human health risks was not possible because of inadequacies of the available data, the committee was to evaluate the scientific information that had become available since the 1980 report and make judgments about the magnitude of the risks. The committee has not addressed risk management, nor any aspects related to policymaking because this was not part of its charge.

In its risk assessment, the committee was to address the following questions:

- Does the subtherapeutic use of penicillin and the tetracyclines in animal feed result in an increased frequency of antimicrobial resistance in pathogens, particularly foodborne pathogens? If so, can the increase in frequency be reliably estimated and compared with the increases associated with other sources of resistance?

- Does antimicrobial resistance increase (or diminish) the ability of foodborne pathogens to cause disease, change the number of foodborne pathogens (dose) needed to produce disease, or alter the severity of disease caused by foodborne pathogens?

- Does the subtherapeutic use of penicillin and the tetracyclines in animal feed result in increased prevalence of pathogens in the animals so fed and in foods derived from them?

- Does antibiotic resistance attributable to subtherapeutic use in feed increase the incidence of foodborne infectious disease in humans or complicate its medical management?

The current committee is well aware of the longstanding uncertainty of the benefits of the subtherapeutic use of antimicrobials in animal feed and its possible restriction in this country and abroad, and it understands the need for a risk assessment as a foundation for risk management in FDA's decision-making (rule-making) regarding the use of feed additives.

It is inherently difficult to relate human morbidity and mortality associated with a specific antibiotic-resistant bacterial pathogen directly to that pathogen's origin in livestock (or poultry) on a farm or in a feedlot and to administration of subtherapeutic amounts (as opposed to treatment amounts) of penicillin and the tetracyclines to the animals. Unequivocal direct evidence linking mortality to the postulated initial events is not available--certainly not in sufficient quantity to establish a cause-and-effect relationship. For want of direct evidence, the committee has

approached its task indirectly by developing a risk model, using the most reliable data available for the individual elements involved, including annual numbers of reported cases of specified infections, fractions of cases due to bacterial strains that show antibiotic resistance, mortality rates, fractions of deaths associated with bacterial strains of farm origin, and fractions of antibiotic-resistant strains of farm origin caused by subtherapeutic use of antibiotics in animal feed. Although some bacterial pathogens (salmonellae, Campylobacter jejuni, enterohemorrhagic E. coli, and Yersinia enterocolitica) are commonly foodborne and of animal origin, salmonella infections are the only ones that have been reportable for many years and for which incidence figures and antimicrobial-susceptibility data have been collected. Salmonellosis has therefore been selected for the risk assessment model, although we acknowledge that several other human infections would also be relevant to our charge.

The committee is particularly conscious of the limitations and inherent weaknesses of the data base used in the risk assessment model. Where an assumption or estimate had to be made, we have stated its basis. We are aware that some estimates used in the model are weaker than others; for example, the fraction of antibiotic-resistant strains of farm origin attributable to subtherapeutic use of antibiotics (or penicillin and the tetracyclines specifically). Because some data for the model were only estimates, we considered a range of values (low, mid-range, and high) for each element and expressed the final risk estimates (deaths per year) as minimum, median, and maximum.

In addition to the risk assessment, the committee has reviewed further new information pertinent to human health that might be related to subtherapeutic use of antibiotics in animal feed. Some of the new information addresses study possibilities identified by the former NRC committee on subtherapeutic antibiotic use in animal feeds. Some of it deals with the biologic impact of antibiotic resistance in bacteria and the use of molecular biologic techniques in identifying clonal features of isolates obtained from farm animals, from foodstuffs derived from livestock and farm animals, and from infected humans. Some of it reflects followup experience in European countries that have, in the last 10-20 years, by regulatory action prohibited use in animal feed of subtherapeutic concentrations of antibiotics that are used in treatment of humans. Much of this information provides only circumstantial evidence bearing on the question under consideration. Some of the facts even appear to be mutually contradictory.

The committee has not addressed any cost-benefit aspects of the issues related to this problem, nor has it made any recommendations regarding regulatory strategies or policies. It hopes that its report on the subtherapeutic use of

penicillin and the tetracyclines in animal feed will be useful to FDA in its consideration of the risk involved and appropriate risk management. The committee stresses the continuing need for more extensive gathering of detailed epidemiologic information to define the human health risks more sharply.

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I

EXECUTIVE SUMMARY

In 1987, the Food and Drug Administration asked the Institute of Medicine to conduct an independent review of the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. The Institute established a committee and gave it a tightly drawn charge: perform "a quantitative risk assessment" of those consequences--to "assess the adequacy of existing human health data and use such data to arrive at an estimate of risk, the basis of which will be justified." If complete quantification of human health risks was not possible because of inadequacies of the available data, the committee was to evaluate the scientific information that had become available since the 1980 NRC report of a similar investigation and make judgments about the magnitude of the risks.

Since the introduction of the first antimicrobials into clinical medicine their use has exerted continuing selective pressure, resulting in an increase in the prevalence of antimicrobial-resistant strains of both primary pathogens and commensal "opportunistic" bacterial species. Succeeding generations of antimicrobials, often possessing broader spectra of activity, have compounded the problem. Over the years, medical practice has yielded abundant examples of the emergence to predominance of strains of common pathogens that bear resistance (often encoded on plasmids) to one or more antimicrobials. In view of this, many have speculated about the need to limit injudicious and unnecessary prophylactic and therapeutic use of antimicrobials in humans. The continued broad, subtherapeutic use of antimicrobials in animal feeds has added to the concern that such practices may contribute to the emergence of resistant strains of bacteria that pose a risk to human health.

Initially, the committee examined epidemiologic studies that employed recently developed molecular fingerprinting techniques capable of directly linking, by clonal characteristics of the etiologic agent, human illness due to a foodborne pathogen (Salmonella) to the same organism with these same clonal characteristics isolated in the food production chain and back on the farm. Although the committee did look at the data available on other infectious bacteria than salmonellae, it was not possible to find a substantial body of direct evidence establishing conclusively

the presence of a human health hazard that resulted from the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds. Nonetheless, the committee believes that important, although as yet sparse, data show the flow of distinct salmonella clones from farm animals medicated with antibiotics in subtherapeutic concentrations, through food products, to humans, who thus acquire clinical salmonellosis. For example, a multiple antibiotic-resistant strain of S. newport originated in farm animals exposed to chloramphenicol, a drug not approved for feed additive use, rather than to penicillin or the tetracyclines. We believe further that application of available methods for clonal analysis of bacterial isolates in future outbreaks of salmonellosis can provide the direct evidence required to relate use of specific antimicrobials on the farm to human infection with antimicrobial-resistant foodborne pathogens; or could show that no relationship exists.

This model estimates risks for salmonellosis, because adequate data were lacking to quantify the risk for other foodborne pathogens, such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli. Furthermore, this report deals only with the risk for mortality from salmonellosis, and does not estimate risk for morbidity due to lack of data.

In contrast to the paucity of direct evidence implicating subtherapeutic use of antimicrobials as a potential human health hazard, the committee found a considerable body of indirect or circumstantial evidence as follows:

o Data on the biologic properties of transposons and R plasmids. The use of antimicrobial agents for either therapeutic, prophylactic, or growth-promoting purposes generates a strong selective pressure for the emergence of drug-resistant bacteria in the environment, and this selection operates at several levels simultaneously by causing molecular expansion of the antimicrobial-resistance genetic elements as well. The basic genetic unit of resistance, the transposon, can occupy almost any location in the genome, but most typically is on a conjugative R plasmid. The ability of the drug-resistance gene to transpose, as a transposon, and to move to other strains and species provides the potential to confer resistance to the selecting antimicrobial on a large population of bacteria.

Studies of bacterial strains differing only in the presence of an R plasmid have generally revealed little difference in virulence. However, the results of R plasmid acquisition can be striking when a conjugative R plasmid contains virulence genes such as ones encoding enterotoxin, aerobactin, hemolysin, or colonization factors in addition to

antimicrobial-resistance determinants. Selection by antimicrobials can promote spread of virulent strains in instances in which R plasmids have incorporated virulence genes or in which virulence plasmids have acquired drug-resistant transposons. Thus, the mere presence of the drug-resistant phenotype may be enough to enhance the disease-producing potential of the pathogen. Most species of Enterobacteriaceae pathogenic for humans and animals carry at least one essential determinant of pathogenicity on a plasmid.

o There is evidence of extensive use on farms and in feedlots of subtherapeutic concentrations of penicillin, the tetracyclines, and other antimicrobials (see Chapter IV). Over 31 million pounds of antibiotics are produced annually in the United States. Although accurate data on antibiotic use in animal feeds are not available, estimates indicate that almost half the total annual production of antibiotics is directed to use in farm animals. The tetracyclines used in livestock and poultry feeds represent almost 50% of the total of antibacterial use in feeds. Almost 90% of all antibiotics used in farm animals and poultry is administered in subtherapeutic concentrations. About 70% of the total of all antibiotics used in subtherapeutic concentrations in animal feeds is given for the purpose of disease prevention (prophylaxis), and the remainder of this amount is administered for growth promotion.

o Ample evidence exists of a high prevalence of antimicrobial resistance among isolates of salmonellae from farm animals. The frequency of resistance to any of the commonly tested antimicrobials among farm-animal isolates of salmonellae ranges from 69 to 80%; of resistance to ampicillin, from 15 to 72%; and of resistance to tetracycline, from 37 to 81%. These frequencies of resistance among animal isolates are 3-5 times greater than those among strains isolated from human beings. Surveys of resistance to various antimicrobials among salmonella and E. coli isolates from farm animals in the United States generally have shown that feeding antibiotics in subtherapeutic concentrations increases resistance to antibiotics. The prevalence of resistance can vary considerably, probably with temporal and regional differences, and differences among farm animal species. The variations may also reflect differences in antibiotic practices, such as the use of specific antibiotics and subtherapeutic vs. therapeutic dosages, the relative proportions of resistance in bacterial isolates from each of the farm animal species, and environmental factors, such as production methods, and stress on the animals.

o Animal and poultry carcasses in meat-processing plants are often contaminated with intestinal pathogens and E. coli (see recommendations in Chapter XI). Contamination with salmonellae has been found in up to 45-50% of samples of ready-to-cook poultry obtained from food markets at different times and in different parts of the United States. Although few data are available on the prevalence of antimicrobial resistance among salmonellae isolated from animal and poultry carcasses at meat-processing plants, it is likely that the prevalence would be similar among isolates from animals on the farm. The limited data available on salmonella isolates from retail-marketed poultry would show about 30-40% are resistant to the tetracyclines and about 65% to one or more antimicrobials.

o Human infection with salmonellae or other enteric bacteria may follow handling and ingestion of improperly cooked, packaged, frozen, or refrigerated meat or poultry contaminated with these organisms.

o The results of several studies have shown the selection of drug-resistance in coliform bacteria due to the use of antibiotics in feed and subsequent spread from farm animals to humans. The potential exposure of members of a farm family to various enteric bacteria indigenous to farm animals has been investigated to determine the temporal frequency with which antibiotic-resistant E. coli strains from such contact spreads to them. Following the use of tetracycline-supplemented feed in flocks of chickens, the intestinal coliform flora in these chickens became largely tetracycline-resistant; within 2 weeks 90% of the coliform isolates, predominantly E. coli, from the chickens were tetracycline-resistant. The prevalence of tetracycline-resistant coliform organisms also increased in the intestinal tracts of the 11 members of the farm family caring for the chickens, but not in members of neighboring families. After 5-6 months of use of subtherapeutic concentrations of the tetracyclines in chicken feed, 31% of fecal samples taken at weekly intervals from members of the family contained over 80% tetracycline-resistant coliform bacteria, in contrast to 7% of the samples from neighbors. Multiple antimicrobial resistance, most likely plasmid-mediated, to unselecting antimicrobials (streptomycin, ampicillin, sulfonamides), and to the tetracyclines, developed in over 50% of the E. coli strains isolated from chickens fed tetracycline-containing feed for over 10 weeks. Another study, described in Chapter VI, indicating that antibiotic-resistant E. coli of farm origin can spread to humans involved the use in pigs of a nonabsorbable antibiotic of the streptothricin group, nourseothricin, which had not been used in humans and therefore could not have spread initially from humans to farm

animals. After 2 years of use of this antibiotic in pig feed, E. coli that contained plasmids bearing nourseothricin resistance were present in the feces of 33% of pigs with diarrheal illness, 18% of workers on the pig farms, 17% of family members of the farm workers, and 16% of outpatients from the same geographic region where nourseothricin had not been used in feed. Even though no nourseothricin had been used in treatment of humans in the region, 1% of the E. coli urinary tract infections of outpatients were due to nourseothricin-resistant strains. In contrast, intestinal E. coli isolated from outpatients in neighboring regions, where nourseothricin had not been used in pig feed, were not nourseothricin-resistant. Although much of the important information needed to evaluate these observations is lacking, the available information does appear to suggest nourseothricin-resistant E. coli from pigs were transmitted to humans and probably from humans to other humans. Whether nourseothricin-resistant isolates are more or less virulent than susceptible ones is not known.

Epidemiologic approaches have been used to address further the question of whether exposure of human beings to antibiotic-resistant bacteria of farm-animal origin enhances the risk of subsequent infection with such strains. In one such study (see Chapter VI), the possible acquisition of E. coli urinary tract infections due to antibiotic-resistant strains of farm origin was examined in a population of about 700 female employees in poultry processing plants. E. coli were isolated in 95% of the cultures from poultry; 96% of these strains were resistant to one or more antimicrobials and 87% were resistant to two or more drugs. Eleven percent of the female workers who had not recently taken antimicrobial therapy had bacteriuria, with E. coli accounting for two-thirds of the isolates. Of the latter, 17% were resistant to one or more antibiotics. The antibiograms of the E. coli from the bacteriuria workers and from the poultry to which they were heavily exposed seldom were similar or identical. The E. coli strains from both sources were examined for commonly occurring R plasmids with restriction-endonuclease-digestion patterns to trace spread of drug resistance from poultry to workers. The presence of a unique plasmid pattern endemic in poultry also found in the human isolates might support the concept of such spread. However, because the same plasmid could be demonstrated in two isolates in only a few instances, the results had too little power to exclude the possibility of spread. Unfortunately, intestinal E. coli from the workers were not studied, and, therefore, possible spread to their gastrointestinal tracts could not be ascertained. The validity of using urinary tract infection as an epidemiologic approach to the question of whether exposure of humans to

antimicrobial-resistant bacteria of farm-animal origin enhances the risk of subsequent infection by such strains can be seriously questioned. E. coli strains causing urinary tract infections appear to represent a selected group of clones not included among those equally likely to colonize animals and humans.

Monitoring of infections among farm workers and their families caused by antimicrobial-resistant salmonellae or other gastrointestinal pathogens of farm animal origin might provide evidence of spread of these organisms to humans with ensuing illness. Extensive data bearing on this issue are not available. In one study, salmonellosis or diarrheal illness did not occur at a higher frequency in farm children regularly exposed to poultry than in a control group lacking such exposure. Although scattered clinical case reports have documented transmission of salmonellae from farm animals to humans, this does not appear to occur commonly, or at least is not often recognized.

In the United Kingdom, since about 1970 all antimicrobials used in humans have been banned for use in animals, thus only selected antimicrobials can be used for animal growth promotion. The Swann Committee report of 1969 made this recommendation and prohibited penicillin, tetracycline, and certain antimicrobials from being used as feed additives. However, these drugs could be used for therapy or prophylaxis of disease only when prescribed by a veterinarian and then only for a limited time period. Alternatively, antimicrobials such as Zinc Bacitracin, Virginiamycin, and Avoparcin have been used as feed additives but not for therapeutic indications. Use of these drugs does not select for bacterial strains resistant to penicillin, tetracycline, or other antimicrobials used in human medicine. Some investigators in the United Kingdom insist that the selection of resistant salmonellae (and other bacterial strains) is due to the therapeutic use of antimicrobial drugs in humans as well as animals. The concentration of 200 grams/ton is a therapeutic or prophylactic dose in the United Kingdom and must be prescribed by a veterinarian. In contrast, this concentration in the USA is considered a subtherapeutic dose when it is administered for 2 weeks or longer and does not require a prescription from a veterinarian. Consequently, comparison of the effects of this concentration of antimicrobials on the selection of resistant enteric pathogens, especially Salmonella, is difficult because of the difference in applications in these two countries. Although penicillin and the tetracyclines have been banned as feed additives in the U.K., both can be used when prescribed for therapy or prophylaxis of disease. The amounts of antimicrobials used in veterinary practice has increased since the Swann Report. The surveys conducted subsequently indicate a fairly constant and low incidence of

resistance among salmonella isolates except for S. typhimurium phage type 204C, an exceptional strain that has become the most commonly isolated strain from cattle, especially calves. Not only has it been reported in increasing numbers since its isolation in 1979, but each year new antimicrobials have been added to the list to which it is resistant. In a few human cases this strain has developed serious treatment problems because of the limited choice of effective antimicrobials. Although in 1985 it was responsible for 4% of all reported cases of salmonellosis in humans, most of them were self-limiting.

It is difficult to assess the effect of banning penicillin and the tetracyclines as feed additives in the U.K. after 1969 when the Swann Report recommendations were implemented. There were no systematic collections of strains prior to the report to serve as a baseline. Also, there have been significant changes in the methods of raising and marketing animals and these have been important factors in the spread of resistant Salmonella, e.g., S. typhimurium phage type 204C. The multiple antimicrobial resistance profile of this particular strain presumably has been caused by the many antimicrobials used therapeutically for scours in calves and not by subtherapeutic doses used in the U.K. Other strains of Salmonella have not shown this tendency to acquire resistance plasmids under similar circumstances. Various other salmonella species, of animal origin, that cause human disease have fluctuated widely in incidence during this same period but there has not been any dramatic increase in resistance to antimicrobials in bacterial isolates. Some experts in the U.K. are convinced the Swann Committee recommendations have had no impact in reducing one hazard this Committee was charged to address, the selection of resistant bacteria by antimicrobials in animal feed.

Because the committee was unable to find data directly implicating the subtherapeutic use of feed antimicrobials in human illness and that much of the available evidence was primarily circumstantial, often ambiguous, and sometimes conflicting, the committee proceeded to develop a risk model and perform a quantitative risk assessment. Even though salmonellosis may contribute only a small portion of the total incidence of possible human disease due to subtherapeutic use of antibiotics in animal feed, our model was restricted to salmonellosis, because adequate data were lacking to quantify the risk for other foodborne pathogens, such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli.

The model consists of a sequence of five mathematically derived quantitative estimates that are linked in a cascade fashion:

(1) Annual number of cases of salmonellosis reported in the U.S. (the committee has used a mid-range estimate of 50,000 cases in developing the model in Chapter VII)

(2) Fraction of salmonella strains from human cases showing resistance to--

(a) any antimicrobial (mid-range estimate, 24%)

(b) penicillin/ampicillin and/or tetracycline (mid-range estimate, 15%)

(3) Death rate associated with infection by salmonella strains with various resistance patterns--

(a) susceptible to all antimicrobials (mid-range estimate, 0.5%)

(b) resistant to any antimicrobial (mid-range estimate, 1.0%)

(c) resistant to penicillin/ampicillin and/or tetracycline (mid-range estimate, 1.0%)

(4) Fraction of deaths due to salmonellosis that are associated with salmonella strains of farm origin (mid-range estimate, 70%)

(5) Proportion of the above fraction (4) resulting from the subtherapeutic use of antimicrobials in animal feed--

(a) any antimicrobial (mid-range estimate, 88%)

(b) penicillin/ampicillin and/or tetracycline (mid-range estimate, 90%

In view of the nature of the link between individual estimates described above, the five estimates can be multiplied to indicate the number of annual deaths. With appropriate modification of the relevant estimates, the number of deaths due to use of antimicrobials in feed only for the purpose of growth promotion, rather than for all subtherapeutic uses (both growth promotion and disease prevention), can be calculated.

It is extremely important to recognize that the numbers of annual deaths estimated by multiplying the five individual estimates listed above are not necessarily excess deaths. It is possible that, even if all subtherapeutic uses of antibiotics were stopped, a like number of deaths might replace those produced by resistant strains as a result of infections by drug-susceptible salmonellae.

It is possible to estimate the number of excess deaths due to subtherapeutic uses of antibiotics by introducing into the risk model the so-called "etiologic fraction." This fraction represents the proportion of cases that would almost certainly not occur in the absence of drug-resistant strains. These excess cases are estimated by taking into account the proportion of the population that is taking antibiotics at any given time and the documented excess risk of infection following such antibiotic administration. Estimates based on inclusion of the "etiologic fraction" are more certain than those estimated without its inclusion in the sense that these represent true excess cases.

Because the data available for use in the risk model are scanty, were likely to have been collected for other purposes and retrospectively, and sometimes require extensive extrapolation, the inherent limitations of the model must be appreciated. Furthermore, it should be recognized that the figures used in the model result in a low estimate of the actual number of deaths, because possible unreported deaths from salmonellosis are not estimated and deaths due to other foodborne intestinal pathogens, such as Campylobacter jejuni, have not been taken into account. Moreover, there is no doubt an additional burden of disease (morbidity) should be considered part of the characterization of risk. Insufficient data were available to allow quantification of this burden.

The committee believes that it has employed the best available data to make a series of low, mid-range, and high estimates for each sequential step in the model. A similar approach is taken to derive three estimates of the etiologic fraction. Because values for five variables (described above) have been multiplied to provide specific risk quantification, and because there are three different estimates of each variable (low, mid, and high), there are 243 different possible estimates of risk for each linked sequence of steps. The committee tends to place greatest reliance on estimates near the mid-range (median) value as being the "likeliest" estimate reported below.

With this risk model, a series of estimates of annual numbers of deaths from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed were made:

- The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion is in the range of 40 per year.

- The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for growth promotion is in the range of 15 per year.

It must be emphasized that neither of the above estimates is certain to represent the true excess number of deaths due to subtherapeutic uses of antibiotics in animal feed. It is possible that stopping such uses will not reduce these numbers. Inclusion of the etiologic fraction allows estimation of the excess numbers of annual deaths:

o The likeliest estimate of excess deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion is in the range of 6 per year.

o The likeliest estimate of the excess deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines only for growth promotion only is in the range of 2 per year.

It is also possible to estimate the numbers of deaths due to "increased difficulty of treatment." These estimates, which are more uncertain than the others, represent cases due to possibly increased virulence of resistant strains, presence of resistance to antimicrobials ordinarily employed in treatment of such infections when they are severe or occur in particularly vulnerable persons, some other factor, or a combination of these. These estimates probably include those in the etiologic fraction estimated above.

o The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for both prophylaxis and growth promotion and arising because of "increased difficulty of disease treatment" is 20 per year.

o The likeliest estimate of deaths attributable to subtherapeutic uses of penicillin and/or the tetracyclines for growth promotion only and arising because of increased difficulty of disease treatment is 8 per year.

The currently available data are an incomplete "patchwork" from a variety of sources; they are not collected systematically for the nation, they are complex, they are frequently of poor quality and require extrapolation for use in risk assessment, and they are not focused on the specific points of direct interest.

Complete evaluation of these risk estimates of mortality from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed requires consideration of the possible benefits to food production that might accrue from such antimicrobial use. Consideration must also be given to the question of whether overall human deaths from salmonellosis (attributable to both antimicrobial-

susceptible and -resistant strains) would be changed by the discontinuance of subtherapeutic use of penicillin and/or tetracycline. The committee believes that, although some deaths attributable to antibiotic-resistant strains may be substituted for by deaths from susceptible strains, the total number of deaths would decrease. However, this cannot be proved at present.

Using all the resources noted above, the committee was unable to find a substantial body of direct evidence that established the existence of a definite human health hazard in the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds.

The committee does not offer recommendations for risk management or policy making, because this was not part of its mandate. However, a series of recommendations for strengthening the data bases for future risk analyses have been made by the committee. Many of these would warrant implementation in order to monitor antibiotic use and the changes that might ensue in drug resistance in isolates from animals and humans with salmonellosis and other foodborne diseases. These recommendations would seem particularly appropriate in view of the fact that debate on the benefits of use of subtherapeutic doses of penicillin and the tetracyclines in animal feed has gone on for over two decades.

II

INTRODUCTION

Subtherapeutic concentrations of antimicrobials in feed have been used for decades in the raising of animals for food production. The concentrations are lower than those usually chosen to treat established infections in animals, but high enough to affect growth of bacterial components of the gastrointestinal flora. Subtherapeutic concentrations are used to improve growth and to prevent infection during periods of increased susceptibility in rearing. In 1980, a committee in the Division of Medical Sciences of the National Research Council prepared a report entitled The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds[2]. The committee concluded that hazards to human health associated with subtherapeutic use of antimicrobials in animal feeds had been neither proved nor disproved; that was not to say that the postulated hazards did not exist. That committee made a number of observations that are still relevant:

o Subtherapeutic uses of antimicrobials in animals increase the prevalence of antimicrobial resistance in some bacteria, such as salmonellae and Escherichia coli. Persons in close contact with animals treated with antimicrobials are more likely to harbor antimicrobial-resistant E. coli than are persons not so exposed. However, in studies of antimicrobial-resistant E. coli in humans with animal contact, the dosage and duration of antimicrobial use in the animals have not been clearly defined. Thus, subtherapeutic use usually cannot be distinguished from therapeutic use.

o Slaughterhouse workers carry some of the same phage types of Enterobacteriaceae as are found in slaughtered animals and in the slaughterhouse environment. However, the relevant studies were not conclusive, because too few persons were examined. In addition, the animals to which the workers had been exposed had probably received both therapeutic and subtherapeutic doses of antimicrobials.

o A link could not be established between illness due to antimicrobial-resistant pathogenic bacteria and contact with animals given only subtherapeutic antimicrobials or ingestion of meat from such animals.

o Therapeutic and prophylactic doses of antimicrobials in humans increase the prevalence of antimicrobial-resistant microorganisms in their bacterial flora and in the flora of their close contacts.

o Data that would allow measurement of the frequency of transfer of R factors (resistance factors) from the bacterial flora of animals to the flora of humans were not available; nor were quantitative data on the frequency of R-factor transfer among components of the human microbial flora.

o Available data were insufficient to determine any relationship in the general human population between ingestion of meat from animals fed subtherapeutic amounts of antimicrobials and the prevalence of drug-resistant E. coli. The limited available data suggested that antimicrobial-resistant E. coli were as prevalent in vegetarians as in meat-eaters.

The purpose of the present study, initiated 7 years later by the Institute of Medicine at the request of the Food and Drug Administration (FDA), was to develop a formal quantitative assessment of human health risk associated with the subtherapeutic use of penicillin and the tetracyclines in animal feed.

HISTORICAL BACKGROUND

It is helpful to review briefly the history of federal policies regarding the use of penicillin and the tetracyclines in animal feeds.[6,7] FDA approved the use of penicillin and chlortetracycline as feed additives in 1951 and the use of oxytetracycline in 1953. In 1972, FDA issued a policy statement regarding the use of antimicrobial drugs in feeds.[6,7] In 1977, the agency issued a Notice of Opportunity for Hearing (NOOH) on penicillin- and tetracycline-containing premixes to help to determine whether the previously approved New Drug Applications for the drugs should be withdrawn because of possible adverse effects on human health.[4,5] In 1978, before any action on these NOOHs, Congress stipulated that the FDA should seek rigorous evaluation of the available scientific evidence of human health hazards associated with the use of the drugs in subtherapeutic concentrations in animal feed. That stipulation led to FDA's request that resulted in the 1980 National Research Council report. Congress, in its fiscal 1981 appropriations hearings, requested that FDA hold any proposed withdrawal proceedings on the New Drug Applications in abeyance until the research recommended in the report could be done and evaluated.

Between the congressional request and the study reported here, the National Resources Defense Council (NRDC) in December 1984 submitted a petition asking the Secretary of Health and Human Services to suspend approval of the drug applications for subtherapeutic use of penicillin and the tetracyclines in animal feeds.[8] NRDC alleged that the use of the drugs presented an "imminent hazard" to the public health. A decision to invoke the imminent-hazard provision would have resulted in immediate withdrawal of the drugs from the market. FDA reviewed the petition and the scientific evidence submitted in support of the claim of imminent hazard. In summary, the FDA stated that it believed that NRDC has not established that antibiotic-resistant Salmonella, whose resistance results from subtherapeutic uses of penicillin and the tetracyclines in animal feed, have a significant impact on the outcome of human salmonellosis. Moreover, the figures used by NRDC to calculate mortality and morbidity rates were derived, in part, from a study that was not designed for that purpose and, therefore, could be biased. The FDA recommended that the Secretary deny NRDC's petition on the grounds that an "imminent hazard" has not been demonstrated.

COMMITTEE APPROACH

The present Committee on Human Health Risk Assessment of Using Subtherapeutic Antibiotics in Animal Feeds has sought data that could be used in making a quantitative assessment of hazards and risk to human health. It has sought the results of peer-reviewed scientific studies on several pathogenic organisms that infect both humans and animals, that cause disease and death in humans, and for which antimicrobial susceptibility testing is commonly performed. The need for data on mortality (reports of human infectious disease reported to state and federal agencies) further limited the bacterial infections that might be included in this study. Few data on morbidity were available. The committee needed a definitive end point (survival or death) that was not afforded in any published evaluations of the effects of salmonellae in causing morbidity in humans. Several other diseases were considered, and these are discussed in Chapter VI, but morbidity and mortality data on the listed diseases other than salmonellosis were unavailable or insufficient in quantity and quality for risk assessment. (Morbidity was not assessed in the present model, because of the lack of data on the cases studied, and the current model has no provisions for morbidity.) Infection with Salmonella species (other than S. typhi) was selected for several reasons: salmonellae are often isolated from farm animals and foodstuffs derived from them; salmonellae are pathogens for

both farm animals and humans, some data are available on antimicrobial resistance among farm-animal and human isolates of salmonellae; the Centers for Disease Control (CDC) conducts a national surveillance for salmonellae and receives numerous human salmonella isolates from all parts of the country for identification and serotyping, and CDC has conducted studies on the incidence of human salmonellosis in selected U.S. urban and rural counties.[1,3] Requisite data related to salmonella infections as either the "underlying cause" or a "contributing cause" of human deaths were sought, and the relevant bacterial isolates were categorized as to antimicrobial susceptibilities. The drug-resistance profiles of the clinical isolates have been examined with a view to determining the sources of drug resistance and whether drug-resistant isolates could be traced to farm origin or, even further, to subtherapeutic use of penicillin or the tetracyclines in animal feed. The task has been formidable, because of the sparseness of data that link drug-resistant clinical isolates to primary sources of infection, whether human or animal, and of data that identify the specific drugs used on the farm, their form of administration (for growth promotion or prevention of infection), and their dosages. The committee has recognized several weaknesses in the data needed for risk assessment and its findings have highlighted these weaknesses.

Bacterial antimicrobial resistance can be natural or acquired, and the growth of bacteria with either type of resistance will be selectively favored when antimicrobials are used in humans, in animals, or in other environments. Natural resistance to an individual antimicrobial pertains to resistance of an entire species to that antimicrobial, e.g., resistance of Pseudomonas aeruginosa to chloramphenicol. Such resistance usually arises from a lack of permeability to the drug or the lack of a susceptible target site for the drug. Acquired resistance occurs as a result of mutation or as a result of transfer of resistance (R) plasmids. Exposure of large bacterial populations to various antimicrobial agents results in selection of antimicrobial-resistant microorganisms, provided that the concentrations of the agents are above the minimal inhibitory concentrations (MICs) for the exposed bacteria. That selection occurs in bacterial cultures in vitro, in humans receiving antibiotics either prophylactically or therapeutically, and in animals whose feed contains antibacterial agents for growth enhancement, for treatment of established infection, or for disease prevention. One would like to know particularly the extent to which the human "pool" of drug-resistant enteric microorganisms is increased by the aggregate of disease-producing pathogenic strains, bowel flora, and gastrointestinal "transients" of animal origin that are

generated by subtherapeutic uses of penicillin and the tetracyclines on the farm and in feedlots.

General questions that might reasonably be raised concerning the importance of the subtherapeutic use of penicillin and the tetracyclines in current problems of human infection with antimicrobial-resistant bacteria include the following:

o What are the relative (quantitative) contributions to drug resistance, in bacterial species pathogenic for humans, of antimicrobial use in humans and in animals?

o What are the relative contributions to bacterial drug resistance of subtherapeutic and therapeutic uses of antimicrobial agents?

o Does bacterial resistance to penicillin and the tetracyclines foster resistance to other antimicrobial agents used in the treatment of animal or human infections? More specifically, what is the relationship of the subtherapeutic use of these drugs in animals to resistance to other drugs?

o If subtherapeutic (but not therapeutic) use of penicillin and the tetracyclines were eliminated, would the frequency of antimicrobial resistance among enteric pathogens be likely to increase, to decrease, or not to change?

The committee hopes that newly developed molecular fingerprinting techniques that pinpoint the sources of infections with antibiotic-resistant pathogens and that trace routes of transmission (animal to human or human to animal) will be used regularly in epidemiologic investigations and that a database will be established for use in future assessment of risk. The pathways of infective bacteria from animals to humans or vice versa and the relationship of the development of antimicrobial resistance in these bacteria to antibiotic use thus might be more clearly identified.

THE REPORT

The committee considered the available data on several bacterial organisms and selected nontyphoidal salmonella infections as the most appropriate infections to consider in making the human health risk assessment, because relevant human case reports, antibiotic resistance profiles in bacterial isolates, and other data needed were available. This report is organized according to individual steps or issues of importance that the committee felt might directly

or indirectly provide insights into current health risks (or might be identified as targets for future investigation).

The report deals with the biologic impact of resistance to antimicrobial agents first, because the mechanisms of antimicrobial resistance, population genetics and the overgrowth of resistant microorganisms in the presence of antimicrobial agents, and bacterial resistance transfer are well-studied topics relevant to antimicrobial drug effects. Furthermore, definition of specific antimicrobial-resistance genes and other genes of plasmids provides a potential means of establishing the common clonal identity of isolates obtained at geographically distant yet epidemiologically related sites.

The next chapter describes antimicrobial production in the Unites States as a means of estimating total antimicrobial use, whether for humans or animals and whether for treatment, disease prevention, or growth enhancement (in farm animals). Particular attention was paid to estimating the use of penicillin and the tetracyclines in livestock and poultry production. To the extent possible, the committee has estimated that portion of the total use of antimicrobials on the farm and in feedlots accounted for by penicillin and the tetracyclines. Such estimates have been analyzed to apportion the total amounts to therapeutic and subtherapeutic use and to growth promotion and disease prevention.

Central to any consideration of possible adverse effects on human health are the patterns and prevalence of antimicrobial resistance in salmonellae and E. coli isolates of animal and human origin. Chapter V discusses possible differences in prevalence and patterns of resistance between isolates from healthy animals and from meat and poultry products and isolates from veterinary institutions that treat ill animals. Studies of the effects of long-term administration of subtherapeutic antibiotics to farm animals (and later discontinuation) on the prevalence of antimicrobial resistance in their E. coli strains are reviewed, and temporal trends in resistance among isolates of human origin are surveyed.

The possible transmission of antimicrobial-resistant pathogens or intestinal commensals of farm origin to humans is examined in the next chapter. Data bearing on the issue have come from experimental field studies, from epidemiologic studies of outbreaks of salmonellosis in which molecular biologic techniques have been used to establish clonality of isolates along the food chain from production to human consumption, and from investigations of the occurrence in farm and slaughterhouse workers of infections due to antimicrobial-resistant microorganisms of farm origin.

The principal focus of this report is the development of a risk model and its use for establishing an estimate of

risk. The model described in Chapter VII is based on human salmonella infection and incorporates data on the following:

- o The numbers of cases reported annually.
- o The fraction of cases due to organisms resistant to more than one antimicrobial or resistant to ampicillin (the penicillin congener that is used in treatment of susceptible human salmonella infections) or the tetracyclines.
- o The fraction of cases that result in death.
- o The fraction of cases of farm origin.
- o The fraction of cases that might be attributable to the subtherapeutic use of antimicrobials in animal feeds.
- o The role of recent ingestion of antimicrobial agents for unrelated reasons in predisposing to infection by smaller inocula of antimicrobial-resistant pathogens than would ordinarily be required to produce disease (the so-called etiologic fraction), i.e., the excess of cases attributable to the effects of previously administered antimicrobial agents.

Several European countries have placed restrictions on the use of antimicrobials in subtherapeutic quantities in animal feeds over the last 2 decades. Their experience regarding use of antimicrobials, patterns of antimicrobial resistance in E. coli and salmonellae, and incidence of disease due to enteric pathogens in livestock and humans has been reviewed for insights into the consequences of such restrictions.

The final chapters of the report present the conclusions reached by the committee and its recommendations for future research.

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III

BIOLOGIC IMPACT OF RESISTANCE TO ANTIMICROBIAL AGENTS

BRIEF HISTORY OF CLINICAL DEVELOPMENT OF DRUG RESISTANCE

The discovery of sulfonamides and antibiotics in the first half of the twentieth century led to at least two biologic "revolutions." The first was the ability to treat infectious diseases. The second was the use of antibiotics to gain insights into the genetics of bacteria. In 1943, Luria and Delbruck[70] first demonstrated that the emergence of bacterial resistance to a single antibiotic after exposure to it was a result of chromosomal mutation (independent of antibiotic exposure), rather than an adaptive change. In the years that followed, it became conventional to consider that the emergence of any drug resistance in any bacteria was due to a selected mutation.

Conventional beliefs concerning the importance of mutation in drug resistance were overturned by events in Japan in the years just after World War II. There, physicians treating patients in epidemics of shigella dysentery used sulfonamides extensively. A substantial proportion of those bacteria soon became resistant to sulfonamides. The widespread sulfonamide resistance caused physicians to switch from sulfonamides to new drugs--streptomycin, tetracycline, and chloramphenicol--to treat shigella dysentery. By the late 1950s, many of the isolates of shigellae were drug-resistant, not only to sulfonamides, but to all drugs. That finding led investigators to question, on theoretical grounds, mutation as the explanation for drug resistance. Mutation had previously been shown to take place in about 1 in 10^7 cells, and it strained credulity to assume that cells of a single strain could spontaneously develop drug resistance to four antibiotics over a short period. Moreover, several serotypes of Shigella spp. were shown to have developed multiple-drug resistance virtually simultaneously, and this multiple-drug-resistance pattern was actually more common than resistance to a single antimicrobial.

Japanese investigators showed in the late 1950s that multiple-drug resistance could be transferred from one bacterial species to another. In light of discovery of bacterial conjugation in the preceding decade, it was possible to demonstrate that multiple-drug resistance was

being transferred by the process of conjugation. Shortly thereafter, other workers demonstrated that these conjugative packages of genetic information, labeled R factors (now called R plasmids), were prevalent not only in Shigella spp., but also in Salmonella spp. and Escherichia coli. Since the time of their initial discovery, R plasmids have been shown--in complementary studies by epidemiologists, molecular geneticists, and bacterial physiologists--to be widely transmissible and specifically selectable when antimicrobial drugs are present in the environment. Increasing drug resistance among human isolates of clinically relevant bacteria during this period has been shown repeatedly to be due primarily to the proliferation of R plasmids.

MECHANISMS OF ACQUIRING ANTIMICROBIAL RESISTANCE IN BACTERIA

CHROMOSOMES

Most drug resistance in clinically relevant bacteria is due to conjugative transfer of R plasmids and their clonal expansion during exposure to antimicrobial drugs. But chromosomal inheritance and the transfer of chromosomal mutant genes by transformation also play a role.

Bacteria can acquire new genetic information in three known ways: conjugation, transduction, and transformation. Conjugation requires that the donor bacterium possess both the means to duplicate part of its genetic information and the means to attach itself to a recipient bacterium for DNA transfer. The donor must mate with a recipient bacterium that is physiologically capable of permitting the new DNA to enter and replicate autonomously as a plasmid or permitting it to become incorporated by recombination into the recipient's chromosome. Transduction, a process much less important for the transfer of drug resistance, depends on bacterial viruses known as bacteriophages to "package" pieces of the chromosome or plasmid of the donor organism and inject the package into the appropriate bacterium for uptake and incorporation of the foreign DNA. Transformation is the process by which DNA in solution is taken up directly by a bacterial cell. Plasmid (circular) DNA is usually taken up more efficiently than chromosomal (linear) DNA, in that many bacteria have enzymes, known as exonucleases, that attack only linear fragments of DNA. Nevertheless, in some organisms, transformation of chromosomal contents has been shown to be capable of transferring drug resistance.

Chromosomal drug resistance, in most cases, is due to mutation of pre-existing DNA. Although there are others, three antimicrobial drugs stand out for their ability to select chromosomal mutants that have acquired drug

resistance: streptomycin, nalidixic acid, and rifampin. Their common characteristic is that they work by binding to protein targets within the bacterial cell. Each of the protein targets provides an important vegetative function for the cell--the streptomycin target is the S12 ribosomal protein, the nalidixic acid target is DNA gyrase, and the rifampin target is RNA polymerase. Those targets perform essential functions for the cell: protein synthesis, the required winding of DNA, and the transcription of DNA into messenger RNA, respectively.

The specific sites of antibiotic binding in the targets can occasionally be altered by mutation in such a way that the antibiotic no longer binds to the target and the target retains most of its function. Thus, the appropriate mutations in the genes for the S12 ribosomal protein, DNA gyrase, and RNA polymerase will lead to resistance to streptomycin, nalidixic acid, and rifampin, respectively. The bacterium pays a price for the mutations, in that it acquires a less than optimal "housekeeping" protein in the process of evading the effect of the antibiotic; thereafter, it is typically not as hardy as its nonmutant parents. Although chromosomal resistance usually involves resistance that is specific for the selecting antimicrobial drug, it is occasionally responsible for simultaneous resistance to several antibiotics of different structures and sites of action, e.g., the *mar* locus in *E. coli* affects uptake of tetracycline, cefoxitin, and chloramphenicol. Extensive epidemiologic investigations of drug resistance in enteric bacteria have yielded little evidence of the importance of chromosomal mutation in the acquisition of drug resistance or of transformation as a means by which drug resistance can be exchanged. Most enteric bacteria have been shown to have low efficiency in taking up DNA (a property known as competence), unless they are treated so as to damage their permeability barriers temporarily.

R PLASMIDS AND TRANSPOSONS

Since their discovery about 30 years ago, R plasmids have been extensively studied epidemiologically and molecularly and have been shown to play a predominant role in drug resistance among bacteria.[7,22-24,27,29,34,55,85,91,92,98] Like other self-replicating nonchromosomal units of DNA, R plasmids carry "machinery" for efficient replication and genes for particular drug-resistance phenotype. By definition, R plasmids carry genes that encode products that confer drug resistance in a bacterium. Often a single R plasmid contains multiple genes, each encoding a different kind of resistance. Some individual resistance genes encode resistance to multiple related antibacterial drugs. Almost

every drug-resistance determinant is carried on a genetic unit (usually small, occasionally large), called a transposon, that can move from its location on the R plasmid to other locations--typically, but not exclusively, other plasmids. That form of DNA rearrangement, or transposition, requires special genes and stretches of DNA that are parts of the transposon.[16,103] It is useful to consider transposons as freely movable genetic modules that can be assorted, reassorted, and added to and subtracted from evolving R plasmids as environmental pressures dictate.

The ability of most drug-resistance genes to transpose provides R plasmids with an extraordinary degree of genetic plasticity. Although it is not clear that the presence of antibiotics in the environment has any influence on the extent of transposition of resistance determinants, antibiotics exert a profound influence on the selection and persistence of R plasmids with multiple drug-resistance determinants. Studies of indigenous soil bacteria in the preantibiotic era showed not only that R plasmids were less prevalent, but also that recovered R plasmids typically contained only one or two resistance determinants each.[24,29] More recently, in sharp contrast, bacteria recovered in environments exposed to antibiotics have had a high prevalence of R plasmids with multiple drug-resistance determinants.[29,34,91,92] It is important to note that these R plasmids are similar in many genetic respects to the preantibiotic-era plasmids that did not have multiple drug-resistance determinants. Transposons, whose drug-resistance genes evolved as "protection" against the natural antibacterial substances in the soil, thus used plasmids already available.[7,29] Moreover, the same transposon can be found in an array of different plasmids; in nature, drug-resistance elements are indeed promiscuous and can locate in a broad spectrum of genomes.

Most bacteria, given the appropriate supplemental genes, are potentially capable of transferring DNA to other bacteria by conjugation. Usually the supplemental genes are carried on a plasmid. When present on an R plasmid, the supplemental genes together make up what is called the resistance transfer factor, or RTF. Most R plasmids contain an RTF, which enables them to be conjugatively transferred between bacteria.[29] Just as the transposon, at the level of the single drug-resistance determinant, is capable of movement to a different R plasmid, the RTF-containing R plasmid is capable of movement to other strains and other species of bacteria. Although initial studies on conjugative R plasmids were limited to facultative gram-negative bacteria,[29] conjugation clearly plays a major role in the transfer of drug resistance among facultative gram-positive bacteria[55,81,105] and among anaerobic bacteria.[15,96,114] Recently, it has been found that some transposons in some

gram-positive bacteria can bypass the need for RTF-containing R plasmids in conjugation.[15] These "conjugative transposons" contain the equivalent of the RTF, as well as the R factor. Other studies involving anaerobic bacteria have shown that sublethal concentrations of tetracycline in vitro actually promote R-plasmid transfer, in addition to selecting bacteria that carry the drug-resistance determinant.[119]

Of equal or greater concern from the standpoint of drug-resistance proliferation is the finding that the tetracycline-resistance determinant (Tc^r) can be conjugatively transferred back and forth between bacteroides (anaerobes) and E. coli (facultative gram-negative bacilli).[119] Inasmuch as anaerobic bacteria, especially species of Bacteroides, are the predominant flora in the mammalian gastrointestinal tract, the presence of back-and-forth transfers suggests that the reservoir for maintenance, persistence, and spread of at least one drug-resistance determinant, Tc^r, is enormous.

The latter example of interspecies conjugative transfer, of which there are many examples (see Odelson et al.[96] for a review), brings up the issue of plasmid host range. In addition to the genetic attributes already discussed, R plasmids contain a vegetative origin of replication, or oriV, which enables them to replicate autonomously in host bacteria. Plasmids can be cataloged into "incompatibility" groups based on their oriV; those with the same oriV cannot coexist within a bacterium, because of competition for identical replication factors.[29] The specific oriV carried by a plasmid constitutes another element of its phenotype: its host range. Narrow-host-range plasmids can replicate in only a few species of bacteria, usually because only those bacteria provide additional factors required for plasmid replication. In contrast, broad-host-range plasmids can transfer to and replicate in a large variety of bacteria. Results of transfer studies in E. coli and bacteroides indicate that antibiotic exposure--in this case to tetracycline--can increase the transfer and selection of at least one form of broad-host-range plasmid resistance to tetracycline.

In early studies of conjugative transfer of R plasmids among bacteria common in the gastrointestinal tracts of humans and farm animals, E. coli was found to act as a good donor and recipient of R plasmids; Salmonella spp. were not as good donors and recipients.[29] In a more recent study involving S. typhimurium and E. coli recovered from calves, strains of S. typhimurium were extremely proficient R plasmid donors--even better than strains of E. coli.[124] Certain plasmids, belonging to a particular group of incompatible plasmids, Inc H2, found in most strains of salmonellae and preferentially in S. typhimurium, showed a peak efficiency of transfer at 30°C and were conjugatively transferred in

calves' feces after excretion.[124] Inc H2 plasmids have other critical features: they carry resistance to both penicillin and the tetracyclines, they transfer to E. coli, and they carry other non-drug-resistance determinants that increase the ability of the bacteria to colonize the gastrointestinal tract.[124] In every general aspect of drug resistance studied (i.e., expression of resistance genes, maintenance of R plasmids, and transfer of plasmids), Salmonella spp. and E. coli have been found to be quite similar.[29] Although Salmonella spp. might behave quite differently from E. coli in the gastrointestinal tract, because of the former organism's ability to invade entrocytes and thereby avoid antibiotics that cannot penetrate cells well (i.e., penicillin and aminoglycosides, but not the tetracyclines or chloramphenicol), evidence suggests that R plasmid transfer occurs with ease in Salmonella spp. in vivo. For example, in an outbreak of gastroenteritis caused by S. typhimurium that affected 1,900 persons who ate contaminated turkey meat, the source strain of bacteria isolated from the meat was antibiotic-susceptible, as were bacterial organisms isolated from persons who had not taken any antibiotics. In sharp contrast, a high proportion of the persons who were given chloramphenicol, ampicillin, or one of several other antibiotics had R plasmids bearing S. typhimurium in their stools; that proves the ability of salmonellae to acquire R plasmids from the human gut.[2a] Thus, strains of salmonellae, as well as E. coli, can act as reservoirs for conjugative R plasmids and thus gain an enormous selective advantage over other bacteria in the face of antibiotics in the environment.

Epidemiologic surveys of R plasmids in bacteria that are clinically important have shown that multiple-drug resistance has increased progressively since the beginning of the antibiotic era.[34,81,91,92] Among individual R plasmids, the number of drug-resistance genes per R plasmid and the likelihood that a given R plasmid is conjugative have also been increasing.[63,89,114] Although early studies had indicated that the problem of drug resistance was most pronounced in the Enterobacteriaceae, the more recent studies show that the problem has spread. Drug resistance has now been found in bacteria of virtually all genera that are important.[15,22,27,34,53,55,81,85,91,92,96,98,102,105,114,119,122]

In summary, R plasmids affect the microbial populations of humans in several ways:

- o R plasmids confer drug resistance on a great number of bacteria, including pathogens and commensals.

- o R plasmids typically confer drug resistance to several antimicrobial drugs simultaneously.

o R plasmids are capable of being transferred among strains of the same species and among different species through the process of conjugation. Transfer can occur from commensal to pathogenic bacteria in the presence of antibiotics.

o R plasmids can carry additional genes, including virulence factors (discussed later).

o Exposure to antimicrobial drugs causes an increase in the number and the spread of R plasmids by preferentially selecting bacterial offspring that are drug-resistant and, in some cases, possibly by increasing the efficiency of conjugative transfer.

ROLE OF ANTIMICROBIAL DOSAGE IN SELECTION OF DRUG-RESISTANT BACTERIAL POPULATIONS

Antimicrobials are used in three ways in agriculture. In the first, high (therapeutic) doses are used for brief periods (usually no longer than about a week to 10 days) for the treatment of infectious disease. In the second, low (growth-enhancing) doses are used for long periods in livestock to promote growth. In the third, low (prophylactic) doses are used for a period up to 2 weeks to prevent disease. Although the duration of antimicrobial use differs between growth-promoting and prophylactic purposes, the dosages for both "subtherapeutic" uses are typically the same, i.e., less than 200 grams/ton. The selection of antimicrobial-resistant bacteria is a consequence of therapeutic use and of both types (growth-enhancing and prophylactic) of subtherapeutic use of antimicrobial agents.

Dosages of drugs that would be classified as subtherapeutic might well produce concentrations in the gastrointestinal tract that are sufficient to inhibit susceptible species of bacteria. In Danish studies examining the incidence of drug resistance in feces of pigs given only intermittent therapeutic courses of antimicrobial drugs (none of the antibiotics was fed), a high proportion (53%) of *E. coli* strains were found to be resistant to at least one commonly used antimicrobial drug.[115] Moreover, 53% of the pigs carried tetracycline resistant *E. coli* despite the fact that none of the animals had been exposed to tetracycline within the past year.[115] Using various methods of analysis, Corpet recently found that continuous subtherapeutic doses of antimicrobial drugs caused a profound alteration in the fecal flora of mice, with a significantly increased proportion of resistant *E. coli*.[17]

Although the data are overwhelming in showing that use of antimicrobial agents promotes the emergence of drug

resistance, the differential effects of the three kinds of use in selecting drug resistance either in animals or in vitro have not been well studied. Nevertheless, in a recently reported study, when three groups of pigs were examined for the presence of drug resistance in their fecal coliform bacteria, a non-antibiotic-treated herd had a lower proportion of drug-resistant bacteria than a herd given antibiotics only intermittently and at a high (therapeutic) dose; isolates from the latter herd had a lower proportion of drug-resistant coliform bacteria than those from a herd exposed continuously to antibiotics at a subtherapeutic dose.[58] Specifically, the proportion of the tetracyclines resistance among the fecal isolates was found to be 26% for the first herd, 76% for the second herd, and 100% for the third herd.[58]

An earlier study by the same group of investigators yielded nearly identical result.[59] The feeding of one of the tetracyclines at subtherapeutic doses resulted in a linear increase in tetracycline-resistant coliform bacteria, and the numbers of drug-resistant bacteria eventually equaled or exceeded those of drug-resistant bacteria from animals treated therapeutically. These studies support the hypothesis that any form of antibiotic exposure increases the prevalence of drug resistance. Moreover, they provide limited evidence that drug resistance is at least as prevalent after continuous subtherapeutic use of antibiotics as after intermittent therapeutic use.

EXPERIENCE WITH ANTIBIOTIC RESISTANCE AFTER ANTIBIOTIC USE IN HUMANS

The experience gained in the use of antimicrobial drugs since the 1940s provides strong evidence of the effects of such agents in the selection of antimicrobial-resistant commensals and pathogens in humans. That experience emphasizes the importance, constantly reiterated in clinical teaching, of avoiding unnecessary, prolonged, inadequate (subtherapeutic dosing), or inappropriate (for the etiologic bacteria) treatment or prophylaxis with antimicrobial drugs.

The current prevalence of antimicrobial-resistant strains of bacteria is a consequence of extensive antimicrobial use in the last 40-50 years. The prevalence was much lower in the pre-antibiotic era.

Baseline Levels of Antibiotic Resistance--the Status in the Pre-antibiotic Era

Insights into the prevalence of antibiotic resistance in the pre-antibiotic era come from examination of bacterial

isolates from primitive societies unexposed to antimicrobial therapy and from study of stored isolates predating the introduction of penicillin.

In an examination of 21 human stool specimens and 19 soil specimens from an "antibiotic-virgin" population in the Solomon Islands in 1968, R plasmids (mediating streptomycin and tetracycline resistance) were found in only two specimens (5%).[35] In a study of human and animal communities in Rhodesia, about 10% of 47 fecal specimens from Kalahari bushmen and 540 from animals contained gram-negative bacilli resistant to one or more antibiotics.[74] Isolates were frequently resistant to only a single drug (often ampicillin), and none contained R factors. However, the initial fecal cultures were streaked on media containing low concentrations of antibiotics, which may have been inhibitory to occasional R-plasmid-containing strains and thus might have caused their prevalence to have been somewhat underestimated.

In a study of another antibiotic-unexposed population, in North Borneo, 50 multiple-antibiotic-resistant strains were found among 1,017 fecal isolates (more than half identified as E. coli) from 128 persons. Of the latter, six strains (all E. coli), contained R plasmids;[24] those strains represented 0.6% of the original isolates. Skerman and Falkow in 1969 studied the incidence of drug-resistant fecal E. coli in members of a "pre-antibiotic" society in Australia and found that 16% of 247 E. coli isolates were antimicrobial-resistant and that less than 1% contained R factors.[30,110]

In a stored collection of enterobacteriaceae from widely scattered parts of the world (Europe, the Middle East, and North America), originally isolated between 1917 and 1954, very little antibiotic resistance was found on susceptibility testing decades later.[45] Of 433 strains, 11 (2.5%) were resistant to antibiotics (9 to tetracyclines, 2 to ampicillin). No transferable antibiotic-resistance plasmids were detected. None of the 210 Salmonella spp. and none of the 32 E. coli strains in the study showed resistance to any of seven antibiotics, to sulfonamides, or to trimethoprim. In contrast, 24% of strains were able to mobilize a nonconjugative plasmid in a recipient strain, indicating the presence, in the host, of a conjugative plasmid that was lacking antimicrobial-resistance determinants. That prevalence is roughly comparable with that (17%) noted in 300 fecal E. coli strains isolated from nonhospitalized persons[65] and that (33%) noted in 60 E. coli strains obtained from human, porcine, and bovine sources.[113]

Thus, it appears that conjugative plasmids were as common among enterobacteriaceae before the introduction of antibiotics as they are in the current antibiotic era. In contrast, under the selective pressure of antibiotic use in

humans and animals, plasmid transfer and gene exchange have markedly expanded the population (strains and species) of resistant bacteria.

Increases in Resistance After Widespread Clinical Use of Antibiotics

Experience over the last 4 decades of antimicrobial use in treatment and prophylaxis of human infections indicates the profound effect of these practices on the prevalence of antimicrobial resistance in a variety of bacterial species. Experience time and again has emphasized the importance of limiting the use of antimicrobials to necessary and reasonable therapeutic indications and, indeed, even to holding some highly effective drugs in reserve. Before 1946, when penicillin became generally available, 85% of clinical isolates of Staphylococcus aureus at the Boston City Hospital were highly susceptible to penicillin.[33] Within 3 years, most of the strains there had become highly resistant to penicillin. In most developed countries today, only about 10% of S. aureus isolates, whether of community or hospital origin, are susceptible to penicillin.

Isolates of another gram-positive human bacterial pathogen, Streptococcus pneumoniae, collected before 1950 were tested years later and found to be uniformly susceptible to penicillin. The situation is entirely different in Barcelona, Spain, where recently about half of a small number of pneumococcal isolates were either moderately or highly resistant to penicillin, and 62% of the same isolates were resistant to chloramphenicol.[60] This high degree of antibiotic resistance might well be related in part to widespread use of those antibiotics, prevalent for many years in Spain.[66]

Problems of antimicrobial resistance in bacterial species associated with widespread antimicrobial use in humans have been evident among gram-negative bacillary species notorious for causing infections in hospitals. For example, whereas serratia strains isolated in the 1950s were uniformly susceptible to kanamycin, nalidixic acid, and gentamicin and a minority of strains were resistant to streptomycin, a large proportion of strains were resistant to all but gentamicin by 1967.[33]

Prophylactic use of antimicrobial drugs, as well as use in therapeutic concentrations, might similarly exert a selective effect in humans, increasing the prevalence of antimicrobial-resistant strains. The experience on the Burn Service at Grady Hospital where prophylactic topical use of gentamicin was extensive from 1964 to 1969, offers a dramatic example.[108] In 1968, more than 1,400 lb of that drug was used topically on burns at that center. From 1965 through

1967, 80-90% of all Pseudomonas aeruginosa isolates from patients in that burn unit were susceptible to gentamicin. By 1969, only nine percent were susceptible. Almost all the resistant isolates were almost all of a single pyocin type--a type that had in the past been seen only infrequently in the burn unit and had previously been susceptible to gentamicin. In mid-1969 routine topical use of gentamicin was discontinued, and it was replaced with other topical drugs that would not have been used in systemic treatment of bacterial infections. In mid-1970, 95% of P. aeruginosa isolates were susceptible to gentamicin--back to baseline values that existed before the routine use of topical gentamicin.[108]

Effect of Reduced Use of Antibiotics in Humans on Antimicrobial Resistance

Quantitative aspects of overall antimicrobial use in humans contribute to selection of resistant strains. Thus, rational choices of drugs for appropriate therapeutic and prophylactic indications are of continuing importance in treating humans. The same rational approach seems warranted in antimicrobial use for animals. If one accepts the concept that populations of bacteria represent a common gene pool with considerable genetic fluidity and that some of the genetic information segregated for much of the time in individual components of the population are capable of being transferred among members of the population, then the effects of antibiotic use on one component (e.g., indigenous flora of farm animals) might be relevant to another component (e.g., commensals and pathogens for humans).

It is not known whether the current widespread prevalence of antimicrobial resistance genes among bacterial isolates from human and farm animal sources is too great to be reduced by any reasonable reduction of overall antimicrobial use; the issue warrants careful consideration. The extensive reduction in gentamicin resistance in P. aeruginosa over a 1- to 2-year period in the Grady Hospital Burn Unit[108] cannot be generalized, because of the relatively short period of use of the drug and the confined location of its extensive use. Since the early 1950s, scattered studies have reported temporal relationships between decreased use of specific antimicrobial agents in a given hospital or hospital ward and decreased prevalence of nosocomial bacterial pathogens resistant to those drugs.[78] However, caution must be exercised in interpreting such data, because many of the reported studies dealt with nosocomial outbreaks. Other epidemic-control measures that were instituted might have contributed as much as the changes in antibiotic use to the decreased prevalence of the resistant strain in some of the

outbreaks. Nonetheless, the data suggest the potential for reduction of antimicrobial resistance by limiting the use of some drugs.

When antimicrobial use in humans has been decreased, usually in a hospital setting, resistant strains did not necessarily disappear quickly. Resistant bacteria can sometimes persist despite the absence of antimicrobial selective pressures. In a study of 56 infants who were known to have been colonized with R-plasmid-containing kanamycin-resistant enterobacteriaceae during a stay in an intensive-care nursery in which kanamycin was extensively used, 89% were still colonized with kanamycin-resistant strains 2 months after their return to the community.[20] Intestinal carriage of such R-plasmid-containing kanamycin-resistant strains gradually decreased, but after 12 months or more, 46% of the infants still harbored the resistant organisms.

EFFECT OF ELIMINATION OF SUBTHERAPEUTIC ANTIBIOTIC USE ON LEVELS OF ANTIBIOTIC RESISTANCE IN FARM ANIMALS

What is the counterpart in animal production of the above-described circumstance of the withdrawal or shift of antibiotic use in humans, and what changes have such shifts made in the prevalence of antibiotic-resistant coliform bacteria in their fecal bacterial population? Results of long-term studies of the responses of the intestinal coliform bacteria of swine to cessation of antibiotic exposure suggest that changes occur slowly (V. Hays, University of Kentucky, personal communication, 1988; also see Langlois et al.[56,57]). In a separated herd of pigs (see additional detail below) maintained on subtherapeutic concentrations of tetracycline (50-100 grams/ton of feed) for 13 years (since 1972), tetracycline resistance averaged over 90% in the fecal coliform population. Such a high level of resistance to tetracycline, almost exclusively R-plasmid-mediated in enterobacteriaceae in this setting, was accompanied by resistance to one or more other antibiotics. Does that high level of resistance indicate such extensive permeation of R plasmids and transposons throughout the intestinal population of *E. coli* and other related coliform species so extensive as to stabilize their predominance and preclude diminution of their major position even if exposure to antimicrobials in feed ceased? On the basis of experience with the use and withdrawal of specific antibiotics in relatively closed populations of hospitalized patients, one might expect a protracted period to elapse before resistance returned to control levels in a comparable population that had not been exposed to antibiotics.

The effects of antibiotic withdrawal on antimicrobial resistance in intestinal coliform organisms in the swine herd

mentioned above has been extensively documented over a 13-year period (V. Hays, 1988, personal communication). This herd of pigs, established in 1963, that received antibiotics routinely as feed additives and in injectable form (when needed for treating sows and pigs), no single antibiotic was used continuously. After 1972, the herd was kept free of any antibacterial agents as feed additives or for therapy. The level of tetracycline resistance among fecal coliform bacteria was over 90% in 1972. The level of resistance subsequently declined, but very slowly. Eight years after cessation of exposure to the tetracyclines and other antimicrobials, the level of tetracycline resistance was still 57%; by 1985, it had dropped to 30%. Those results certainly indicate that an early postwithdrawal "snapshot" should not be the basis for evaluation of the effects of discontinuation of antibiotic use.

Why did reversion to lower levels of antibiotic resistance in the coliform flora take so long in the experiments in swine just described? First, some strains (O-serotypes) of E. coli appear better constituted than others to persist and multiply in the colon, perhaps by virtue of specific plasmid carriage and particular surface antigens.[42] In piglets, the presence of the plasmid K88 confers adhesive properties on some strains of E. coli, facilitating attachment to brush borders of intestinal cells.[49] Similarly, in calves, a comparable plasmid, K99, is important in enteric disease.[113] In a situation where over 90% of coliform organisms start out with R-plasmid-mediated resistance to the tetracyclines (or other antimicrobials), strains with a selective advantage, such as the presence of colonization factors or nutrient sequestration, are preferentially retained. Thus, once R plasmids and transposons are extensively established in the coliform flora, they can be retained by selective advantages other than those provided by antibiotic use.

Second, persistence of R-plasmid-containing resistant strains might be facilitated by environmental factors. In the pigs that showed only very slow loss of antibiotic-resistant intestinal coliform bacteria over 13 years, the isolation of the experimental animals from other animals could have played a role in the slowness of replacement of such bacteria. The quarters of the animals were undoubtedly fouled initially with their own excreta, which contained resistant coliform bacteria. Reintroduction of the same bacterial flora from a constantly soiled environment must have occurred repeatedly over the years. Exposure of the animals to untreated animals whose intestinal flora contains coliform bacteria that colonize efficiently but are susceptible to antibiotics, might be required if the antibiotic-resistant strains are to be "diluted out" more rapidly.

Chickens fed subtherapeutic doses of oxytetracycline rapidly began to excrete an intestinal population in which over 90% of coliform bacteria were tetracycline-resistant; despite frequent cleaning of the cages, the chickens continued to excrete high concentrations of tetracycline-resistant organisms.[64] The resistance determinants might have been plasmids that had established stable relationships with the host bacteria. Insofar as conjugative plasmids help to mediate chromosomal gene recombination and genetic transfer among members of a population, population fitness, rather than individual bacterial fitness might be enhanced by these plasmids.[62] The proportion of resistant coliform bacteria decreased only when the chickens were mixed with other chickens excreting antibiotic-susceptible coliform bacteria or when they were moved to different cages.

Plasmids might acquire multiple resistance determinants when the host bacteria are exposed to a single drug in an animal's intestinal tract. The long-term use of tylosin, a macrolide antibiotic, as a feed additive in pigs resulted in the evolution of an intestinal streptococcal population that was multiple-drug-resistant.[14] Such multiple-drug-resistant plasmids appeared to differ from streptococcal plasmids present in control antibiotic-free pigs only in the presence of added resistance determinants.

The acquisition by conjugative plasmids of individual or multiple resistance transposons from smaller, nonconjugative plasmids under the influence of antibiotic exposure might facilitate subsequent dissemination of the resistance determinants. Whether persistence of antibiotic resistance in intestinal coliform bacteria after cessation of antibiotic exposure indicates the presence of resistance determinants cannot be known, but such persistence could be important in determining the results of cessation. If determinants are present, whether they are on conjugative or non-conjugative plasmids might affect the ultimate rate of spread of resistance. Although the level of resistance to tetracycline remained relatively high after discontinuation of the tetracyclines as feed additives in Great Britain, there was an observable decline.[111]

The prevalence of antimicrobial resistance to one or more drugs in human isolates of salmonellae is 16-31% (8-22% resistance to tetracycline, 5-19% to ampicillin) (see Chapter V), and prevalence of resistance to ampicillin and tetracycline in human isolates of *E. coli* is 23-32% and 25-29%, respectively.[3] These data suggest that the dispersion of resistance genes might not yet have gone so far as to be irreversible.

SPECIFIC MECHANISMS OF RESISTANCE TO ANTIMICROBIAL DRUGS IN PATHOGENS FROM ANIMAL SOURCES

RATIONALE AND EFFICACY OF MOLECULAR GENETIC TECHNIQUES IN RELATING HUMAN BACTERIAL ISOLATES TO FARM ORIGINS

A pivotal issue concerning the potential spread of animal-borne pathogens to humans is the ability to determine that a given pathogen is "the same" in both locations. Because bacteria divide asexually, each parent gives rise to two daughter cells that, assuming no major acquisition or loss of genetic material (e.g., an R plasmid), would be genetically and biochemically identical with the parent. The daughter cells are "clonal" with respect to each other and to the parent. By definition, clonal bacteria have the same recent origin and are therefore identical or nearly identical genetically and biochemically. Clonality cannot be absolutely ensured in the field or in the laboratory, but its existence can be shown with statistical analytic procedures. In this analysis, two bacteria are labeled operationally clonal if the test comparison shows so many similarities that the probability that the bacteria examined are different approaches zero.

The demonstration of clonality, with some degree of statistical certainty, is necessary but not sufficient for proving that a given pathogen has been transferred from an animal source to humans (either through the food chain or by some other means). Also required is evidence that the pathogen in question is not normally a part of the human flora. With consistently pathogenic bacteria, that confounding factor, which would otherwise present substantial background noise, is not usually a problem. Modern epidemiologic techniques for investigating human outbreaks of infectious disease usually make it possible to determine whether a pathogen has entered the human environment from an outside source. The same techniques can be applied to each prior source in the linear chain of contagion, so that, assuming the rarity of a given pathogen in a given setting, the primary source can be determined with reasonable certainty.

The power of the epidemiologic approach rests squarely on the ability to prove clonality. A number of recent detailed reviews have examined the various methods available and have analyzed their resolving strength.[1,32,41,107,131] Other reviews have examined the evolutionary stability of bacterial chromosomes and plasmids, with the greatest attention focused on *E. coli* and *Salmonella* spp.[12,43,82,100,105,117,121,125,126] The consensus is that, for large classification projects involving hundreds of strains of a given species, simple, inexpensive techniques, such as isoenzyme analysis, are capable of yielding adequate

discrimination of strains.[1] This biochemical approach has been useful in following the evolution and dissemination of clones of a single species, such as E. coli.[107]

Many of the simple biochemical approaches are not useful for identification, because their discriminatory power is insufficient. When small numbers of isolates are to be identified the use of molecular genetic techniques to "fingerprint" plasmids with DNA-probe technology[122] or restriction-fragment-length polymorphism is extremely useful for exactly the reasons that make these techniques worthless for the large numbers of isolates from population studies gathered over several years. Most plasmids--especially R plasmids, because of their genetic plasticity--are unlikely to be invariant over time. Moreover, because of their conjugative nature, plasmids are spread horizontally between strains (and species) of different clonal origin. Those effects are minimal in short-term outbreaks, so identity of plasmid profiles, particularly in the background of substantial plasmid diversity, is strong evidence of clonality.

In some instances, the use of molecular genetic techniques to analyze plasmids has achieved great certainty of strain identification.[8,43,100,104,117] In situations in which either the bacteria have no plasmids or the plasmids are particularly stable (e.g., those needed to preserve strain virulence), the use of cloned, random chromosomal sequences as probes to identify clones of Salmonella spp. has been valuable in research settings.[125]

R PLASMIDS OF VEGETABLE ORIGIN AND ANIMAL ORIGIN AND THE EMERGENCE OF DRUG RESISTANCE

In evaluations of the origin of drug-resistant bacteria in humans, investigators have analyzed fecal bacterial isolates from meat-eaters and of vegetarians and have compared the flora for resistance. The frequency of drug-resistant bacteria in vegetarians is at least as high as, if not higher than, that in omnivores. No studies have used the molecular epidemiologic techniques described above to demonstrate the origin of the drug-resistant bacteria in vegetarians, but cross-contamination of vegetables, which have been shown to carry high numbers of bacteria,[18,99] presumably occurs in the environment. On the basis of the preceding discussion, increased drug resistance in the environment may be said to reflect the presence of increased amounts of substances with antibacterial activity, probably from any source.

EFFECTS OF RESISTANCE TO ANTIMICROBIAL DRUGS ON BACTERIAL VIRULENCE

Two mechanisms enable bacteria to change from a drug-susceptible to a drug-resistant phenotype: mutation of chromosomal genes or acquisition of new genetic information, typically in the form of R plasmids. The former often involves the alteration of a target of the antibiotic (e.g., the S12 ribosomal protein and streptomycin or the RNA polymerase and rifampin). The antibiotic target is usually required to perform a vital vegetative function of the cell, so the effect of the mutation must be limited specifically to the antibiotic-binding domain. Occasionally, point mutations have pleiotropic effects and can subtly or profoundly alter the ability of the organisms to exist in their environment. Insofar as those vegetative functions are required for the infective stage of the pathogen, the virulence of the pathogen will naturally be attenuated.

Perhaps in part for that reason, nearly all drug resistance in bacteria, particularly in pathogenic species, is due to R-plasmid acquisition, as reviewed above. Most drug-resistance genes act either by inactivating an antibiotic or by excluding it from its target site.[22,23,29,87] As opposed to the mutation of chromosomal genes, the acquisition of new genes (on the R plasmid) might be expected to have little if any effect on bacterial virulence. Presumably, no vegetative function has been altered. Any pleiotropic consequences to the bacterial cell might be expected to be limited to the effect of the small additional burden of replicating new genes and synthesizing new gene products. To balance that theoretical deleterious effect on cell physiology, it should be pointed out that R plasmids can carry genes in addition to those essential for drug resistance. Thus, the selection of R plasmids that also contain toxin genes or other virulence factors would indirectly select for the acquisition of new virulence factors.[25,28,31,37,39,40,70,71,73,77,85,130]

Two approaches have been used to analyze the effects of R-plasmid acquisition on the virulence of a bacterial strain. Some investigators have begun with a strain known to be virulent in an experimental infection model, genetically transferred a given R plasmid into the strain, and then compared the parent and the recipient for virulence in the model.[5,10,19,50,54,85,112,123] Others have surveyed isolates in the field (e.g., a hospital setting), determined which strains were drug-susceptible and which drug-resistant, and then evaluated the amount of disease that the two classes of bacterial strains were capable of producing.[44,47]

Studies comparing bacterial strains that differ only in whether they contain an R plasmid have generally revealed little difference in virulence, although a few exceptions are

notable. Two early reports of the effects of an R plasmid on S. typhimurium[112,123] showed decreased virulence in the strain that had acquired the R plasmid by conjugative transfer. The molecular basis of those results was not pursued; but they might be explained by more recent studies that have shown many clinical isolates to be relatively poor recipients in conjugative crosses. The efficiency of mating, though, can be overcome with a mutation in the smooth lipopolysaccharide (LPS), which concomitantly reduces bacterial virulence.[72] A single mutation is sufficient for that effect, so a single back-mutation to the virulent wild type readily appears in an appropriate setting, such as the animal gastrointestinal tract.

The consequences of R-plasmid acquisition can be dramatic, in contrast, when the R plasmid contains, in addition to drug-resistance determinants and RTF, a virulence gene, such as enterotoxin[25,37,40] or hemolysin,[130] or when the R plasmid is capable of mobilizing[85] or recombining with[77] a virulence-encoding plasmid. Thus, in special cases in which R plasmids are linked with virulence genes, selection by antibacterial agents might promote spread of virulent strains. For example, aerobactin plasmids in E. coli strains isolated are commonly associated with resistance to one or more antimicrobials, such as the tetracyclines and ampicillin.[48] The broad epidemiologic studies suggest that R plasmids do not interfere with bacterial pathogenicity and might significantly increase the severity of disease. The best examples include cases of plasmid coinheritance of drug-resistance factors and various enterotoxins, as in clinical isolates of Staphylococcus aureus[73] and E. coli.[5,25,37,69] Even without specific evidence of a coinherited virulence factor, it is clear that drug-resistant bacteria are culprits in major outbreaks of disease.[47] A recent review examined 175 published and unpublished reports evaluating the effects of resistance to specific antibiotics on the outcome of bacterial infections in both community and nosocomial settings.[44] Regardless of the setting, the drug-resistant bacteria, compared with drug-susceptible bacteria, were found to be associated with illness that was significantly more severe; in particular, the mortality, the likelihood of hospitalization, and the length of hospitalization were at least twice as great. Further analysis determined that the underlying causes of the worse outcome in hospitalized patients infected with a variety of drug-resistant bacteria were twofold: drug resistance led to a high incidence of antibiotic failure, and drug-resistant pathogens emerged in the superinfection that followed prior antibiotic treatment for a different disease.

In summary, R plasmids have been shown repeatedly to increase the virulence of bacteria, both when specific mechanisms can be elucidated[28] and when they cannot.[44] The

few experimental situations in which reduced virulence after R-plasmid acquisition has been demonstrated are unlikely to reflect the importance of selective pressures in nature. Any environmental factors that select for spread of R plasmids in bacterial populations (such as use of antimicrobial drugs) are likely to coselect for spread of coinherited virulence factors.

THE SPREAD OF ANTIMICROBIAL-RESISTANCE GENES THROUGH BACTERIAL POPULATIONS IN STAGES

Bacteria live in the world not in pure culture, but as mixed strains and species competing in ecosystems, many of which are carried by animals or humans.[129]

Bacteria isolated from humans a half-century ago had resistance plasmids, but not the resistance genes found in comparable isolates today.[21] The use of each new antibacterial agent since than has commonly led eventually to the emergence and spread on plasmids of genes that encode resistance to the agent.[93] The recent appearance of new resistance genes and of older ones in pathogens that had been free of them indicates that the process of emergence and spread is continuing.[26,80,83,86]

We review here evidence that the use of antimicrobial agents advances the emergence and spread of antimicrobial-resistance genes through bacterial populations in discrete stages triggered by specific events than are increasingly identifiable.

A previously unknown resistance gene can emerge by becoming mobilized from an obscure strain or by evolving from an ancestral gene. Use of an antimicrobial agent could mobilize a resistance gene from an obscure strain by selecting for the strain's overgrowth and thus increasing its contact with other strains. Use would similarly favor evolution by selecting for increasingly resistant mutants of the ancestral gene. Mobilization and evolution would each be likely to progress through stages, and some resistance genes might progress through stages of both.[36,127]

Broad-spectrum β-lactamases able to hydrolyze newer β-lactam antibiotics, for example, have recently emerged apparently by two-step mutations of β-lactamases (SHV-1 or TEM-2) that had emerged earlier.[52,116] Use of the older β-lactams had presumably mobilized the older resistance genes to stages of sufficient prevalence for their very rare mutants that were resistant to the new β-lactams to occur often for selection by use of the new agents and thus the beginning of the new stage.

Antibacterial agents can affect bacterial populations directly only by inhibiting susceptible strains. A bacterial ecosystem so depleted by an antimicrobial agent can be

repopulated by the progeny of a single bacterium that has a gene that encodes resistance to the agent. Overgrowth of a clone in the face of such selection can generate overnight a billionfold amplification of the number of copies of the resistance gene. That amplification might be transitory, however, because the isogeneic bacteria that carry these copies might prove less fit for the diverse niches of the ecosystem than would their heterogenic predecessors or successors.

SIGNIFICANCE OF PLASMIDS

Once inserted on a plasmid, a resistance gene can replicate and persist in the niches of all the strains to which the plasmid can be transferred. Recombination by various mechanisms, such as transposition or site-specific recombination, moveover, can move the resistance gene to other plasmids capable of transfer to additional strains and thus to additional niches, beyond the host range of the first plasmid.[6,75]

LINKAGE AND DISSEMINATION OF RESISTANCE GENES

Recombination of a resistance gene with additional plasmids and coresidence of the recombinant plasmids with additional plasmids and chromosomes in new strains would also tend to link or associate the resistance gene with other genes in the presence of different selection. Such other genes would include other resistance genes in the presence of selection by other antimicrobials, other adaptive genes on chromosomes, and genes that were maintained on plasmids and had unknown survival values before antibacterials began to be used.[21,109] Selection for any of these would preserve or amplify the resistance gene in the absence of the agents to which it encodes resistance.

Spread of plague and cholera across continents showed long ago that the world's bacterial ecosystems interconnect.[38] Accordingly, dissemination of an emerged resistance gene through them would await only sufficient amplification, recombination and transfer of the gene to get it into intercolonizing strains. Studies of resistance-gene phenotypes and more recently of their nucleotide sequences are, in fact, revealing few examples of parochial resistance genes confined to one area, and those examples may prove to have been premature.[46,79]

In recent years, moreover, new technology has made possible the detection, not just of distinctive resistance genes, but of distinctive plasmids carrying them that have

become widely distributed among bacterial genera, animal and human hosts, and widely separated geographic areas.

Two small multicopy plasmids that encode resistance to sulfonamide and streptomycin and that share homology over half their length were found in escherichia and salmonellae from animals and humans in many parts of the world.[4,128] Three distinctive multiple-resistant plasmids were found in collections of salmonella isolates from both animals and humans in the United States; one of them was endemic in cattle in 20 states and sporadic in humans in at least two other states.[95] A plasmid in an isolate from Venezuela was found also to carry one of the earliest gentamicin resistance genes in seven genera of enterobacteriaceae from eight widely separated medical centers in the United States.[94] A plasmid that encodes resistance to trimethoprim and other agents was in isolates of E. coli from pigs on a farm and from patients in a medical center 40 miles away.[13]

It has become possible to recognize identical or closely related specific transposons in plasmids from bacteria isolated in different parts of the world.[101,118] One large (20-kilobase) transposon, Tn 21, has been found in plasmids from some of the earliest multiple-resistance shigellae isolated in Japan and in a remarkable number of different-looking plasmids from other genera in different parts of the world. They include nearly all of the varied plasmids that first brought gentamicin resistance to 20 medical centers in Germany and a number of plasmids from various locations that carry different β-lactamase genes.[61,106]

A lineage has been proposed for the evolution of Tn 21 and its progressive acquisition of different resistance genes.[120] Tn 21 appears to have specific recombination sites that permit exchange of new resistance genes between its progeny.[11] If Tn 21 continues to be found in additional resistance plasmids, it will raise the possibility that resistance might have progressed in the world quite differently if it had not evolved.

SPREAD OF RESISTANCE GENES IN STAGES

The preceding observations indicate that resistance genes emerge and spread in successive stages.[90] The initial mobilization or evolution of the gene, insertion into a plasmid, transfer to strains within the plasmid host range, recombination with other plasmids, and insertion into other transposons and consequent linkage with other genes in the presence of different antibiotic selection, as well as the parallel evolution of the plasmids and transposons themselves (e.g., Tn 21) toward fitness in more niches, are all discrete steps in the dissemination of the gene.

Each of the stages sketched above can be seen to have the qualities that characterize the steps in an enzymatic cascade: amplification and irreversibility. At any stage, a given amount of antibacterial use would select more copies of the resistance gene than it would have at the preceding stage. Similarly, the use needed to maintain a new stage would be less than the threshold amount needed to initiate it.[76]

Each new stage begins with a mutation, a chance encounter of rare strains in vast populations, a conjugative transfer, or a recombinational event. Each such event has a low probability of occurrence, and the ultimate prevalence of a resistance gene depends on the product of the probabilities of the events that inaugurate each of its stages of spread.

We know, for example, that recombination of DNA segments that encode the TEM-1 β-lactamase with plasmids resident in strains of Hemophilus influenzae and Neisseria gonorrheae was the final step in the disastrous emergence of resistance to penicillins in these two pathogens 15 years ago. We can only guess, however, at the chain of preceding events that required 30 years of penicillin use before those final stages could occur.[9,133]

A resistance gene is an essential participant in each of the improbable events cited above, therefore, the chance that any of them will occur is a function of the prevalence of the resistance gene in the preceding stage.

EFFECT OF ANTIMICROBIAL USE ON SPREAD OF RESISTANCE GENES

Because use of antimicrobial agents can greatly amplify the prevalence of genes that encode resistance to them at each stage, such use can be seen as the main force driving the progression of resistance genes through their stages of spread.

If resistant bacteria arose only through frequent single-stage mutation of susceptible strains, as was once thought the genesis of a resistant strain isolated from any patient might be due entirely to antimicrobial use in that patient or a neighbor. If, however, the resistance in each such isolate is encoded on a complex genetic element that has been assembled in sequential stages, each triggered by a rare event, the lineage of that resistance might be traced back to almost anywhere. Antimicrobial use by the patient might have produced overgrowth and manifestation of the resistant strain in that patient, but the evolution of the strain's resistance genome and its spread to the patient required much greater use elsewhere.

The emerging connection between the molecular evolution of the genomes that carry a resistance gene and its stage of

spread suggests that even very little interchange between bacterial populations might be sufficient for wide dispersion through them of a resistance gene that had been selected to an advanced stage by intensive antimicrobial use in the first population. The analogy would be to a virus that evolves to high contagiousness on one continent and becomes epidemic on a second continent when taken there by a single tourist.

The growing information reviewed above is enough to show that there is a global epidemiology of antimicrobial-resistance genes and genomes, but not enough to measure them. A range of models can be considered.

At one extreme, a model would propose that antimicrobial use in bacterial populations in one region would have no effect on later resistance-gene prevalence in distant bacterial populations. At the opposite extreme is a model that proposes that the prevalence of resistance genes in a bacterial population is a function of total antibacterial use in all bacterial populations, however remote.

Neither of those extreme models appears to be appropriate. All the above evidence indicates some effect of use in one population on prevalence of resistance in others. For a more specific example, we have good reason to believe that current prevalence of penicillinase-producing Neisseria gonorrheae (PPNG) in a number of American cities is a consequence of antibiotic use in areas of the Far East 15 years ago.[29]

There appears to be (see Chapter V) gradients of prevalence of resistance from high to moderate to low as one moves from populations of intense to moderate to no use of antimicrobials. The critical question is: How much greater is the prevalence of resistance (and how much sooner is it attained) in populations of moderate or no use than would be the case if there had been no (or fewer) populations of intense use.

IMPLICATIONS FOR ANTIMICROBIAL USE

The models indicate the potential effect of the use of antimicrobial agents in animal bacterial populations on the prevalence of resistance genes in human bacterial populations. Nearly half the antimicrobial use in the United States is in animals. The pool of resistance genes in animal flora in the United States may be estimated (see Chapter V) to be 10 times that in the total human flora. Animal bacterial populations are not distant from human bacterial populations, in as much as continuous samples of the animal populations flow on slaughtered carcasses through food distribution chains to most households in the United States.

Although recent work with new technology has elaborated many of the observations presented above, the global effect

of antibacterial use on bacterial resistance has long been grasped intuitively by workers studying resistance in the laboratory and by clinicians coping with resistance in their patients.[29,134] Many have urged that antimicrobial use be minimized whenever possible and have identified use of antimicrobials in animal feed as the largest use category that could be reduced in the United States.[67]

Their concerns and recommendations, however, were based on indirect evidence. The complexity of the processes outlined above and the hugeness of the bacterial populations in which they operate had precluded reconstructing the entire chain of use that led to the presence of a particular resistance gene in the isolate from a particular patient.[68]

DUAL ROLES OF SALMONELLAE AS PATHOGENS AND TRACERS

The first direct evidence that antimicrobial use in animals led to resistance in bacteria in humans came from an epidemic of multiple-resistant salmonellae among calves in Britain, apparently augmented by the antimicrobial use, that spread to infect humans. It became the occasion to enact legislation banning routine addition of antibiotics to animal feed in Britain and countries of the European Common Market (see Chapter V).[2]

Some observers felt that the epidemic in Britain had been exceptional, ascribable to peculiar husbandry practices there, and not pertinent for the United States.[51] When the National Research Council Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds met in 1980, it could still be argued that in the United States animal and human bacterial resistance genes were in separate pools that did not interchange.[88] The committee judged that hazard to human health associated with the subtherapeutic use of antimicrobials in animal feeds was neither proved nor disproved.[88]

A report published two years later found three different examples in which isolates of salmonellae from animals and humans in the United States carried resistance plasmids that had the same distinctive restriction endonuclease fragments. Several later studies used the same method to trace spread of resistant strains of salmonellae from animals to humans in recognized food-borne outbreaks in the United States (see Chapter V).

All the direct evidence comes from salmonellae, because they are peculiarly traceable. Relatively rare in human flora, they signal their presence by producing a conspicuous illness. Laboratories routinely find them with selective media even when they are sparse in stool flora and send them to reference laboratories, where their serotypes are discriminated from more than a 1,000 possible types. More

than 40,000 strains are winnowed out of human flora in the United States by that elaborate system each year, and their serotypes are recorded. A parallel system exists for salmonellae isolates from animals.

Although their relative rarity in human flora helps to make salmonellae traceable, it also leaves them a very small part of the antimicrobial-resistance problem. It is probably reasonable to estimate that, of all the courses of antimicrobial therapy administered to humans in the United States, including all the expensive and sometimes toxic second and third generation agents used to circumvent resistance to older agents, much less than 1% are directed against infection by salmonellae.

DUAL LINES OF EVIDENCE OF FEED-ADDITIVE HAZARD

Two lines of evidence of hazard associated with antimicrobial use in animal feed additives have been developing in the last several years.

One line of evidence, as outlined here, arises from the growing understanding that the resistance genomes in bacterial isolates of humans are the products of extensive evolutionary development that required long exposure of vase bacterial populations to antibacterial agents. Where the populations might not be critical, given the growing evidence of their interconnection. Which antibacterials were used might also not be critical, given the close linkages observed between resistance genes and the ease with which some plasmids, once made prevalent by use of an antimicrobial, appear to acquire new resistance genes by site-specific recombination.[97]

The second line of evidence arises from epidemiologic observations, supported by molecular fingerprinting of plasmids, of outbreaks and other surveys of salmonella infection in the United States in recent years. They provide valuable examples for the first line of evidence, but also direct evidence of the effect of antibacterial use in animals on the outcome of specific types of human infection.

The first line of evidence addresses all resistance in human bacterial flora, but still cannot yield enough direct evidence for development of a quantitative risk assessment model. The second line of evidence addresses a subset of human bacterial infections, but can yield direct evidence for a risk assessment model, as developed in Chapter VII.

SUMMARY OF THE BIOLOGIC IMPACT OF DRUG RESISTANCE

Although present at low incidence in the preantibiotic era, drug resistance has burgeoned since the wide use of

antimicrobial drugs began 4 decades ago. Most drug resistance is due to the presence of transposable genetic elements, called transposons, that are found on R plasmids, which are usually conjugative. Because of the flexibility of this mechanism of genetic expression, bacteria can acquire resistance to a given antimicrobial agent quite easily and at minimal cost in terms of growth rate and general hardiness. Because of the ability of R plasmids to add additional transposons in modular fashion, the conjugative acquisition of an R plasmid typically confers drug-resistance phenotypes in addition to that specifically selected by the presence of antibiotic in the environment.

R factors make a direct contribution to the ability of bacteria to cause disease. Unfortunately, however, R factors occasionally carry virulence factors as well. The mere presence of the drug-resistance phenotype has been shown, in epidemiologic surveys, to increase severity of illness. The mechanism of the latter is twofold: antimicrobial resistance leads to delays in the selection of an appropriate therapeutic agent and permits the differential outgrowth of the resistant organism during antimicrobial therapy or some other infection or presumed infection.

With greater understanding of the molecular biology of drug resistance, R-plasmid passage and strain dissemination have become better understood as well. R plasmids are genetically plastic and in many cases are transmissible to a wide variety of strains and species. Therefore, the outbreak of disease due to a clonally expanded strain can often be followed with great precision by following its plasmid molecular profile, as deduced from restriction fragment-length polymorphism. With this sophisticated technology, R-plasmid-containing strains of salmonella have been traced unequivocally from the farm through the food chain to clusters of human patients. Outbreaks have provided strong evidence of the deleterious role that antimicrobial use in the environment has played in the selection and propagation of antimicrobial resistant bacterial pathogens of humans.

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IV

QUANTITATION OF ANTIBACTERIAL AGENTS USED IN LIVESTOCK AND POULTRY FEEDS

Exposing large numbers of bacteria to an antibacterial agent at concentrations that inhibit growth is the most effective means of selecting antimicrobial-resistant strains (or species, if the initial population comprises a mixture of species). The concentration of the antimicrobial chemical to which bacteria are exposed determines both the number of resistant organisms that may be isolated initially and the magnitude of resistance to that antimicrobial chemical. The duration of exposure to an antimicrobial compound has an important role in the elimination of susceptible strains or species in mixed populations, e.g., in the lower gastrointestinal tracts of humans and animals.

Antibacterials are used in livestock and poultry in several dosages: at high (therapeutic) concentrations to treat established infectious diseases and at low (subtherapeutic) concentrations for enhancement of growth and for disease prevention (prophylaxis). The Food and Drug Administration (FDA) approves separately each label claim for efficacy for each dose and animal species. The Feed Additive Compendium summarizes all FDA-approved feed additives, label claims, doses, animal types, and federal regulations. It is estimated that approximately 75% of dairy calves, 60% of beef cattle, 75% of swine, and 80% of the poultry marketed have received one or more antimicrobial drugs in their feed at some time.[1,2]

Twenty antimicrobial drugs have been approved by FDA as feed additives (Table IV-1). Penicillin and chlortetracycline were approved in 1951 and oxytetracycline in 1953. Nonantibiotic antimicrobial drugs, such as nitrofurans and sulfonamides, are used in the same fashion. In addition, ionophores, such as monensin, which have little antibacterial activity, are extensively used as coccidiostats in the poultry industry. Combinations of antimicrobials such as chlortetracycline and hygromycin or chlortetracycline, penicillin, and sulfathiazole--are approved as feed additives.

POPULATION OF LIVESTOCK AND POULTRY

It is useful to know the population of each species of animal for calculating the amounts of penicillin or the

TABLE IV-1

COMMON ANTIBACTERIAL FEED ADDITIVES
APPROVED BY U.S. FOOD AND DRUG ADMINISTRATION

Additive	Year Approved
Sulfaquinoxaline (sulfonamide)	1947
Roxarsone (arsenical)	1951
Chlortetracycline	1951
Penicillin	1951
Bacitracin	1953
Sodium arsenate (arsenical)	1953
Furazolidone	1953
Oxytetracycline	1953
Erythromycin	1955
Hygromycin B	1957
Neomycin	a
Novobiocin	1961
Tylosin	1961
Sulfamethazine[b]	1963
Sulfamerazine[c]	1967
Oleandomycin	1968
Lincomycin	1970
Sulfathiazole[b]	1971
Bambermycin	1973
Virginiamycin	1974

Source: U.S. Food and Drug Administration, Division of Animal Feeds, Center for Veterinary Medicine, December 1987 (personal communication).

[a] Never officially approved; has been marketed since before 1958.

[b] For use in combination only.

[c] For use in fish.

tetracyclines used to medicate them. The food-animal population in the United States is very large--more than 20 times the human population (Table IV-2). In 1971 and 1985, for example, the total U.S. food-animal population was 3,522 and 5,122 million head, respectively (Table IV-2). The number of head of livestock (exclusive of poultry) for the same 2 years was 237 and 206 million. In the intervening 14 years, production of red meat declined somewhat, and poultry production increased. The relationship of the amount of red- to white-meat food produced is important in considering the magnitude of human exposure to meat or poultry products contaminated with pathogenic bacteria of farm-animal origin. An understanding of the magnitude can be had by inspection of per-capita consumption figures for meat and poultry in this country. The consumption of red meat per capita ranged from a high of 168 lb in 1971 to a low of 153.2 lb in 1985. In the same period, the amount of poultry consumed increased from 49.0 to 69.7 lb.[6]

ANIMAL CONSUMPTION OF FEED AND BACTERIAL CONTENT OF GASTROINTESTINAL WASTE

To understand the number of coliform organisms potentially exposed to antimicrobials in animal feed, one need only examine Table IV-3, which shows the large amounts of feed consumed per head of livestock or poultry annually. (The amounts of medicated feed consumed are discussed later.) Multiplying the amounts of feed by numbers of animals in production in the United States (Table IV-2) yields an estimate of the large amounts of manure produced. Animal manure is a direct source and a vector of bacterial contamination of the farm, of farm animals, of forage crops and feed, of farmers, of slaughterhouse workers, and of meat or food by products (such as milk and eggs) consumed by humans.[3] The spread of bacteria can be enhanced by the coprophagic habits of some animal species. As an example of the magnitude of the potential bacterial contamination problem associated with farm and feedlot exposure of animals, the numbers of coliform organisms and the large amount of manure produced by beef cattle can be cited. A single 900-lb feedlot steer produces about 9 lb (4.1 kg) of manure solids daily; each gram contains approximately 10^7 coliform organisms, for a total of 4.14×10^{10} such organisms per day.[5]

TOTAL ANTIBIOTIC PRODUCTION

Accurate analysis of the impact of antimicrobials on farm animals (on their infectious bacterial flora, health, or

TABLE IV-2

ANIMAL PRODUCTION IN UNITED STATES
(Thousands of Head Marketed or Produced for Marketing)

Year	Cattle	Calves	Sheep	Lambs	Chickens	Broilers	Turkeys	Hogs	Hogs/pigs[a]
1987	49,900	10,564	-	-	-	-	-	-	-
1986	49,995	10,477	-	-	216,938	4,646,312	207,216	-	-
1985	48,739	10,488	1,610	6,456	251,957	4,478,749	185,282	86,583	52,298
1984	50,682	10,253	1,821	7,007	234,769	4,282,391	171,296	87,344	54,073
1983	48,089	10,443	1,820	7,104	231,821	4,183,660	170,723	89,129	56,694
1981	46,647	10,383	1,510	7,013	223,721	4,147,521	170,875	95,986	58,698
1979	48,358	10,151	1,347	6,336	225,066	3,951,291	156,457	92,499	67,318
1977	56,378	12,621	1,450	7,361	235,856	3,939,897	136,890	80,939	56,539
1975	54,315	12,239	1,812	9,039	238,576	2,950,099	124,165	73,627	49,267
1973	48,369	11,652	2,198	10,879	236,710	3,008,667	132,231	82,410	60,614
1971	49,143	12,086	2,202	12,627	220,195	2,945,348	119,657	98,644	62,412

Source: U.S. Department of Agriculture.[6a]

[a] Combination of pigs and overweight market hogs.

TABLE IV-3

FEED CONSUMED BY LIVESTOCK AND POULTRY 1971-1983
(Feed Consumed Per Head, Pounds, Equivalent Feeding Value of Corn)

Year (Beginning October)	Dairy Cattle		Beef Cattle[a]	Sheep and Lambs	Poultry				Hogs (per 100 lb)
	Milk Cows	Other			Hens and Pullets	Chickens	Broilers	Turkeys	
1971	11,370	5,859	9,198	1,008	99	32	9.3	96	954
1973	11,570	5,774	7,708	2,021	100	32	9.7	94	660
1975	11,540	6,303	7,593	1,260	101	26	7.7	71	566
1977	12,129	8,864	6,524	1,442	103	24	8.6	79	633
1979	12,978	6,800	8,122	1,373	107	26	9.4	76	688
1981	12,181	6,248	8,759	1,409	108	26	10.0	84	620
1983	12,648	5,795	7,848	1,723	105	28	9.3	82	605

Source: Adapted from U.S. Department of Agriculture.[6a]

[a] Feed consumed divided by the number of cattle on feed January 1.

rates of growth) requires reliable data on the total amounts of penicillin and the tetracyclines used annually in animals (as feed additives, for growth promotion, for prophylaxis, and for treatment of infections) and medical use in humans. The same information on other common feed-additive drugs would be useful for purposes of comparison, but such detailed information is not available. Although gross estimates of production and use have been made, there is not good agreement between the figures from different sources. However, the figures that are available to the committee provide a general basis for estimating the breadth of use of antibiotics in animals reared for human food.

The U.S. International Trade Commission provides information on the annual production of antibiotics in the United States (Table IV-4). The data show total pounds produced for medicinal and nonmedicinal use in humans and animals. From 1950 to 1986, the total annual antibiotic production in the U.S. increased by a factor of about 49 (from 0.9 to 44.4 million pounds). Production figures for 1986 show an atypical annual increase (39%) over the preceding year and may represent an aberration. However, the figure for 1985 (31.9 million pounds) is within the range for the previous 4 years (30.4-32.5 million pounds) and represents an increase by a factor of about 35 over production in 1950.

The data in Table IV-4 show that the percentage of total production directed to animal feed and other uses increased from 16% in 1951 to 38% in 1959. In the 1960s, an average of about 40% of the total antibiotic production was directed to animal feed and other uses. By the late 1970s, 42-48% of antibiotic production was directed to animal feed and other uses. Although information on the actual amount of antimicrobial production used in animal feed rather than other use is not available, an assumption can be based on information presented later in this chapter--that the nonmedicinal represented the predominant use. In Table IV-5, for 1981-1986, the total production figures are available for classes of antimicrobials, but a direct breakdown into medicinal and nonmedicinal uses is not available. Although Tables IV-4 and IV-5 are not directly comparable, they do show that large amounts of antimicrobials were used as feed additives. In 1983, for example, 31.9 million pounds of antibiotics was produced, of which 22.5 million pounds (71%) was tetracyclines plus other non-β-lactam antibiotics. In the same year, 75% of the antibiotics (other than penicillins, cephalosporins and tetracyclines) were directed to feed additive and other uses (Table IV-4). Thus, 36% of the entire antibiotic production for 1983 consisted of antibiotics (other than β-lactams and tetracyclines) that were directed to feed additive and other uses.[7] Considerable amounts of tetracycline and penicillin go into animal feed,

TABLE IV-4

U.S. ANTIBIOTIC PRODUCTION 1950-1986[a]

Year	Total		Medicinal Use	Nonmedicinal Use		
	U.S. Production, 10^6 lb	Annual Change, %	In Humans and Animals, 10^6 lb	Added to Animal Feed and for Other Uses, 10^6 lb	Annual Change, %	Portion of Antibiotic Production Added to Animal Feed and Other Uses, %
1986	44.4	+39	b	b	b	b
1985	31.9	+ 5	b	b	b	b
1984	30.4	- 5	b	b	b	b
1983	31.9	- 2	b	b	b	b
1982	32.5	+ 6	b	b	b	b
1981	30.6	+24	b	b	b	b
1980	24.6	- 2	b	b	b	b
1979	25.2	- 2	14.6	10.7	-13	42
1978	25.7	+11	13.4	12.3	+22	48
1977	23.1	+13	14.0	10.1	+ 1	43
1976	20.5	+12	10.4	10.0	+12	48
1975	18.3	-11	9.4	8.9	+20	48
1974	20.5	- 1	13.2	7.4	-10	36
1973	20.8	+25	12.6	8.2	+21	39
1972	16.6	- 7	9.8	6.8	- 4	41
1971	17.9	+ 6	10.8	7.1	- 3	40
1970	16.9	+28	9.6	7.3	+26	43
1969	13.2	+28	7.4	5.8	+35	43
1968	10.3	+ 8	6.0	4.3	+ 2	42
1967	9.5	- 2	5.2	4.2	0	45
1966	9.7	29	5.4	4.2	+50	43
1965	7.5	+15	4.7	2.8	+ 8	37
1964	6.5	- 3	3.9	2.6	+ 4	40
1963	6.7	+ 6	4.2	2.5	+ 9	37
1962	6.3	+24	4.0	2.3	+28	36
1961	5.1	+ 9	3.3	1.8	+ 6	35
1960	4.7	+27	3.0	1.7	+21	36
1959	3.7	+ 6	2.3	1.4	+56	38
1958	3.5	+ 9	2.6	0.9	0	26
1957	3.2	+19	2.4	0.9	+12	26
1956	2.7	+29	2.0	0.8	+60	28
1955	2.1	- 9	1.6	0.5	0	25
1954	2.3	+10	1.8	0.5	+25	21
1953	2.1	+24	1.6	0.4	+33	21
1952	1.7	+13	1.5	0.3	+50	15
1951	1.5	+67	1.3	0.2	-	16
1950	0.9	-	0.9	b	-	-

TABLE IV-4 FOOTNOTES

a 1950-1978 data from National Research Council (NRC) report, The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds[4a] (NRC data is based on reports of the U.S. International Trade Commission [ITC][7]) 1979-1986 data culled from ITC reports[7] (animal-feed antibiotic data breakdown not presented by ITC for 1980-1986).

Amounts of sulfonamides produced not included. Only chemicals included in ITC production figures; not included are finished pharmaceutical preparations and products in form of pills, tablets, and capsules.

In ITC data, amounts of antibiotics produced differ from reported amounts sold; e.g., in 1986, 44.4 million pounds of antibiotics produced and 11.3 million pounds sold. Difference between reported production and sales attributed to inventory changes, losses in processing, and captive consumption converted into ethical and proprietary pharmaceutical products by primary manufacturer. Many pharmaceutical manufacturers not included in ITC reports if not primary producers of medicinal chemical (i.e., their drug requirements are met by purchases from U.S. or foreign producers).

Amounts of antibiotics produced annually assumed to provide better estimate of amounts used than ITC estimates of amounts sold, because finished dosage-form products made under captive use not included in estimates of amounts sold.

b Figures not available.

TABLE IV-5

U.S. PRODUCTION OF INDIVIDUAL CLASSES OF ANTIBIOTICS, 1981-1986

	Production, Millions of Pounds					
	1981	1982	1983	1984	1985	1986
Cephalosporins	1.1	1.1	1.4	1.4	a	1.7
Penicillins	7.4[b]	7.4[b]	6.1[b]	a	6.8[c]	7.7[c]
Semisynthetic penicillins[d]	2.3	2.1	1.9	2.0	-	-
Tetracyclines	6.8	7.2	7.2	a	a	a
Others	13.0	14.7	15.3	27.0	25.1[e]	35.0[f]
Total sulfonamides	3.9	3.1	2.8	a	a	a
Total	30.6	32.5	31.9	30.4	31.9	44.4

Source: Adapted by the committee from U.S. International Trade Commission (ITC) Reports.[7]

a Figures not available.

b Penicillins other than semisynthetic penicillins.

c Includes all penicillins.

d Includes ampicillin, amoxicillin, dicloxacillin, cloxacillin, and oxacillin.

e Includes tetracyclines and cephalosporins.

f Includes tetracyclines.

so it is clear that, on the basis of production data, the true figure for the percentage of antibiotic production going into feed additives probably lies somewhere between 42% and 48% (Table IV-4).

Penicillins and tetracyclines together make up 42% of the total 1983 antibiotic production. Of the other antibiotics, which account for 58% of the total production for the same year, only a few are approved for use as feed additives (see Table IV-1). Total sulfonamide production of 2.8 million pounds in 1983 was not included in the previously cited antimicrobial-use data. Information is not available as to what portion of sulfonamide production was directed to animal feed uses.

Although precise figures on the amounts of individual antibiotics used in feed are not available from the ITC data, it appears that a large percentage (42-48%) of the total antibiotic production in the United States is used in animal feed; penicillins and tetracyclines represent a sizable fraction thereof. FDA, in using 1979 data from ITC, has estimated that approximately 55-60% of the penicillin and tetracycline consumed in the United States is used in animal feed in subtherapeutic dosages; an additional percentage is used for therapeutic purposes (FDA, Personal Correspondence, Office of Planning and Evaluation, 21 May 1986).

ANTIBIOTIC USE IN FARMS AND FEEDLOTS

Actual percentages of each type of antibiotic used in farms and feedlots would be more informative than the foregoing calculations of total antibiotics used.

A summary analysis of amounts used in farms and feedlots for the period 1980-1985 has been attempted (see Table IV-6) with data from IMA America, Ltd., private organizations (on purchases of feed antibacterials by feed manufacturers, livestock supply stores and distributors, etc.), the Animal Health Institute (AHI), ITC, researchers, trade associations, and the companies that manufacture drugs for use in animals. The figures in Table IV-6, the best available to this committee, show little variation in this period in total feed use (amount sold to the feed trade) of antibacterials: 9.7-11.7 million lbs/yr. Tetracycline accounted for 57% of this volume in 1980 and 49% in 1984 and 1985. Penicillin accounted for only 5-8% of the total. The total use of tetracyclines and penicillin in feed in this period gradually declined. The foregoing figures on annual sales of antibacterials for livestock and poultry feeds can be related to the total annual production of all antibiotics (for all uses) and to the total annual production of the tetracyclines and penicillin. For example, in 1983, 31.9 million pounds of antibiotics was produced and 9.9 million pounds was sold to

TABLE IV-6

ANNUAL SALES OF ANTIBACTERIALS FOR LIVESTOCK AND POULTRY FEEDS, 1980-1985

Year	Production for all Uses[a]	Annual Sales for Livestock and Poultry Feeds											
		Total			Tetracyclines			Penicillin			Others		
	10^6 lb	10^6 lb	10^6 kg	%[b]	10^6 lb	10^6 kg	%[c]	10^6 lb	10^6 kg	%[c]	10^6 lb	10^6 kg	%[c]
1980	24.6	11.2	5.1	46	6.4	2.9	57	0.9	0.4	8	3.9	1.8	35
1981	30.6	9.7	4.4	32	5.2	2.4	54	0.7	0.3	7	3.8	1.7	39
1982	32.5	10.8	4.9	33	5.3	2.4	49	0.7	0.3	6	4.9	2.2	45
1983	31.9	9.9	4.5	31	5.1	2.3	52	0.6	0.3	6	4.2	1.9	42
1984	30.4	11.7	5.3	38	5.7	2.6	49	0.7	0.3	6	5.3	2.4	45
1985	31.9	11.0	5.0	34	5.4	2.4	49	0.6	0.3	5	5.1	2.3	46

Source: Modified from a presentation (unpublished) to the committee by H. W. Jamison of the Animal Health Institute's Antibacterial Research Criteria Task Force, March 21, 1988, entitled "Estimating the Volume of Antibacterials Used in Livestock and Poultry Feeds."

[a] From Table IV-4.

[b] Percent of total antibiotic production.

[c] Percent of total annual sales for livestock and poultry feed.

the feed trade for use in livestock and poultry feeds. Tetracyclines and penicillin accounted for 58% of the 9.9 million pounds. In the same year, of the 7.2 million pounds of tetracyclines produced, 5.1 million pounds (71%) was sold for use in livestock and poultry feeds (Tables IV-5 and IV-6).

SUBTHERAPEUTIC USE OF ANTIBACTERIAL DRUGS IN ANIMAL FEED

The current FDA-approved uses of the tetracyclines and penicillin in animal feeds are for growth enhancement, disease prevention, and treatment of disease. In the first two of these categories (commonly designated "subtherapeutic"), these antibiotics are used at 200 grams or less per ton for 2 weeks or more.[4] Some combinations with other antibacterials (tetracycline at 100 g/ton [g/ton = grams drug per ton of feed], sulfamethazine at 100 g/ton, and penicillin at 50 g/ton) have been approved by FDA for use in pigs that weigh up to 75 lbs. The Center for Veterinary Medicine (see Footnote #1, page 1 of Preface) considers any extended use of antibiotics in feed at 200 g/ton or less beyond 2 weeks as "subtherapeutic use," whether it is for growth enhancement or disease prevention. "Use levels are generally 200 g/ton or less of penicillin or tetracycline, but dosage units will vary by species. Levels approved for claims of growth promotion and disease prophylaxis are usually lower than those approved for disease treatment; however, there is some overlap in the claims for dose levels of 200 g/ton or less." There is more concern in the agency with the length of time the antibiotic is used in feed than in the level of drug. (FDA, personal correspondence, April 26, 1988.) Although the need for such information is critical, there are no actual data (only estimates) on the amounts of penicillin or tetracycline used subtherapeutically in animal feed.

Experts in veterinary medicine from the Animal Health Institute and elsewhere testified to this committee that use of the prophylactic dosage of antibacterial agents is common and important for successful and profitable rearing of livestock. It appears that hog farmers use antibacterial feed additives rather consistently during the early periods in raising swine. That involves such use of antibacterial additives for about 10 weeks in feeder pigs (approximately one half of their life span), divided into several stages. The first stage often consists of the tetracycline-sulfamethazine-penicillin combination or a tylosin-sulfamethazine combination. After that stage, tetracycline (25-50 g/ton), tylosin alone, bacitracin, or bambermycin is used. In cattle it is common to feed a tetracycline-sulfamethazine combination per head (350 mg of each drug to

each animal) per day for 4-6 weeks as the cattle go into feedlots. The intent of such administration is primarily "prophylactic." Although penicillin and the tetracyclines are approved by FDA for use in several classes of poultry, they are not used commonly in broilers.

In its efforts to obtain broader and more up-to-date information about the use of antibiotics by the different animal industries, the committee made inquiries to the National Broiler Council (NBC), the Texas Cattle Feeders Association (TCFA), and the National Pork Producers Association (NPPA). NBC in 1984 surveyed 30 companies representing 77.8% of the industry's broiler production for that year. None of the companies reported using penicillin or the tetracyclines to increase rate of growth and feed efficiency. However, they did indicate that those antibiotics were widely used in disease prevention and treatment programs. Of the 30 companies surveyed, 18 (60%) reported using penicillin in disease prevention or treatment programs, 28 (93%) reported using chlortetracycline, 23 (77%) reported using oxytetracycline, and 10 (33%) reported using tetracycline. The use of the antibiotics for disease prevention is summarized in Table IV-7.

A survey by the TCFA in 1986 involved 102 feedyards that produce 75% of the feed cattle in the TCFA area (New Mexico, Oklahoma, Texas). Responses from nutritionists responsible for more than 11 million head of cattle (approximately 42% of all the feed cattle produced annually in the United States, assuming a total feed cattle production of 26 million a year) indicated that none of the animals receives continuous low-dose tetracyclines in feed (penicillin is not approved for such use). However, the survey did not address the use of subtherapeutic concentrations for disease prevention.

The NPPA has not conducted a recent survey on the use of penicillin or tetracyclines in swine feed. However, Virgil Hays (personal communication, 1988) has calculated the amount of tetracycline used in swine feed for the committee (Table IV-8). According to U.S. Department of Agriculture figures, approximately 86.5 million pigs (see Table IV-2) with an average weight of 110 kg were marketed in 1985. On the basis of the numbers in Table IV-8 and the survey estimate of 6.6 g of tetracyclines per pig, it is possible to derive a figure for the total amount of tetracyclines used in the rearing of swine in that year: 86.5 million (head of swine) x 6.6 g (tetracyclines per pig) = 0.57 million kilograms (1.25 million pounds) of tetracyclines. If all swine feed were medicated with tetracyclines, the total would be 1.7 million kilograms (3.7 million pounds). Because 5.4 million pounds of tetracyclines were estimated to have been used in livestock and poultry feeds in 1985 (Table IV-6), the figure of 1.25 million pounds used in pig production represents approximately 23% of the total tetracyclines used in animal

TABLE IV-7

USE OF PENICILLIN OR TETRACYCLINES FOR DISEASE PREVENTION IN POULTRY, 1984

Drug	No. Companies[a]	Route	Dosage	Duration of use, days
Penicillin	3/14	Feed	50-100 g/ton	3-14
Penicillin	4/18	Water	100-160 KU/gal[b]	3-14
Chlortetracycline	9/28	Feed	100-500 g/ton	2-7
Chlortetracycline	9/28	Water	100-400 mg/gal	2-7
Oxytetracycline	6/23	Feed	100-200 g/ton	3-14
Oxytetracycline	8/23	Water	100-400 mg/gal	3-14
Tetracycline	5/10	Water	200-1,000 mg/gal	3-7

Source: Data from J. P. Pretanik, National Broiler Council, 1988 (personal communication).

[a] No. Companies = (number of companies reporting this use)/(number of companies reporting).

[b] KU = thousand units.

TABLE IV-8

ESTIMATES OF TETRACYCLINE USED IN REARING SWINE

Use	Feed, kg	Tetracycline, g[a]	
		All Diets	Survey Estimate
In sow breeding and gestation: estimate used 21-day breeding period	44	2.7	0.17
In lactation: assumption of 100 g/ton	15	1.6	0.17
Starter: assumption of 100 g/ton	23	2.5	0.47
Grower: assumption of 50 g/ton	96	5.3	1.92
Finisher: assumption of 25 g/ton	280	7.7	3.87
Total per pig	458	19.8	6.60

Source: Data from Virgil Hays, University of Kentucky, 1988, (personal communication).

[a] As baseline for comparison, amount of tetracycline is calculated for medication of all diets. On basis of surveys done in the state of Illinois and those done by University of Nebraska in 1983, and more recently by Hays (1988) of seven major suppliers of feed for swine, figures have been calculated in right-hand column. The committee recognizes that accuracy of figures cannot be validated in practice. However, in absence of recent comprehensive survey data, the committee views these as best estimates of amounts of tetracycline used in swine.

and poultry feed. According to Virgil Hayes (personal communication, 1988), triple-drug medication that includes the tetracyclines in the feed of growing swine is used routinely, thus subtherapeutic dosing is frequent and widespread.

Estimates of antimicrobial consumption indicate their use in farm animals is predominantly in subtherapeutic concentrations. In December 1984, Gustafson testified before the Subcommittee on Investigations and Oversight of the U.S. House of Representatives Committee on Science and Technology. Gustafson presented estimates (Table IV-9) derived primarily from industry sources, that, of the 8,316,000 kg (18.3 million pounds) of antibiotics used in animal production, 88% was used in subtherapeutic concentrations; of the latter amount, 28% was for growth promotion. Of the 2,640,000 kg of the tetracyclines administered to farm animals (Table IV-9), 2,403,000 kg (91%) was used in subtherapeutic concentrations. Only 7% and 2%, respectively, of all the tetracyclines administered to cattle and swine were for therapeutic purposes, but 85% of the tetracyclines given to poultry was used therapeutically. In addition to reviewing the above estimates made by Gustafson in 1984, the committee sought and received estimates made by AHI that would have involved data from the same sources as were available to Gustafson (Table IV-6). In those estimates, the total sales of antibacterials for livestock and poultry feeds was 5.0 million kilograms in 1985, contrasting with the figure of 8.3 million kilograms reported by Gustafson (Table IV-9). The difference is substantial and is not readily explicable. However, it should be noted that the total figures for annual use (or sales) of tetracyclines in livestock and poultry are very similar: 2.6 million kilograms in the Gustafson estimates and 2.4 million kilograms in the AHI estimates, prorated for total U.S. antibiotic production in 1985 (Tables IV-6 and IV-9). The AHI estimates are related to "antibacterials" and the Gustafson estimates to "antibiotics," so the differences might be due to inclusion in the latter category of antibiotics that are coccidiostats (e.g., monensin), rather than antibacterial agents. As a result of the aforementioned differences, the committee is uncertain as to which estimates should be used as a basis for comparison of trends in annual use.

The estimates of tetracycline use in feed for individual animal and avian species show some possible discordances. The estimates by Hays of tetracycline use in swine indicate that 1.7 million kilograms would be used if all swine feed were medicated with this drug. This figure would be close to the 1.65 million kilograms in the 1985 estimate of Gustafson. However, not all swine are medicated with tetracyclines during growth. In addition, the previously noted NBC and TCFA surveys both indicate recent marked reductions in the

TABLE IV-9

ESTIMATED 1985 ANNUAL ANTIBIOTIC USE IN THERAPY, DISEASE PREVENTION, AND GROWTH PROMOTION

All Antibiotics, thousands of kilograms

		Subtherapeutic Use		
Therapeutic Use		Disease Prevention	Growth Promotion	Total
Cattle	458	1,100	340	1,898
Swine	250	3,578	1,391	5,219
Poultry	304	580	315	1,199
Total	1,012	5,258	2,046	8,316

Tetracyclines (Chlortetracycline and Oxytetracycline), thousands of kilograms

		Subtherapeutic Use		
Therapeutic Use		Disease Prevention	Growth Promotion	Total
Cattle	50	589	130	769
Swine	30	950	701	1,651
Poultry	187	33		220
Total	267	1,572	831	2,640

Source: Correspondence from R. H. Gustafson (American Cyanamid Company) to E. Eastman (Subcommittee on Investigations and Oversight, House Committee on Science and Technology), January 24, 1985 (personal communication).

use of tetracyclines for growth promotion. However, the committee believes that the tetracyclines are widely used in poultry production for disease prevention. In cattle-raising, data on the extent of use of prophylaxis are not reported. Although use of tetracycline for growth promotion in poultry and cattle has reportedly decreased, it is unknown whether the prophylactic use of subtherapeutic concentrations has remained constant or has increased correspondingly. Thus, it is very difficult to quantify current subtherapeutic use of tetracyclines (and penicillin) on U.S. farms and feedlots.

In summary, exact data on antibiotic use in animal feed are not obtainable. The best available estimates indicate that 31-46% of the total annual production of antibiotics (31.9 million pounds) in the United States is used in animals for all purposes. (Even higher figures have been suggested). A range of 9.7-11.7 million pounds (4.4-5.3 million kilograms) was used annually from 1980 through 1985 (Table IV-6). In the same years, tetracycline use in livestock and poultry totaled 5.1-6.4 million pounds, and penicillin use 0.6-0.9 million pounds. Although data are limited, it appears that about twice the amount of antibacterials is used for disease prevention as for growth promotion.

The committee is aware that strict compliance with the regulations in the United States governing subtherapeutic use of antimicrobials is not always achieved in common practice. On the farm, planned brief periods of antimicrobial use and the amount of medication and duration of its administration might sometimes be below or exceeded the specifications. For example, concentrations of antimicrobials actually achieved in feed might vary widely from the specified regulatory limits, because of miscalculation or improper mixing of drug and feed either on the farm or by the feedmill. The true period of medication might be longer or shorter due to the time taken to consume the amount of feed in storage or for other reasons. For purposes of analysis, the committee has considered all use of antimicrobials as specified for both growth enhancement and disease prevention as being in the category of subtherapeutic use. That is also the view taken by FDA (M. F. Lowe, 1988, personal communication). The approach seems reasonable, in that, with the exception of the report of Gustafson, there is little quantitative information to distinguish use for growth promotion from use for disease prevention. Gustafson's estimates indicated that 88% of all antibiotic use in livestock and poultry was subtherapeutic.

Tetracyclines account for almost 50% of total antibacterial use in livestock and poultry feeds (Table IV-6). In the estimates of Gustafson (Table IV-9), subtherapeutic use of tetracyclines accounts for 29% of all antibiotic use in cattle, swine, and poultry for all purposes. If data in Tables IV-4, IV-5, and IV-9 are

combined, it appears that subtherapeutic use of tetracyclines (2.4 million kilograms) accounts for 17% of total annual production of all antibiotics (approximately 14 million kilograms in 1983) and 73% of all tetracyclines produced for all purposes (3.3 million kilograms in 1983). Data on subtherapeutic use of tetracyclines for the last 3 years are not available, and it is not possible to calculate the percentages of total antibiotic production or of total farm and feedlot use that they account for today.

Antibiotic use in animals, in quantitative terms, is an important pressure for selection of antibiotic resistance in enteric bacteria on the farm and in feedlots. Subtherapeutic use in animal feed appears to be greater in quantative terms in this regard than antibiotic use for therapy, and tetracycline accounts for about half the amount of antibacterials used in this fashion. Although distinction between the growth-promotion and disease-prevention uses of subtherapeutic concentrations would be helpful, no accurate data is available. Furthermore, disease-prevention uses apparently are routine when there is suspicion of disease in some animals in a group or the durations of medication might be prolonged beyond specified regulatory time periods in severe cases or simply because of mistakes.

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V

ANTIMICROBIAL RESISTANCE IN HUMANS AND ANIMALS

FREQUENCY OF DRUG RESISTANCE IN CLINICAL ISOLATES OF SALMONELLA SPP. FROM HUMANS AND ANIMALS IN THE UNITED STATES

Thousands of clinical laboratories throughout the United States isolate occasional strains of salmonellae from humans, test their susceptibility to antimicrobial agents, and send the isolates--but not the susceptibility test results--to state reference laboratories for serotyping. Similarly, a parallel set of veterinary service and reference laboratories process animal isolates of salmonellae similarly. This whole elaborate salmonella reporting apparatus lists about 45,000 total isolates per year. These salmonella test results, however, are scattered through the files of thousands of laboratories, and even the reference laboratory files rarely have both susceptibility and serotype results. Thus, an expensive system obscures a major part of its only product, epidemiologic information.

To survey the prevalence of resistance in salmonellae, it is therefore necessary to repeat work that has already been done, to collect serotyped isolates from reference laboratories to retest their susceptibility and to file the results together. Table V-1 summarizes an example of such work carried out 8 years ago as background for a study of resistance plasmids in salmonellae. Susceptibility testing was performed on several thousand human isolates of salmonellae collected and serotyped by the Massachusetts State Laboratory and several thousand animal isolates collected and serotyped from all parts of the United States by the National Veterinary Laboratory, in Ames, Iowa.

In this chapter and throughout the report the committee has included data on the antibiotic resistance profile of bacterial isolates collected from various veterinary laboratories throughout the United States over several years. The history of antibiotic exposure was not available in most cases. The committee has made the assumption that the antibiotic resistance profile of the isolates is indicative of exposure of the animal host to the specific antibiotics listed in the profile. There is an inherent problem in this assumption that is clearly recognized; the antibiotic resistance profile of an isolate cannot be used with absolute certainty to determine direct exposure of a

TABLE V-1

RESISTANCE TO ANTIMICROBIAL AGENTS IN SALMONELLA ISOLATES (1979-1980)

HOST	SALMONELLA SEROTYPE	NO. (%) ISOLATES	NO. (%) ISOLATES RESISTANT TO: tetracycline	chloramphenicol	streptomycin	sulfonamide	ampicillin	any
	agona	141 (5)	19 (13)	0 (0)	26 (18)	6 (4)	4 (3)	34 (24)
	anatum	24 (1)	6 (25)	0 (0)	6 (25)	1 (4)	0 (0)	9 (37.5)
	blockley	55 (2)	0 (0)	0 (0)	5 (9)	0 (0)	3 (5)	7 (13)
HUMAN	enteritidis	884 (31)	15 (2)	0 (0)	12 (1)	2 (0)	12 (1)	28 (3)
(A)	heidelberg	121 (4)	20 (17)	2 (2)	53 (44)	26 (21)	28 (23)	68 (56)
	infantis	127 (4)	3 (2)	0 (0)	12 (9)	2 (2)	0 (0)	13 (10)
(MASS.	montevideo	61 (2)	6 (10)	0 (0)	2 (3)	2 (3)	0 (0)	8 (13)
STATE	newport	84 (3)	8 (10)	4 (5)	10 (12)	8 (10)	7 (8)	10 (12)
LABORATORY)	oranienburg	34 (1)	2 (6)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6)
	St. paul	75 (3)	17 (23)	3 (4)	15 (20)	8 (11)	14 (19)	22 (29)
	typhimurium	959 (34)	119 (12)	3 (0)	134 (14)	86 (9)	52 (5)	188 (20)
	" v.copen.	261 (9)	32 (12)	1 (0)	41 (16)	23 (9)	23 (9)	48 (18)
		2826	247 (8.7)	13 (0.5)	316 (11.2)	164 (5.8)	143 (5.1)	437 (15.5)
	agona	197 (11)	52 (26)	30 (15)	67 (34)	43 (22)	38 (19)	79 (40)
	anatum	167 (10)	81 (49)	2 (1)	102 (61)	79 (47)	9 (5)	130 (78)
ANIMAL	blockley	36 (2)	0 (0)	0 (0)	10 (28)	0 (0)	1 (3)	9 (25)
(B)	enteritidis	55 (3)	1 (2)	0 (0)	2 (4)	1 (2)	1 (2)	2 (4)
	heidelberg	240 (14)	110 (46)	0 (0)	178 (74)	110 (46)	40 (17)	183 (76)
(NATIONAL	infantis	68 (4)	15 (22)	3 (4)	24 (35)	9 (13)	5 (7)	29 (43)
VETERINARY	montevideo	56 (3)	5 (9)	0 (0)	19 (34)	15 (27)	12 (21)	21 (37.5)
LABORATORY)	newport	45 (3)	8 (18)	1 (2)	13 (29)	10 (22)	10 (22)	14 (31)
	oranienburg	42 (2)	0 (0)	0 (0)	7 (17)	2 (5)	2 (5)	7 (17)
	st. paul	131 (8)	84 (64)	2 (2)	49 (37)	86 (66)	7 (5)	108 (82)
	typhimurium	502 (29)	345 (69)	32 (6)	357 (71)	275 (55)	243 (48)	391 (78)
	" v.copen.	206 (12)	143 (69)	1 (0)	158 (77)	142 (69)	108 (52)	162 (79)
		1745	844 (48.4)	71 (4.1)	986 (56.5)	772 (44.2)	476 (27.3)	1135 (65)
OTHER SEROTYPES	choleraesuis	290 (41)	37 (13)	1 (0)	289 (100)	285 (98)	37 (13)	289 (100)
IN ANIMALS	derby	95 (13)	22 (23)	2 (2)	31 (33)	16 (17)	6 (6)	40 (42)
(C)	dublin	125 (18)	100 (80)	3 (2)	106 (85)	25 (20)	93 (74)	116 (93)
(NAT. VETERINARY	pullorum	95 (13)	12 (13)	0 (0)	56 (59)	8 (8)	11 (12)	60 (63)
LABORATORY)	san diego	106 (15)	63 (59)	0 (0)	69 (65)	42 (40)	15 (14)	79 (75)
		711	234 (32.9)	6 (0.8)	551 (77.5)	376 (52.9)	162 (22.8)	584 (79)
B+C TOTAL		2456	1078 (43.9)	77 (3.1)	1537 (62.6)	1148 (46.7)	638 (26.0)	1697 (69)

Source: Adapted from data compiled by the committee.

human or an animal to any specific antibiotic, nor to the dosage, nor to the route of administration (oral or parenteral). This could result in errors in judgement, e.g., the inability to differentiate bacterial resistance to an antibiotic administered either parenterally or via the feed (per os). (Note: Penicillin/ampicillin is used as terminology to include penicillin G to reflect the fact that, although this is the form of the β-lactam administered parenterally to livestock, susceptibility testing of salmonellae isolates from humans is performed with ampicillin as the β-lactam drug.)

The prevalence of several serotypes can be seen to differ greatly between the human and animal isolate collections (See Table V-1). Strains of S. enteritidis constitutes 31% of human but only 3% of animal isolates; S. choleraesuis, S. derby, S. dublin, S. pullorum, and S. san diego were found mostly in the animal isolate collection.

Overall, resistance to each of the antibacterial agents tested or to any of the agents was more frequent in the animal than in the human collections. Resistance was also disproportionately prevalent in isolates of several serotypes (e.g., S. typhimurium, S. heidelberg, S. anatum, S. agona, and S. st. paul) in both animal and human isolates. The frequency of resistance to the different antibacterial agents had the same rank order of resistance in both animal and human isolates as follows:

(streptomycin>tetracycline>sulfonamide>ampicillin> chloramphenicol).

Table V-2 arranges animal isolate data similar to that in Table V-1 by serotype and antibiotype. Each antibiotype that was found in 2% or more of the isolates of any serotype is shown in Table V-2. The table shows that much of the resistance in the isolates of a given serotype is clustered into a small number of antibiotypes, and that these prevalent antibiotypes vary greatly from serotype to serotype. When collections of isolates representing several of the prevalent serotype-antibiotype combinations were tested (e.g., the antibiotype TSKUHA for S. typhimurium var. copenhagen) it was found that resistance in many of the isolates was due to a common resistance plasmid which they shared, even when the isolates came from different states or from both animals and humans.[15]

The results shown in Table V-2 also illustrate a problem encountered in surveys of the prevalence of resistance in Salmonella spp. The resistance tends to be discontinuous, that is, clustered in groupings of multiple resistance in particular serotypes. Any survey that includes, for unsuspected epidemiologic reasons, a disproportionate number of isolates belonging to one of these clusters will not

TABLE V-2

PERCENTAGES[+] OF ANIMAL ISOLATES OF EACH COMMON SEROTYPE OF SALMONELLA THAT TESTED RESISTANT TO VARIOUS COMBINATIONS OF ANTIMICROBIAL AGENTS

ANTIBIO-TYPE*	SEROTYPE																									
TCSKUHAGX	Agona	Anatum	Blockley	Bredeney	Cerro	C-suis Kzf.	Derby	Dublin	Eimsbuettel	Enteritidis	Havana	Heidelberg	Infantis	Johannesburg	London	Montevideo	Newport	Oranienburg	Pullorum	Reading	St. Paul	San Diego	Senftenburg	Thompson	Typhimurium	Typh. Copenhag.	Worthington
none	52	22	70	61	74		60	11	14	96	72	19	52	86	57	62	64	78	34	40	17	22	42	72	23	18	42
T	4	5			5		5								4												21
C																											
S	8	15	26	12	13		10	4	9		14	22	25		9	4	9	14	49	9	6	5	13	17	10	9	12
K					3											3					3						
U		3									3				2				2	5	10	8					
H																											
A																											3
G																											
X																											
T S	4	5					6					5	9								5	35	9	3	3		3
SK		5										2			2												3
T U		4																			25				2		
S U		7				84					3								4	4	4		3	3			
S G																											3
U A														3													
TCS									3																		
T SK								3				2			2					7	3						6
T S U		12		16		3	3					7			2					5	8	7	4		4	4	
T S A								8							11					14					3		
SKU												5															
S U A					3									7													
T SKU		17		4			4					10				3					11	6	4		6	8	3
T SK A								39											3					3	3		
T S U A						3		4									3					5			3		
T UHA																	3										
T S HA								3							2												
SKU G									9																		
SK HA																							6				
S UHA																											3
T SKU A					3	2		9						3	6		13		4	4		3			16	14	
T SK HA								3																			
T S UHA								4														3			6		
SKUHA															2												
T SKUHA																									6	37	
SKU AG																22		3									
SKUHAG									6																		
T SKUHAG									60			9															
TCSKU AG											7																
T SKU G												4										2					
TCSKUHAG	20																										
TOTAL PATTERNS	28	26	11	15	12	24	20	26	14	12	12	40	19	13	17	17	18	13	15	22	26	21	28	13	48	28	16
NUMBER ISOLATES	282	232	43	69	38	473	111	158	35	76	29	337	112	29	47	74	67	59	125	57	167	134	78	29	717	287	33

*AGENTS TO WHICH RESISTANT

T = tetracycline
C = chloramphenicol
S = streptomycin
K = kanamycin
U = sulfonamides
H = cephalothin
A = ampicillin
G = gentamicin
X = sulfatrimethoprim

[+]Percentages less than 2 not shown

Source: Adapted from O'Brien et al.[15]

accurately represent the prevalence that might be found in a larger or more broadly-based sample.

Table V-3 summarizes in chronologic order the prevalence of resistance in collections of salmonella isolates from humans and from animals in the United States. Among the human isolates, the first two reports, from the late 1960s, show similar prevalence of resistant strains at 21-22%.[20,29] Four reports of studies done in the 1970s were based on reference laboratory collections--those of Cherubin et al.,[5] Saad and Farrar,[19] O'Brien et al.,[16] and MacDonald et al.[14] --the results in these surveys were in close agreement; percentage of resistant strains ranged from 15.5 to 17.4. The higher percentage reported during this period was the survey of Bissett et al.,[2] that showed 31% resistant strains.

The report of Lorian,[13] covering the decade from 1975 through 1984, was produced by a different method of surveillance than those mentioned above. This method was not based on collections of reference laboratories, but on analysis of computer-data files from several hundred hospital laboratories. Although these computer files did not give serotype identification, there was broad geographic representation in this very large data file. Lorian's analysis of these data did not attempt to determine the percentage of isolates resistance to at least one of the tested antibacterial agents. However, we can project that the percentage of isolates resistant to any of the agents would be around 30%, since the resistance to either tetracycline or to ampicillin alone was 22% and 18%, respectively, assuming a distribution of groupings of resistance similar to that seen in the other studies. Thus, we are left with the puzzle of why these hospital-based survey data showed approximately twice the percentage resistance as the reference-laboratory-based surveys of approximately the same time period.

One possible explanation is that the different reporting method for the data in the Lorian survey[13] might have accounted for some of the observed difference in prevalence of resistance. These data did not include the primary measurements of the extent of resistance (inhibition zone diameter or MIC), but only reported susceptibility rather than resistance, and this has been projected here to make the results comparable with those of the other studies. In Lorian's method the percentages in the intermediate range, which are not reported here, were reported as resistant, while in the other studies, they would have been reported as nonresistant. The intermediate percentages for the antibacterial agents reported in the other studies, are too small to account for the difference between them and the Lorian data, also there was no information on quality control

TABLE V-3

RESISTANCE TO ANTIMICROBIAL AGENTS AMONG ISOLATES OF SALMONELLAE FROM HUMANS AND ANIMALS IN THE UNITED STATES

HOST	PERIOD	REGION	NO. ISOLATES	PERCENT RESISTANT TO tetra-cycline	chloram-phenicol	strepto-mycin	sulfon-amide	ampi-cillin	any	INVESTIGATOR
HUMAN	1967	NATIONAL	400	12.5	0	14.2		8	22.2	SCHROEDER ET AL[20]
"	1968-1969	NORTHEAST	292	8.2	0	15	11.5	13.5	21.6	WINSHELL ET AL[29]
"	1970	NORTHEAST	315	11.7	0	14.9		9.5	17.4	CHERUBIN ET AL[5]
"	1971-1972	CALIFORNIA	2246	20.3	1.5	26	19.5	18.5	31	BISSETT ET AL[2]
"	1973-1974	GA AND SC	305	10	0.3	11	6	8	16	SAAD AND FARRAR[19]
"	1975-1984	NATIONAL	20708	22	4			18	?30	LORIAN[13]
"	1979-1980	MA	2826	8.7	0.4	11.2	5.8	5.1	15.5	O'BRIEN ET AL[16]
"	1979-1980	NATIONAL	511	8.6	0.8	12	8	8	16	MACDONALD ET AL[14]
"	1984-1985	NATIONAL	485	13	2	12.2	7	9	24	" " "
		TOTAL	28088							
ANIMAL	1973-1974	GA AND SC	152	10	0	16	4	4	21	SAAD AND FARRAR[19]
"	1979-1980	NATIONAL	2456	43.8	3.1	62.5	46.7	25.9	69.09	O'BRIEN ET AL[16]
"	1980-1981	NATIONAL	3500	45	12	66	57	31	80	BLACKBURN ET AL[3]
BOVINE	1981-1987	S. DAKOTA	207	80				62		PERSONAL COMMUNICATION
"	1985-1986	TEXAS	621	47				39		FROM VETERINARY
"	1981-1987	S. DAKOTA	596	81				42		LABORATORIES
PORCINE	1985-1986	TEXAS	51	37				23		(COLE, THAYER,
"	1986-1987	GEORGIA	223	69				72		LIBAL, WHITFORD)
POULTRY	1981-1987	S. DAKOTA	124	43				15		" " "
		TOTAL	7930							

Source: Adapted by the committee from data by Blackburn et al.[3], Bissett et al.[2], Cherubin et al.[5], Lorian[13], MacDonald et al.[14], O'Brien et al.[16], Saad and Farrar[19], Schroeder et al.[20], Winshell et al.[29], and personal communications (Cole, Libal, Thayer and Whitford, Veterinary Laboratories).

in the hundreds of laboratories that generated the data reported in Lorian's large survey.[13]

The survey results reported by MacDonald et al.[14] showed a rise from 16 to 24% from 1980 to 1985 for human isolates of salmonellae resistant to any of the tested antimicrobial agents. These percentages are important, because they indicate that the antibacterial resistance found in human isolates of salmonellae has increased. Accordingly, the latter value (~24%) would be the more nearly contemporary percentage to use in the risk assessment model.

The validity of the results of the 1985 survey by MacDonald et al.[14] is supported by the geographic representation incorporated in its design and by its comparability to the survey performed 5 years earlier. Also, the percentage of resistance (16%) found in the earlier (1980) survey reported by MacDonald agrees closely with the percentages mentioned in the three other surveys of that time period. Given the complexity of the problem, however, a sample of 485 isolates is not large. This illustrates the committee's earlier concern about the inadequacy of surveillance of resistance in isolates of salmonellae in the United States; when we consider that the 485 isolates on which this survey was based were from nearly 200,000 human isolates of salmonellae serotyped by reference laboratories in the United States since the earlier survey.

The report by Saad and Farrar[19] of the percentage of resistant salmonellae in animal isolates from Georgia and South Carolina during 1973 and 1974 shows a relatively low percentage of resistance, in contrast with the two later studies of animal isolates (Table V-3). This probably is due to regional differences and small-sized samples, and thus does not represent a true national secular trend. Studies by O'Brien et al.[16] covering the period 1979-1980 and by Blackburn et al.[3] covering 1980-1981--were both based on the same data source, namely, the large collection of animal isolates at the National Veterinary Laboratory in Ames, Iowa. Although the results of the Blackburn et al.[3] study showed somewhat higher percentage of resistance than the O'Brien study, more recent data would be needed to determine whether these results represents a trend. In addition to these three surveys, veterinary laboratories in several states have provided results of susceptibility tests done on salmonellae isolated in recent years. Yearly values for percentages of resistance showed no clear trends over time, so values for all years were averaged for each state and host species, as shown in the lowest six lines of Table V-3.

Table V-3 essentially amplifies the observations of Table V-1. The percentage of isolates resistant to any antibacterial agent for which data are available in any of the nine collections of animal-isolates, except for the small group reported by Saad et al., exceeds the percentage

reported in any of the human isolate collections. The percentages reported for resistance to tetracycline are greater than those resistant to ampicillin in eight of the nine collections of human isolates and in all nine collections of animal isolates. The average percentage of resistance of salmonellae isolates to ampicillin or to tetracycline in the animal collections is 3 to 4 times greater than in the human collections.

FREQUENCY OF DRUG RESISTANCE IN CLINICAL ISOLATES OF E. COLI FROM HUMANS AND ANIMALS IN THE UNITED STATES

The data in Table V-4 shows the percentages of isolates resistant to antimicrobial agents in collections of isolates of E. coli from humans and animals in the United States. The data in the first row, reported by O'Brien, is from all isolates tested during 1986 by the laboratory of a large general hospital in Boston (O'Brien, 1988, personal communication). It is the committee's belief that these percentages are similar to those commonly found in United States hospital laboratories, because they are comparable to the percentages in the second row reported by Atkinson and Lorian from isolates tested over a decade in several hundreds hospitals in the United States.[1] The percentages in the latter large study by Atkinson might be slightly inflated in comparison with the percentages in other collections, because they were reported as "percent nonsusceptible"--that is the total of resistant isolates plus isolates in the intermediate resistant category.

The data in the first two rows of Table V-4, reported by O'Brien, Atkinson, and Lorian, are representative of data that are commonly reported by hospital laboratories. Data from this source are considered biased, because most of the isolates are from patients who are hospitalized presumably due to an illness, thus constituting a small group having more isolates that are resistant than the human population at large. The E. coli isolates in the third row reported by Lester et al. from the general human population probable give a better representation of the resistance profile from the human population at large. These data are from a collection of 10 random colonies of E. coli from stool cultures from each of 38 healthy children who had not been treated with any antimicrobial agents for at least 4 months before culture. The percentages of resistance reported in these community-based isolates of E. coli are about one-third of those reported for hospital-laboratory isolates.

The lower part of Table V-4 shows percentages of resistant isolates in various collections of animal isolates of E. coli. The first three rows are from earlier studies by

TABLE V-4

RESISTANCE TO ANTIMICROBIALS AMONG ISOLATES OF E. COLI FROM HUMANS AND ANIMALS IN THE UNITED STATES

HOST	SOURCE	PERIOD	REGION	NO. OF ISOLATES	% RESISTANT TO: tetracycline	chloramphenicol	streptomycin	sulfonamide	ampicillin	INVESTIGATOR
HUMAN	HOSPITAL	1986	BOSTON	3757	26	8		27	29	O'BRIEN ET AL[16]
"	" "	1971-1982	U.S.	1.8 mil	28	5		29	28	ATKINSON AND LORIAN[1]
"	COMMUNITY	1985	BOSTON	388	12	1		9	10	LESTER ET AL[12]
ANIMAL	PORCINE	1974	ILLINOIS	530	90	1.7	93	83	53	SIEGAL ET AL[21]
"	BOVINE	1974	" "	106	49	0	50	29	13	" " "
"	" "	1974	MONTANA	431	0	2	1	1	1	" " "
"	ANIMAL	1980	U.S.	100	80	6	72	70	29	O'BRIEN ET AL[15]
"	BOVINE	1981-1987	SOUTH DAKOTA	366	76		69		38	PERSONAL COMMUNICATION
"	" "	1985-1986	TEXAS	405	71				49	FROM VETERINARY
"	" "	1986-1987	GEORGIA	265	57		55		35	LABORATORIES
"	PORCINE	1981-1987	SOUTH DAKOTA	1015	96		93		44	(COLE, THAYER,
"	" "	1985-1986	TEXAS	107	93				77	LIBAL, WHITFORD)
"	" "	1986-1987	GEORGIA	405	93				62	" " "
"	POULTRY	1981-1987	SOUTH DAKOTA	30	83		80		31	" " "

Source: Adapted by the committee from data by Atkinson and Lorian[1], Lester et al.[12], O'Brien et al.[15,16], Siegal et al.[21], and personal communications (Cole, Libal, Thayer and Whitford, Veterinary Laboratories).

Siegal et al. of E. coli in fecal samples from pigs and cattle on farms in Illinois and from range cattle in Montana.[21] The isolates from range cattle were remarkable, in that scarcely any of them were resistant to any of the antimicrobial agents tested.

Isolates from animal hosts reported in the fourth row of Table V-4 shows the resistance in 100 E. coli strains chosen randomly from among the large collection of animal isolates sent to the National Veterinary Reference Laboratory for serotyping. The data in the other seven rows show the percentage resistance in the collections of E. coli isolates from different animal host species tested by the same veterinary laboratories that provided the data on salmonellae isolates in Table V-3. In all collections of E. coli isolates resistance to tetracycline was more frequent than resistance to ampicillin, with the exception of those from range cattle.

In addition to the data in Table V-4, data from other studies provide information about the resistance to antibacterials in salmonellae and E. coli isolated from animals in the United States. Fagerberg and her associates[6] surveyed fecal samples from production cattle, broilers, and swine at various slaughter plants in the United States. Salmonellae were isolated from 5% of the broilers, 5% of the production swine, 9% of the beef units, and 60% of the swine at the slaughter plants. The resulting 199 salmonella isolates were of 27 serotypes; 82% of the strains were drug resistant. The survey results showed 1,563 strains of E. coli, of which 95% were drug-resistant: 72% resistant to tetracycline, 60% resistant to streptomycin, 84% resistant to sulfadiazine, and 13% resistant to ampicillin.

Gustafson et al.[7] found differing rates (10 to 84%) for the isolation of Salmonella from healthy market hogs taken at slaughter plants in Pennsylvania, Iowa and Georgia and different percentages (0-24%) of drug resistance among the isolates, although only 24 isolates out of 1491 from 658 swine were resistant to multiple antibacterials.

A study by Søgaard[22] involved pigs without clinical signs of illness which had not been fed any antibiotics in their feed but were given therapeutic dosages of antibiotics. A prevalence of 74% resistance was reported to one or more antibiotics. The incidence of resistance to sulfonamides, tetracyclines, and streptomycin was high. Most strains were susceptible to ampicillin and chloramphenicol. Pigs never given antibiotics either subtherapeutically or therapeutically showed a prevalence of resistance among E. coli of 53%. Thus, the therapeutic use of antibiotics in swine accounted for an absolute 21% increase (from 53% to 74%) of organisms resistance to various antibiotics. Langlois et al.[10] found tetracycline resistance in 76% of the fecal coliform organisms in swine fed no subtherapeutic

antibiotics but receiving only therapeutic antibiotics, 26% among isolates from swine never having received antibiotics and in 100% of the isolates from swine continuously exposed to subtherapeutic levels of antibiotics. Langlois et al.[11] found less of a decrease in antibiotic-resistant coliform organisms in pigs given therapeutic levels of antibiotics compared to pigs fed continuous subtherapeutic levels of antibiotics once the drugs were withdrawn. Therefore, Langlois et al.[11] has suggested therapeutic doses of antibiotics may have a more marked effect than subtherapeutic doses of antibiotics. They found bacterial isolates from pigs that received therapeutic doses of one of the tetracyclines for only 14 days (total of 22.0 μg/g) had more antibiotic resistance than those that received subtherapeutic doses (total of 27.5 μg/g) for 85 days.

Variability in the frequency of antibiotic resistance of salmonellae isolated from healthy poultry has been noted. Salmons[19a] has summarized several industry-sponsored studies. In the survey of chickens, "by far the predominance of Salmonella isolates were sensitive to the broad spectrum of antibiotics used in human or animal therapy. However, isolates from turkeys were 22-23% resistant to streptomycins, tetracycline, panamycin and neomycin."

Other reports have found similar variations between the prevalence of resistance among species, by years and by geographical locations.[2,3,13,14,16,19] They were unable to correlate level of antimicrobial drug use and percentage of resistance in most of these studies; percentage of resistance was reported for tetracycline as 50% to 85% and for ampicillin as 16% to 80% which agrees with the results submitted to the committee. Two labs also reported a decreased percentage of resistant organisms.[14,16]

In conclusion, there have been several reports and surveys of resistance of various enterobacteriaceae to various antibiotics in cattle, swine, and poultry within the United States. These reports generally agree that feeding subtherapeutic antibiotics to animals or therapeutically treating animals with various antibiotics causes an increased in the frequency of isolation of Salmonella spp. and E. coli that are resistant to those antibiotics. However, there appears to be a regional and temporal difference in the percentage of resistance and some variation of resistance expressed between animal species. These results probably reflect the difference of usage both subtherapeutically and therapeutically of the various antibiotics between poultry, cattle, and swine specimens submitted to these labs. Varying production methods, stress, and management practices could also explain some of the differences and reported decreases in resistance.

Also, these data indicate that the percentage of resistance to antimicrobial agents in isolates of salmonellae

from animals in the U.S. is 3 to 5 times greater than that in isolates from humans. Greater differences are seen in the data for isolates of E. coli in humans or animals, if we assume that hospitalized patients or range cattle represent a small portion of the total human or animal populations, respectively. Since farm animals outnumber humans in the U.S. (see Chapter IV), they harbor in their intestinal flora a reservoir of resistance genes that may be an order of magnitude larger than that of the flora in the total human population.

EFFECT OF BANNING THE USE OF SUBTHERAPEUTIC DOSES OF ANTIBIOTICS

To assess the impact of subtherapeutic use of antibiotics on the selection of antimicrobial-resistant bacteria isolated from animals that may also cause human disease, it is critical to review the experience in England and the other countries where growth promoting use of antibiotics has been prohibited. In 1969, Swann et al.[24] were appointed to the Joint Committee on the Use of Antibiotics in Animal Husbandry and Veterinary Medicine in England to obtain information about an increase in resistant strains; it produced a report that attempted to explain in simple and straightforward terms how the use of antibiotics in animals may affect both humans and animals.

Other concerns that influenced the Swann committee included the presence of trace amounts of antibiotics in meat or poultry products consumed by humans and their potential for causing allergic or toxic reactions and allowing the selection of resistant bacteria from among the nosocomial flora, and the possibility that S. typhi would develop resistance to chloramphenicol, the drug of choice at that time.

Recommendations adopted included the division of antibiotics for agricultural use into two classes: therapeutic antibiotics, for use in treating bacterial infections in animals and available only by prescription from a veterinarian; and "Feed" antibiotics used in subtherapeutic doses for growth promotion that is available to the farmer without a prescription through feed merchants or farm stores. It was recommended that the latter class be restricted to those drugs that have no use in human medicine. Thus, Zinc Bacitracin, Virginiamycin, Avopracin have been used and apparently do not select strains that would be resistant to the tetracyclines, penicillin, and other antibiotics. Penicillin and the tetracyclines have not been used for feed additives or growth promotion. Although those two antibiotics could be added to animal feed if the purpose was for treatment or prevention of a bacterial infection, neither

could be included at low concentrations for promoting growth. Such use of an antibiotic in feed is prescribed by a veterinarian for a particular disease episode, generally for no longer than 4 weeks.

The subtherapeutic dose (200 g/ton) of antimicrobials in the United States is considered a therapeutic dose in the United Kingdom. Therefore, it is difficult to compare the impact due to the use of this concentration of antibiotics in each of these two countries on the selection of resistance because the applications for this concentration have been different. This difference in application of dosage is critical to our understanding in the United States about the position of the British scientists who feel that 200 g/ton is a concentration that is important in selecting resistance in infectious bacterial strains.

Many changes have occurred in animal husbandry since 1969, and there was no systematic collection of data before that date, so the effect of the Swann Committee recommendations cannot be accurately assessed.[18,27,28] Interested investigators and government groups have gathered data on the number and types of some resistant organisms in animals and humans in the British Isles, but no comprehensive prospective study has been initiated to evaluate the effect of the recommendations. Antibiotic use apparently had increased in both humans and animals since the Swann report (see Table 1 in Braude[4]). Also, human use of antibiotics in the United Kingdom increased rapidly; it was 17 times the veterinary use in 1980, but before the Swann report, the human use was only 1.4 times the veterinary use.[30]

Walton and other researchers have become convinced that the therapeutic use of antibiotics in humans, as well as in animals, causes the selection of resistance in the bacterial strains in humans.[18,25,27,28] They contend that concentrations of antibiotics achieved in animals receiving subtherapeutic concentrations of antibiotics (presumably less than 200 g/ton of penicillin or the tetracyclines) did not reach the critical points necessary for the selection of resistant strains.[28] Although there is concern that some bacterial strains found in animals have multiple antibiotic resistance that could be a hazard to human health, the situation has not worsened despite increasing antibiotic use in animals. Furthermore, it is the general feeling of some scientists in the United Kingdom that the Swann Committee recommendations have had no impact in reducing this hazard.

The available data are sufficient for an assessment of the changes in resistance patterns as well as assessment of the numbers of isolations of various species of salmonellae in England since 1970. Sojka et al.[23] have periodically reported similar results over the period from 1972-1986. Those surveys clearly indicate that the recommendations in the Swann report did not stop the development of antibiotic

resistance, especially resistant to penicillins and tetracyclines. Chloramphenicol resistance has steadily increased in some isolates, despite the prohibition of the use of this antibiotic in feed. The authors of those surveys conclude that the therapeutic use of antibiotics in animals, combined with poor animal handling and management practices, especially regarding calves, does continue to promote the development of resistant strains.[18,25,27,28]

Resistance of salmonellae to penicillins and tetracyclines in animals varies with the animal; those of bovine origin are less likely to be sensitive to either kind of drug than those from poultry. In all isolates of resistant S. typhimurium, predominately phage type 204C, and related types 49 and 204--accounted for most of the resistant strains. Those phage types appeared in calves in 1979, spread widely in the next 2 years, and they remain the predominant types in cattle (59% in 1985). Phage type 204C has also caused enteritis in humans as observed in 4% of the patients in 1985. The disease has usually consisted of mild to moderate diarrhea, but several of the 677 patients with S. typhimurium infection in 1977-1984 had to be hospitalized for severe diarrhea. The cases in two outbreaks might have been due to consumption of raw milk, while most of the other cases were thought to be farm workers, but that has not been confirmed. Most people with infections had no farming connections. The bacterial strains isolated from these cases were resistant to ampicillin, chloramphenicol, gentamicin, tetracycline, and trimethoprim. Strain of 204C phage type accounts for 77% of all the Salmonella strains isolated from calves in 1985.[9] Apparently, infected calves stop shedding S. typhimurium before they reach slaughter weight and, therefore do not serve as a source of infection to humans because they do not enter the food chain in great numbers. The spread of phage type 204C probably occurred because of the practice of selling colostrum-deprived calves from broker to broker several times during the first 56 days of life. Calves apparently are susceptible to Salmonella infection during this early period and poor management practices contribute to the problem during frequent trips to market, whereby they acquire salmonellae from other animals. It is speculated that the resistant salmonellae became resistant because of futile attempts to treat calf scours with numerous antibiotics.[25] These salmonellae also appear to have a predilection for acquiring plasmids. Each year since isolation the resistance pattern of phage type 204C has broadened; gentamicin is the most recent antibiotic to which the strain has developed resistance.[23] The plasmid that codes for resistance to gentamicin also confers resistance to netilmicin, tobramycin, and apramycin. The last named is an aminoglycoside that is used to treat salmonella infections in calves, its use is probably the reason that resistance to

apramycin and gentamicin appeared. Phage type 204C has also appeared in the Netherlands[26] and Denmark,[8] and was imported into the European countries via veal calves.

The appearance among bacterial isolates of S. typhimurium phage type 204C with multiple antibiotic resistance has been an isolated event in England. Other Salmonella species have not shown the same rapid increase in acquiring resistance. There have been dramatic shifts in the number of isolations of various other resistant Salmonella species. For example, S. agona appeared in the early 1970s in England and the United States, having been imported from Peru with contaminated fish meal (to be used as poultry feed).[9] By 1975, there were 1,821 human isolates, the peak number. The number fell to around 450 for the years 1979-1983; reasons for the decline are unknown. S. hadar appeared in 1971 and peaked in 1979 at 2,480 isolates. This strain was isolated from turkey breeding stock, and the meat from contaminated, undercooked large birds caused outbreaks.[9] In 1984, only 496 isolations were reported; again, the reasons for the decline are unknown. Those two examples demonstrate that the presence of antibiotics in animals did not cause the strains to proliferate or to develop resistance to antibiotics, such as occurred with S. typhimurium phage type 204C and related types.

Walton noted several lessons that were learned from the United Kingdom's experience with the Swann Committee recommendations.[27,28] Antibiotics, such as the tetracyclines and penicillin used in the production of meat products, did not become ineffective, despite the development of resistance by bacteria. Those two classes of drugs continue to be used and to be effective prophylactically and therapeutically. Food animals have short life spans--broiler chickens, 35-56 days; pigs, 3.5 to 5 months; and cattle 2.5 years--and the rapid turnover results in the destruction of large numbers of bacteria.[27,28] When large batches of animals leave their quarters, cleaning is carried out with high-pressure water, which not only removes most gross amounts of offal but also dilutes and kills bacteria. Better control of antibiotics has been instituted because of the requirement that veterinarians write prescriptions for the use of antibiotics in feed.

One item that has not been clear from the discussions presented above is the human health hazard associated with the increasing number of salmonellae isolated in the United Kingdom. Figure V-1 shows the incidence of salmonellosis in England and Wales in 1941-1984. This shows a significant increase in the numbers of incidents during these years. Interpretation of the trends indicates an epidemic of S. typhimurium infection in the United Kingdom. Total figures for mortality caused by salmonellae is not available to the committee. However, some figures have been obtained;

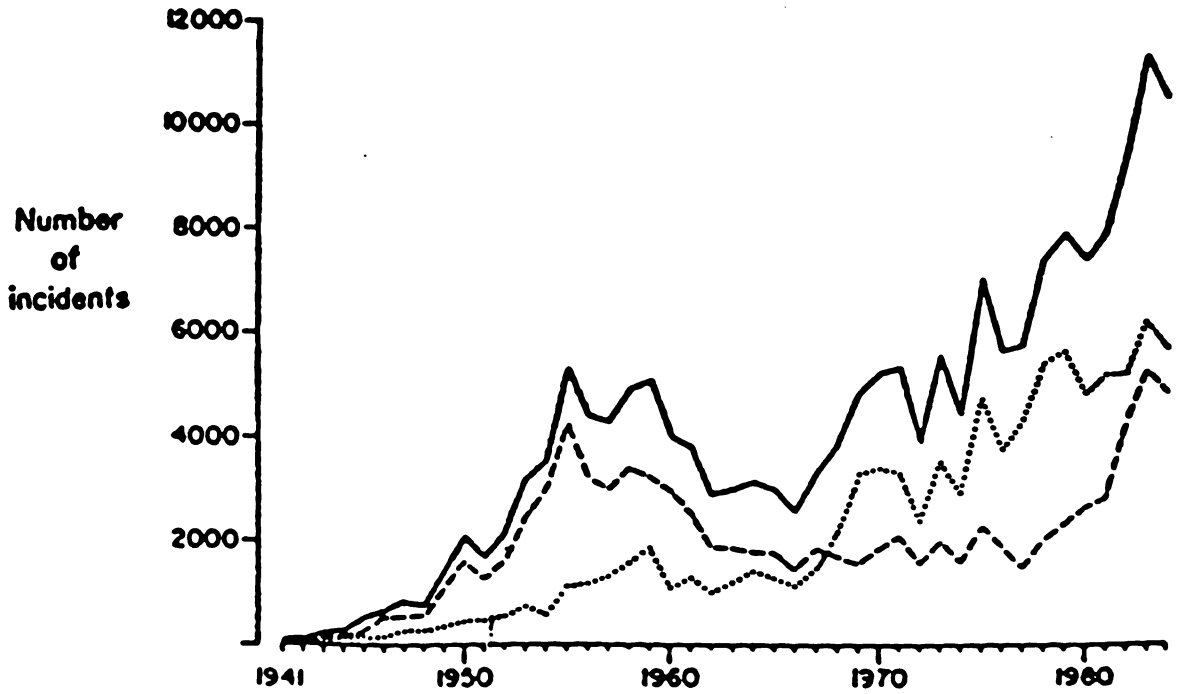

Figure V-1. Salmonellosis, England and Wales, 1941-1984. Reprinted from Palmer and Rowe.[17]

——— Total Salmonellae
- - - Salmonella typhimurium
····· Other salmonella serotypes

a major hospital outbreak in 1984 involved about 350 patients and 50 staff members; 19 patients died. S. typhimurium was the causative organism. Other less striking examples of the deaths during outbreaks listed were as follows: 2 of 654 patients infected by S. typhumurium from raw milk in 1981; none of 500 patients infected by S. montevideo from chicken in 1981; none of 245 patients infected by S. napoli from chocolate in 1982; 2 of 766 patients infected by S. enteritidis from a spicy glaze in 1984; 4 of 274 patients infected by S. virchow from cooked meats in 1985; and 1 of 60 patients infected by S. ealing from infant dried milk in 1985. Those are selected outbreaks and do not represent a thorough survey. The resistance of these bacteria to various antimicrobial drugs was not reported. No great increase in mortality occurred inasmuch as the authors who reported on the incidence of the disease and the apparent increase in numbers of bacterial isolates did not indicate any increase in mortality.[18,25,27,28]

Walton[27,28] also suggested in 1985 that the 15 years of antibiotic controls in the United Kingdom as recommended by the Swann Committee and similar controls in Europe had provided guidance for other countries that wanted to develop antibiotic control policies. Other authors such as Rowe and Threlfall[18] appeared to concur, with the following suggestion: Total control of antibiotic use is neither possible nor even necessary. Rather a redefinition of the current policy is needed, plus updated practical measures to assess the most effective use of the drugs.

In summary, the United Kingdom's experience with restricting the use of antibiotics in feeds has shown that resistance in bacteria probably develop in spite of the controls on "feed" (subtherapeutic concentrations) antibiotics not used in humans. Thus, prohibition of subtherapeutic doses of antibiotics in animals has not prevented or even affected the prevalence of resistant bacteria in the United Kingdom.

In conclusion, it is impossible to ascertain the effectiveness of the Swann Committee recommendations, because agricultural practices have changed substantially and because the therapeutic use of antibiotics--is a more important stress in the selection of resistant organisms than subtherapeutic. Resistant strains of salmonellae and other bacteria have persisted; some have increased in incidence, and others have decreased. The reasons for the changes are unknown, but do not appear to be related solely to the presence of antibiotics in the gastrointestinal tract. Human health hazards persist, perhaps they have increased. Human cases of salmonellosis have increased, but whether mortality from this disease has changed cannot be ascertained.

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VI

EVIDENCE OF TRANSMISSION OF PATHOGENS OF FARM ORIGIN TO HUMANS

Evidence of transmission of bacteria from farm-animal-origin to humans has been found in two genera of bacteria: Escherichia and Salmonella.

ESCHERICHIA COLI

Escherichia. coli and other enteric bacteria resistant to multiple drugs have been found to spread from farm animals into farm workers, their families, and the nearby community has been investigated. Such studies have, in general, indicated that multiple-drug-resistant E. coli organisms do indeed colonize farm workers, and to a lesser extent their families, and at times even spreads to nearby non-farm populations. No evidence has suggested, however, that multiple-drug-resistant E. coli of farm origin is associated with a higher risk of serious infection than E. coli of non-farm origin.

Perhaps the first systemic study of the change of coliform organisms from susceptible to multiple-drug-resistant in farm animals and in eleven members of the family on this farm. This was a prospective study carried out by Levy and Associates.[11] The systemic fecal sampling showed an increase in resistant E. coli within a week after start of the feeding of tetracycline-supplemented feed to a flock of chickens. The numbers of tetracycline-resistant intestinal coliforms also increased in the eleven members of this farm family, but not in their neighbors. Within 3-5 months after medicating the chickens, 31% of the fecal samples taken each week from each member of the farm family yielded bacterial populations of which 80% of the coliform bacteria colonies were tetracycline-resistant, compared with 6.8% of the samples from neighbors. About 6 months after the tetracyclines had been removed from the animal feed, the percentage of resistance organisms in farm dwellers' fecal samples that yielded coliform organisms over 80% of which were tetracycline-resistant had decreased to approximately the magnitude found before use of the tetracyclines was started. The rapidity with which commercially processed poultry is marketed precludes a study over a long time period of the change in the percentage of tetracycline-resistant

coliform bacteria isolated from chickens after discontinuation of tetracycline-supplemented feed.

The potential for spread of antimicrobial-resistant E. coli between farm animals and from farm animals to farm workers and the environment was further demonstrated in very recent experiments by Levy and Marshall.[11a] In this study a calf was fed a marked strain (containing both chromosomally mediated nalidixic acid resistance and a large plasmid encoding multiple antimicrobial resistance including that of tetracycline) capable of colonizing the human as well as bovine intestinal tracts. In the absence of any antimicrobial administration the marked strain was detected in the feces of the calf, and of another bovine kept in an adjacent stall, for at least 3 months. The same market strain was also present in the excreta of mice kept caged in the stall with the calf and in flies trapped in the farm. In addition, two farm workers caring for the bovines began excreting the marked strain in the stools 4-7 days after the experimental strain had been fed to the calf. Colonization of the intestinal tract of these two farm workers, who were not receiving antimicrobials continue for 30-45 days.

That antibiotic-resistant coliform organisms of farm origin sometimes can cause disease in humans was suggested by Hummel and colleagues.[10] They studied a pig-farming in a defined territory in which the streptothricin antibiotic nourseothricin was added to pig feed to promote growth. After 2 years of nourseothricin use in pig feed, they reported that coliform organisms containing plasmids encoded for nourseothricin resistance were found in 33% of the isolates from fecal cultures from pigs with diarrheal disease, in 18% in those from employees of the pig farms, 17% among isolates from families of employees, and 16% in those of outpatients living in nearby communities. Although no nourseothricin had been used in the human population in the territory, 1% of the isolates from urinary tract infections of outpatients were nourseothricin-resistant E. coli. Examination of cultures from pigs, farm employees, and outpatients in neighboring territories that did not use nourseothricin in pig feed revealed no nourseothricin-resistant E. coli.

Much of the important information needed to evaluate the results of the study of Hummel et al. is lacking. The dates of the study were not specified, nor were demographic data on the territories studied well defined. There was no information on the density of the pig population or the human population, and it has been difficult to assess the degree of contact with pigs on different farms in the various populations studied. Similarly, the timing of cultures and their study thereof for nourseothricin resistance in the various populations was not specified. However, the available data do suggest that nourseothricin-resistant

E. coli was transmitted from pigs to humans and from humans to other humans. Once the human gastrointestinal tract had been found to be colonized, it was not surprising that the organisms were occasionally found in urinary tract infections; however, it was not determined whether the organisms were more or less virulent than E. coli not resistant to nourseothricin.

Parsonnet and Kass[14] compared the antibiotic-resistance patterns of E. coli isolated from the urine of bacteriuric female slaughterhouse workers with those of E. coli from poultry in the processing line. E. coli was found in 95% of the cultures from poultry; 96% of them were resistant to antibiotics, and 87% were resistant to more than one antibiotic. The microorganisms isolated from the bacteriuric women's urine, however, only infrequently showed similar resistance patterns or identical patterns with those of the microorganisms from the poultry to which they were heavily exposed. Unfortunately, the bacteria in the women's feces were not studied, so direct spread from the processing line to their gastrointestinal tracts could not be determined.

Such a direct analysis of antimicrobial resistance patterns in fecal E. coli strains (rather than strains causing urinary tract infections) would seem necessary to document spread from animal foodstuff to humans, since E. coli strains causing human urinary tract infections represent only a small nonrandom group of clones, not found with equal probability among those which colonize the intestinal tracts of humans and animals. E. coli strains causing urinary tract infections in individuals without underlying microbiologic abnormalities belong to a limited number of O.K. serogroups and possess specific virulence factors.[10a,24a]

The antibiotic-resistance plasmids of the poultry and slaughterhouse workers from the latter study were examined for matching restriction endonuclease digestion-fragment patterns (T. F. O'Brien, 1988, personal communication). If a plasmid had been endemic among the poultry isolates, as found earlier among cattle isolates of Salmonella typhimurium var. copenhagen, its presence or absence in the human isolates would support or argue against the spread of drug resistance from the poultry to the workers.[10,11] In fact, the same plasmid could be found in two isolates in only a few instances, so the result had little power to exclude the possibility of spread. Extensive spread, however, might have been expected to yield human isolates with higher rates of resistance or more antibiotypes closely matching those of the poultry isolates.

The human health hazards attributable to infection with multiple-drug-resistant E. coli of animal origin were studied more directly by Habte-Gabr and colleagues in Iowa.[8] In 1972-1973, they studied 148 Iowa families: 51 families exposed to livestock given antibiotic-supplemented feed, 43

rural families with no exposure to livestock, and 54 urban families. Multiple-drug-resistant E. coli was found in 15% of the stool cultures from members of animal-exposed families, in 6% of those from members of rural families not exposed, and in 7% of those from members of urban families. A follow-up health survey was conducted 12 years later with 126 of the original 148 families. The incidence of serious infections was 6% in members of rural families exposed to livestock, 13% in members of rural families not exposed to livestock, and 12% in members of urban families. Thus, colonization by multiple-drug-resistant E. coli of farm origin did not appear to be a factor in infection in members of those populations. More extensive bacteriologic studies were not carried out and the populations studied were not large, so the study could "detect" only a high level of transmission of multiple-drug-resistant E. coli of farm origin that caused serious infections. If such spread of infection occurs at all, it is likely to be infrequent.

SALMONELLAE

Most evidence linking human disease to multi-resistant bacteria of farm origin has been found in salmonellae. Data detailing the incidence and associated morbidity and mortality of salmonella infections in farmers, slaughterhouse workers, and their families are not available. Comparison of case reports on farmers who used subtherapeutic antibiotics as livestock feed additives with those on farmers who did not might be particularly informative. The only information available is in the form of case reports or descriptions of small numbers of outbreaks in farmers and their families, but not in slaughterhouse workers.

The paucity of reports might suggest that the occurrence of salmonella infection in the rural or urban population is rare, indeed. In a 10-month study of 279 second-grade farm children in a rural county of Virginia, 149 episodes of diarrheal illness occurred in 97 children; salmonellae were isolated from only one of over 400 stool samples in the 149 cases of diarrhea.[5] The children were in two groups: 92 lived on commercial poultry farms, and 187 did not. The occurrence of diarrheal episodes was almost identical in the two groups. Despite the high prevalence (27%) of salmonella infection among the poultry flocks, only one culture-proven case of salmonella gastroenteritis (antibiotic susceptibilities not known) occurred, and it was in a child who did not live on a poultry farm.

Williams[25] described two veterinarians with pustular forearm lesions due to salmonellae (S. dublin and S. typhimurium) that occurred several days after they delivered an infected stillborn calf or cleaned a cow that had recently

aborted. But they did not describe the antimicrobial susceptibilities of the isolates.

Through 1980, five outbreaks of human salmonellosis directly linked to contact with farm animals have been reported. In the mid-1960s a multiple-antibiotic-resistant strain of S. typhimurium (phage type 29) caused a large outbreak of bovine infection in Great Britain.[1] Infection occurred in farmers, their families, and veterinarians who treated infected calves; spread from animals to humans was implicated. Spread of infection to dairy cows led to 59 human cases of milk-borne salmonella gastroenteritis. Prophylactic use, in healthy animals, of antibiotics to which the epidemic Salmonella strain was resistant might have favored infection with the pathogen by reducing the numbers of competing nonpathogenic antibiotic-susceptible intestinal bacteria.

Salmonella gastroenteritis occurred in a 12-year-old Canadian farm boy who cared for an infected dairy cow and its new calf.[7] The strains of S. typhimurium isolated from the cow and the boy were of the same phage type and antibiotic-resistance pattern (resistant to tetracycline and chloramphenicol). Spread of infection from the cattle to the child was considered most likely. Administration of antibiotics to the sick cow by the farmer 5-6 days before his son became ill might have led to selection of salmonellae with the aforementioned resistance pattern. However, possible use of subtherapeutic doses of antibiotics in feed was not mentioned.

An outbreak of salmonellosis involving several newly arrived calves on a Connecticut farm occurred in 1976.[12] S. heidelberg that was resistant to chloramphenicol, sulfamethoxazole, and tetracycline was responsible. The farmer and his pregnant daughter cared for the sick animals and became infected themselves. The daughter gave birth to a son 9 days after the calves arrived on the farm; 3 days after delivery, her newborn infant developed salmonella gastroenteritis and bacteremia. Infection spread in the nursery to two other babies, most likely by contact with nursery staff. The strain of S. heidelberg isolated from three calves and the farmer had identical antimicrobial susceptibilities, and those isolated from the farmer's daughter and the three infants were very similar (resistant to chloramphenicol, sulfamethoxazole, and tetracycline), but lacked resistance to neomycin, streptomycin, and kanamycin. Information on subtherapeutic or therapeutic use of antibiotics in the calves is not available.

In the late 1970s, numerous outbreaks of salmonellosis due to multiple-antibiotic-resistant S. typhimurium of phage types 204 and 193 occurred among calves on over 300 farms throughout Great Britain.[17,24] The two strains of S. typhimurium made up 28% of all S. typhimurium isolates from

cattle that were sent to the Central Public Health Laboratory for evaluation in 1978. The same two strains were later isolated from 211 human infections, including one that ended fatally, in the British Isles. In most human cases, no apparent connection with cattle could be found, but the same strains were later isolated from minced meat and sausage, suggesting entrance at some point into the human food supply. However, 30 of the human infections occurred in persons on farms where outbreaks of bovine infection with the multiple-drug-resistant strains of S. typhimurium were occurring or had previously occurred.[17]

Before 1977, the predominant-antibiotic resistance pattern in S. typhimurium of phage type 204 responsible for several outbreaks of salmonellosis in cattle and humans in Great Britain consisted of resistance to sulfonamide (nontransferable) and tetracycline (not directly transferable, but mobilizable by F-like plasmids). In 1977, a strain of phage type 204 that had gained an additional transmissible R plasmid (H2 compatibility group) bearing resistance to chloramphenicol (C), streptomycin (Sm), sulfonamide (Su), and tetracycline (T) was responsible for a small outbreak of salmonellosis on a farm in Leicestershire. The farm was involved extensively in calf-trading, which resulted in wide distribution of calves infected with the multiple-drug-resistant strain. It was thought that acquisition of the new H2 plasmid probably resulted from selective pressure introduced by the use of chloramphenicol in treatment of a calf infected with a type 204 strain that had the original R plasmid (SuT), which was predominant before 1977. Alternatively, the multiple-drug-resistant (C Sm Su T) plasmid might have been brought in with a newly purchased, already infected animal that could have been introduced into the herd shortly before the outbreak.[24]

An outbreak of multiple-drug-resistant salmonella infections involving three of four members of a family who worked on a dairy farm in Kentucky occurred in 1977.[4] Infection appeared to have been transmitted through ingestion of unpasteurized milk.

These data are not sufficient to support any conclusions concerning the relative incidence of infections with salmonellae (either antibiotic-susceptible or -resistant) in farm workers or their families, compared with other population groups. Data are not available to this committee on the frequency or severity of infections with salmonellae (either antibiotic-susceptible or -resistant) in slaughterhouse workers. In the five salmonella outbreaks described above, there are no data on the role of subtherapeutic use of antibiotics in feed, although the use of therapeutic dosages of antibiotics in the first four was considered to have important effects.

Since 1980, several additional outbreaks of multiple-

drug-resistant salmonellosis provide some evidence, and in one case compelling evidence, that the resistant salmonellae originated in farm animals fed antimicrobial drugs. Holmberg et al.[9] reported on an outbreak of S. newport resistant to ampicillin, carbenicillin, and tetracycline that occurred in several midwestern states. Food histories and plasmid profiles of the organisms isolated from both humans and animals led the authors to conclude that the resistant organisms infecting the patients were of animal origin and that the probable source was contaminated hamburger, the meat of which was derived from a single herd. The subtherapeutic use of chlortetracycline in this herd was admitted by the farmer, but this has not been analyzed or proven. Although the editorial that accompanied the report of Holmberg et al.[9] suggested that the study provided the "important missing link" between human disease and resistance in the infecting bacteria due to the feeding of subtherapeutic antibiotics to animals, the evidence is incomplete. (Note: Dr. Holmberg's comments, in personal communication, about this article are inserted parenthetically below.)

First, as pointed out by DuPont and Steele,[5] the pathogenic bacterial strain was not recovered from the slaughterhouse or from the hamburger (all the hamburger meat had already been consumed and none of the slaughterhouse animals were available for study), and no cases of S. newport disease occurred in the cattle or in the people associated with the farm that reared the animals or the processing plants (living cows remaining on the farm were excreting S. newport). Second, another processing plant in another state received half the carcasses from this herd of cattle and had no apparent problem (actually there were only 12 animals out of the 105 animal herd sent to another state; that cases traceable to these were not uncovered may only mean that some or all of these 12 animals were not infected or that ill persons were not ascertained or reported). Third, the only S. newport isolated from an animal and of a strain identical with the outbreak strain was isolated from a calf that died in an adjacent dairy herd. That calf might have been the source of the infection (this calf was not the only animal from which S. newport was isolated, as stated above, some of the cows on the farm were excreting the bacterium).

More recently, an outbreak of multiple-drug-resistant S. newport in California in 1985 convincingly demonstrated the entire chain of transmission.[22] The outbreak strain was resistant to chloramphenicol, tetracycline, kanamycin, ampicillin, and sulfisoxazole and was characterized by a single large plasmid. Epidemiologic studies identified ground beef as the suspect food vehicle, and many of the patients had consumed the ground beef at fast-food restaurants. Microbiologic and epidemiologic studies traced

the epidemic strain through the hamburger, back to meat processing plants, and ultimately back to the farms from which the animals were sent for slaughter. The isolates were from ill calves and cows at a number of dairies in important dairy-farming areas. Isolation of chloramphenicol-resistant salmonellae was associated with chloramphenicol use at those dairies. Such use of chloramphenicol as a feed additive is not approved by the Food and Drug Administration.

Several recent milk-borne outbreaks of multiple-drug-resistant salmonellae provide additional information, but do not directly link the organism to a farm source, or to subtherapeutic use of antibiotics. Tackett et al.[23] reported an outbreak of multiple-drug-resistant S. typhimurium that occurred in Arizona caused by the ingestion of raw milk. This bacterial strain was isolated from the raw milk samples. Further investigations into the source of contamination was not done because the implicated dairy withdrew the product from the market and would not permit any examination of the facility, its employees, or its animals.

The largest outbreak of salmonellosis ever recorded in the United States occurred in Illinois and Wisconsin in 1985 and involved over 16,000 bacterial-culture-confirmed cases.[18] In these studies, the estimates of cases derived from telephone surveys estimated the actual number of people infected was about 175,000. The epidemic strain was resistant to ampicillin, tetracycline, carbenicillin, streptomycin, sulfisoxazole, erythromycin, and penicillin. The outbreak ultimately was traced to two brands of pasteurized milk produced by a single dairy plant. The source of the infecting bacterial organism was presumed to be the dairy cattle, although this could not be demonstrated conclusively because no isolates either from the dairy-animals or the farm had exactly the same plasmid profile as the strain isolated from the milk.

One might speculate on whether those outbreaks due to multiple-drug-resistant salmonellae might still have occurred had the salmonellae been fully susceptible. The cause of each of the outbreaks appeared to be defects in food processing or inappropriate food preparation, rather than being due to the fact that the salmonellae were multiple-drug-resistant. Such defects would allow the persistence of any salmonellae, whether antibiotic susceptible or resistant. Hence, it would be difficult to argue that the outbreaks would not have occurred at all had the salmonellae been fully susceptible.

A recently reported outbreak of egg-associated fully-drug-susceptible S. enteritidis infections underscores the ease with which salmonellae can enter the food chain, in spite of the usual food processing and food preparation safeguards. Epidemiologic data suggested that, rather than the usual mechanism of contamination of the shells,

especially cracked shells, by salmonella-containing chicken feces, the mode of transmission was transovarial, with infection of the yolk before shell deposition. That mechanism would thwart the usual method to decontaminate eggs; and any use of such eggs that involved little or no cooking--e.g., use with hollandaise sauce, eggnog, or Caesar salad dressing--would likely result in cases of human salmonellosis.

The evidence of farm-to-human spread of salmonellae derived from the study of outbreaks should be put into the broader context of the overall epidemiology of human salmonellosis. In a study of farm children regularly exposed to poultry, Marx[13] could find no evidence of a greater occurrence of salmonellosis and diarrheal illness than in a control group. In a multivariate analysis of clinical and epidemiologic features of multi-drug-resistant salmonellae causing salmonellosis in humans, Riley and colleagues[16] found no evidence that exposure to animals or pets was a significant risk factor. Risk factors identified in their investigation included the recent use of antimicrobial agents by patients, a Hispanic ethnic background, regular antacid use, and age over 60 years. Thus, although transmission of salmonellae from farm animals to humans has been documented in several instances, it is not frequently recognized. It would appear that the best protection against multiple-drug-resistant salmonellae is the same as that against fully susceptible salmonellae; that is, accepted sanitation and sterilization (cooking) techniques of food processing and food preparation.

OTHER ENTERIC PATHOGENS

The available information on three enteric pathogens--enterohemorrhagic *E. coli*, *Yersinia enterocolitica*, and *Campylobacter* spp.--is sparse, but they are responsible for important clinical infections and should be mentioned. The committee did not search the data files of clinical or diagnostic laboratories in the United States for information on those organisms, but has relied on published summaries. The committee acknowledges that the hazard associated with transmission to humans of these bacteria that might have originated on the farm cannot be evaluated.

Enterohemorrhagic *E. coli* was recognized in 1982 as a major etiologic agent of the syndrome of hemorrhagic colitis, a diarrheal syndrome characterized by rapid progression from watery to bloody diarrhea and marked by severe morbidity.[3,15,19] Signs of enteroinvasive infection, such as fever or the presence of fecal polymorphonuclear leukocytes, are usually lacking or are not prominent. A particular serotype of *E. coli*, O 157:H7, is especially associated with

the syndrome and has been found to produce cytotoxins similar to the shiga toxin of Shigella dysenteriae type 1.

Outbreaks of hemorrhagic colitis due to enterohemorrhagic E. coli have occurred in persons of all ages, but have been prominent in elderly residents of nursing homes. Deaths were frequent in nursing home outbreaks. Several outbreaks have been traced to the consumption of beef and dairy products, and the organisms have been isolated from cattle. Thus, cattle are suspected of being a major reservoir. There is no comprehensive information on the epidemiology of the syndrome, and national surveillance data do not yet exist.

Antibiotic resistance of enterohemorrhagic E. coli has not been an issue. Indeed, the role of antibiotics is paradoxical; their use appears to be a risk factor for development of hemorrhagic colitis if they are given during exposure; but they appear to have little therapeutic value in the disease, in that the pathogenesis is toxin-mediated, rather than enteroinvasive infection.[15]

Infection caused by Yersinia enterocolitica, although moderately common in some European countries, is rarely recognized in the United States. There might be serious underdiagnosis of infection caused by this species, but far more extensive data would be needed to establish yersiniosis as an important clinical problem in this country. Most isolates are susceptible to tetracycline, although ampicillin resistance is common. The committee knows of no nationwide database that permits estimation of the incidence of infection with Y. enterocolitica.

Results of recent surveys in several areas of the United States suggest that Campylobacter spp. might cause at least as much illness and death as salmonellae. However, nationwide data on infections caused by campylobacters are not available yet, and antimicrobial resistance is not a recognized issue in the treatment of infections with them.[2,6,20,21]

In summary, studies have indicated spread into farm workers and their families of E. coli originating in farm animals and poultry. If a drug-resistant enteric flora is selected in farm animals or poultry by the use of antibiotic supplemented feeds, the drug-resistant enteric flora might spread into the farm workers, their families, and ultimately, to some extent into the community at large. There is no evidence, however, to suggest that drug-resistant E. coli of farm origin are more infective or more virulent than drug-susceptible E. coli of non-farm origin. Farm workers and their families have not been found in limited studies to have an increase in serious infections with diarrheal diseases, as compared to the population at large.

There is evidence, derived from the study of food-borne outbreaks of salmonellosis, that the causative salmonellae

were of farm origin, and entered the human food chain. In a number of outbreaks of multiple drug-resistant salmonellosis, an animal or poultry source was implicated, and the multiple drug-resistance was believed to be due to the use of antibiotics in animal feeds. In only one such outbreak was the evidence compelling, with full documentation of the entire chain of transmission from infected cattle to infected humans.

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VII

THE RISK MODEL: OVERVIEW OF THE PROBLEM AND NEED FOR A MODEL

The committee took as its principal charge the quantitative assessment of hazards to human health from the subtherapeutic administration of penicillin/ampicillin and the tetracyclines to farm animals. The committee deliberately chose to consider the tetracyclines and penicillin G together, rather than separately, for several reasons:

o Antimicrobial resistance in salmonellae and *E. coli* to each of these drugs is predominantly plasmid-mediated.

o Simultaneous resistance to both ampicillin and the tetracyclines is commonly found in the same individual animal isolates of salmonellae. Of 717 isolates of *S. typhimuriun* (see Table V-2), 52% had tetracyclines resistance, and 37% had both tetracycline and ampicillin resistance.

o Exposure to either penicillin G or a tetracycline of *E. coli* or salmonella strains bearing R plasmids that encode both tetracycline and ampicillin resistance markers selects for such R-plasmid-containing strains in a mixed population. Thus, exposure to either of the two antibiotics would enrich the population of microorganisms resistant to the other, as well as resistant to it itself.

o The tetracyclines far exceed penicillin G in use in livestock and poultry feeds. For example, in 1985, tetracycline accounted for 49% of annual sales of antimicrobials for animal feeds, and penicillin accounted for only 5% (Table IV-6). Because penicillin use was only 10% of that of the tetracyclines, it did not seem to the committee that performing a separate risk analysis for penicillin G would provide useful information.

o In performing the risk assessment, the committee could not find evidence sufficient to justify the use of different death rates for strains resistant to ampicillin (penicillin), as opposed to the tetracyclines.

This task requires the study of broad questions regarding the effects of drug resistance on the epidemiology of various pathogens and diseases and the effects of feeding subtherapeutic doses of antimicrobial agents on (a) the

prevalence of carriage of various pathogens by farm animals; (b) the antimicrobial susceptibility patterns of these pathogens; (c) the prevalence of infections caused by these pathogens in humans.

Data do not exist to answer directly the principal question posed to the committee even for a well-recognized pathogen such as Salmonella spp. Indeed, the data are sparse and conflicting even with regard to the subordinate questions cited above. To illustrate some of the problems confronting the committee, Figures VII-1 and VII-1A provide a summary of current information about the impact of drug resistance of salmonellae on the epidemiology of salmonellosis in human or animal populations exposed or not exposed to antimicrobial agents. In constructing Figures VII-1 and VII-1A, it was assumed that there is a gradual shift of strains from drug susceptibility to drug resistance. The committee believes that drug resistance is a manifestation primarily of exposure of bacteria to antimicrobial agents for long periods with ultimate selection of resistant strains; thus, it is anticipated that with increasing time of exposure, the prevalence of resistant strains in the animal and human populations will increase. For certain elements in both Figures VII-1 and VII-1A, no data are available, as indicated in parentheses after the item. The studies cited in Figures VII-1 and VII-1A are limited in applicability by the fact that they were not done as part of a cohesive attempt to address the overall issues posed to this committee, but rather were done to address more limited aspects of the problem.

As illustrated in the first horizontal line of Figure VII-1, the "majority" of the reports show that salmonellae in the fecal flora of farm animals are resistant (i.e., resistant to at least one antimicrobial) and a minority are susceptible (meaning susceptible to ampicillin or the tetracyclines). By contrast, the majority of the reports show that human isolates are still susceptible to commonly tested antimicrobials (Figure VII-1A). The prevalence of resistance appears to be rising both for E. coli and Salmonella spp.

No data prove directly that administering antimicrobial agents in subtherapeutic doses to farm animals increases the prevalence of carriage of susceptible salmonella in farm animals; the argument (a highly unlikely one) would be indirect by analogy with the effect of antimicrobial agents on infections by enterohemorrhagic E. coli (EHEC) in humans.[7] By contrast, the 1980 NRC report[21] cited various studies showing that the feeding of antimicrobial drugs to farm animals enhanced the rate of elimination of susceptible strains of Salmonella spp.; this effect would result in a decrease in the prevalence of these susceptible isolates.

	Susceptible Strains	Resistant Strains
1. Current prevalence	Minority of strains	Majority of strains
2. Effect of subtherapeutic administration on prevalence	+ (no data except by analogy with EHEC** strains in humans, see text) -- (1980 NAS [21])	++ (numerous studies of E. coli in animals [see text] but for Salmonella, and extra polation from "etiologic fraction" concept in humans) - (no data) o (Fagerberg [13])

FIGURE VII-1. Potential Effects of Antimicrobial Use on Prevalence of Antimicrobial-Susceptible and -Resistant Salmonella Strains in Farm Animals. Figure prepared by the committee.

+ represents mild increase in prevalence of strains, in degree of virulence, or in the characteristic specified; ++ represents a moderate increase; and +++ represents a major increase.

- represents a mild decrease in prevalence of strains, in degree of virulence or in the character specified; -- represents a moderate decrease; and --- represents a major decrease.

o represents no change.

** EHEC = Enterohemorrhagic E. coli.

	Susceptible Strains	Resistant Strains
1. Current prevalence	Majority of strains	Minority of strains
2. Virulence for humans (ability to colonize and cause disease)	Current level	+ compared to susceptible strains (see Chap III)
	+ (by analogy with EHEC)**	+++ ("etiologic fraction"; see text)
3. Virulence for humans taking antibiotics for other reasons	-- (by analogy with effect on clearance in animals per 1980 NAS report [21]	- (no data)
		--- (great difficulty if "wrong" drug chosen, presumably uncommon)
4. Effiency of treatment of infections	Current effectiveness	

**EHEC = Enterohemorrhagic E. coli.

FIGURE VII-1a. Potential Effects of Antimicrobial Characteristics on Antimicrobial-Susceptible and -Resistant Salmonella Strains in Humans. Figure prepared by the committee.

+ represents mild increase in prevalence of strains, in degree of virulence, or in the characteristic specified; ++ represents a moderate increase; and +++ represents a major increase.

- represents a mild decrease in prevalence of strains, in degree of virulence or in the character specified; -- represents a moderate decrease; and --- represents a major decrease.

o represents no change.

There are few data on animals concerning the effect of feeding subtherapeutic doses of antimicrobial agents on the carriage rate of drug-resistant strains. However, Bohnhoff and colleagues showed many years ago that the feeding of a single oral dose of streptomycin to mice markedly increased their susceptibility to infection by a streptomycin-resistant strain of salmonellae administered orally.[5a] Similar findings were later reported by Meynell,[20a] Bohnhoff and Miller,[5b] Meynell and Sabbaiah,[20b] and Miller and Bohnhoff.[20c] Furthermore, by extrapolation from the "etiologic fraction" in humans (the proportion of infections that would not have occurred but for the resistance of the infecting bacterial strain to the antimicrobial administered), one would expect a marked enhancement of infectivity and hence of prevalence. No data support a diminution in the prevalence of drug-resistant strains as a result of feeding subtherapeutic doses of antimicrobial agents. One study by Fagerberg[13] indicates no difference in the clearance rates of tetracycline-resistant strains of salmonellae between animals given tetracycline and those given another antibacterial drug.

In assessing the second, third, and fourth elements of Figure VII-1A, which deal with the impact of salmonella infections in humans, the committee had the opinion (see below) that the majority of strains of salmonellae that find their way into humans are transmitted from food products which originate on the farm. The second element deals with the effect of antimicrobial resistance on the virulence of salmonellae for humans. Various authors have used the term "virulence" in different ways. Some have restricted the term to the ability to cause disease, particularly toxin-mediated disease, whereas others have incorporated the ability to colonize and to cause disease by any mechanism. The committee decided to use the second definition for this assessment and to use the terms "virulence" and "infectivity" interchangeably. If drug-susceptible strains are considered as a baseline, there is evidence (see Chapter III) that drug resistance may be associated with either a decrease or an increase in virulence. On balance, the committee decided that the data were more compelling for either no change in virulence or an increase in virulence than they were for a decrease in virulence, although the data are weak and rather inconclusive.

In terms of the virulence of Salmonella spp. for humans who are taking antibiotics for other reasons (element 3 in Figure VII-1A), there is strong evidence that drug resistance of the salmonellae facilitates infection. In persons in this category, whose disease is included in the "etiologic fraction," drug-resistant strains are able to colonize the gastrointestinal tract and cause disease even in inocula too small to cause infection in other circumstances, presumably

because the antimicrobial drugs inhibit the normal competing flora (see below). By contrast, the committee is aware of no data indicating that drug resistance diminishes the infectivity of Salmonella spp. for humans. The committee also is unaware of any data on the effect of taking antimicrobial agents on the infectivity of drug-susceptible strains. However, by analogy with data in animals noted under element 2 in Figure VII-1, the administration of antimicrobial agents could enhance the rate of elimination and hence reduce the infectivity of drug-susceptible strains for humans. Nevertheless, using infection by enterohemorrhagic E. coli (EHEC) as an analogy, a study in 1987 by Carter[7] of EHEC infection in a nursing home showed a higher rate of secondary infection among patients who were taking antimicrobial agents to which EHEC was presumably susceptible (isolates of EHEC appear to be almost uniformly susceptible to ampicillin, tetracyclines, chloramphenicol, and trimethoprim-sulfamethoxazole,[22a,23,29a] as opposed to a control group that was not taking such antimicrobial agents. This suggests a facilitating effect of antimicrobial agents even upon infection by susceptible strains.

Finally, the committee considered the effect of drug resistance on the treatability of salmonella infections in humans. In principle, resistance should lead to increased difficulty in treatment. However, considering the epidemiology and the population at greatest risk of death i.e., neonates and the very elderly--it seemed likely to the committee that many patients who die of salmonellosis never receive specific antimicrobial treatment and that failure of treatment because the wrong drug was chosen may be uncommon. However, some patients may be treated inadvertently because their physician does not recognize the infection as salmonellosis. Treatment that prevents bacteremia, a rare event anyway, might lead to an unrecognized benefit. Taking these considerations into account, the committee considered that drug resistance is an uncommon cause of treatment failure.

Overall, the committee concluded that the major consequences of feeding antimicrobial agents to animals or humans are likely to be: 1) a tendency to increase the prevalence of drug-resistant strains; 2) an effect on both the pathogen and the fecal flora that might alter their usual interaction; and, thus, the relative infectivity of the pathogen.

The number of reported cases of salmonellosis in the U.S. has risen progressively over the past three decades, a period during which the practice of subtherapeutic administration of antimicrobial agents to farm animals has been steadily increasing (Figure VII-2). However, this observation does not prove that the increase in salmonellosis is related to antibiotic use because other potentially

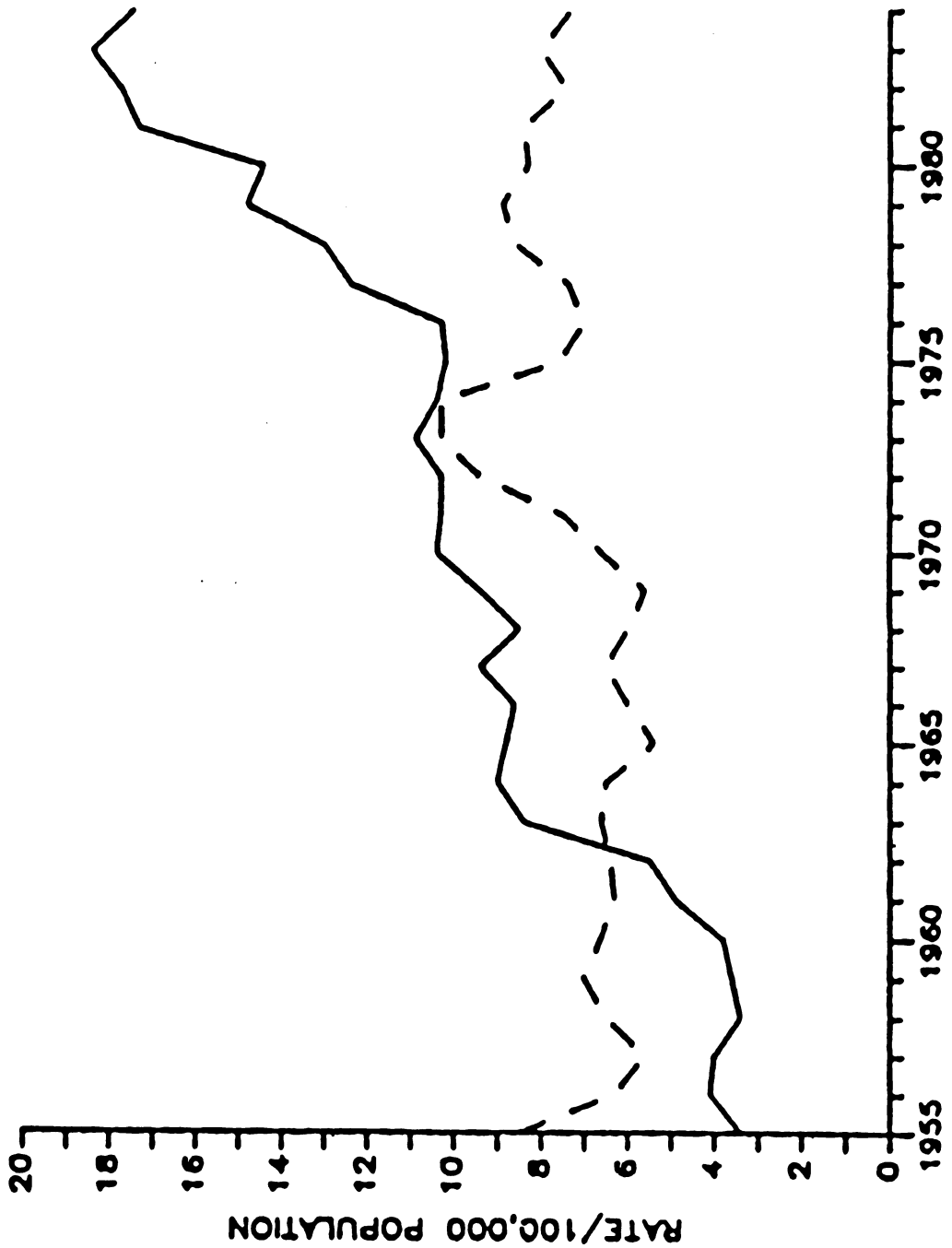

FIGURE VII-2. Salmonella (-) and Shigella (--) infections reported to the Centers for Disease Control, 1955-1984. Rates are per 100,000 population in the United States. Salmonella rate excludes infections due to Salmonella typhi. Reprinted from Chalker and Blaser.[11]

confounding variables have occurred in the same interval, including the increasing use of convenience foods and prepared "fast foods." That the increase in reported cases of salmonellosis in humans over the past 30 years is not an artifact of reporting is suggested by the fact that the number of infections caused by Shigella spp. has remained fairly constant during this period (Figure VII-2). Thus, there is direct evidence of an increase in salmonellosis in humans. Some of this increase might be attributed to some of the elements shown in Figure VII-1A. Furthermore, it is difficult to determine directly the contribution of drug resistance to fatality from salmonella infection because neither the published CDC data for reported cases nor the NCHS figures for deaths from salmonellosis make note of the drug susceptibility or resistance of the pathogen.

After consideration of the concepts illustrated in Figure VII-1 and the limited data available pertaining to the issues, the committee concluded that it was impossible to arrive at a firm answer to the important question of whether or not the administration of antimicrobial agents in subtherapeutic doses to farm animals has led to an overall change in the total number of cases of salmonellosis in humans. Accordingly, the committee decided to approach the problem indirectly by devising a risk model that focuses upon one aspect of the problem, namely, the number of deaths which can be attributed to the subtherapeutic administration of antibiotics to farm animals.

STRUCTURE AND LIMITATIONS OF THE MODEL

As summarized above, questions about the size of the human risks from low-level farm uses of antibiotics cannot be answered by the direct interpretation of data on this matter. Therefore, the committee developed and adopted a conceptual approach, or model, in which some information is available at each step. In devising the model, the committee chose to deal only with salmonellosis because this was the only pathogen for which there were data available in quantity and quality that the committee could use in quantifying the risk. Nevertheless, the committee recognized that there are other infectious organisms that may account for at least as large a part of the overall problem of human illness attributable to the subtherapeutic use of antibiotics on the farm.

The model includes a sequence of five quantitative estimates, each dependent on the prior estimates. In steps 2, 3, and 5 (see below), the estimates were calculated separately for resistance to any antibiotic and for resistance to at least penicillin/ampicillin or the tetracyclines. These estimates are illustrated in parentheses by the committee's mid-range estimate for each

step with respect to resistance to penicillin/ampicillin or the tetracyclines, as in the following:

1. Annual number of cases of salmonellosis reported in the U.S. (50,000).

2. Fraction of human cases due to bacterial strains showing resistance to penicillin/ampicillin or the tetracyclines (15%).

3. Death rate (1.0%) among cases with drug-resistant salmonellosis.

4. Fraction of these deaths associated with infection by bacterial strains of farm origin (70%).

5. Proportion of this fraction resulting from subtherapeutic use of penicillin/ampicillin or the tetracyclines in animal feed (90%).

Since these estimates are linked in stepwise fashion, and each is developed to be statistically conditional on all that precede it, they can be multiplied to estimate the number of deaths. This chapter develops the risk model and explains the committee's choices of the quantitative inputs the model requires, and Chapter VIII uses the model to develop estimates of excess deaths while reflecting the uncertainty of those estimates.

FOCUS ON SALMONELLAE

The model has several limitations. One limitation is that it assesses only the hazards of infection with *Salmonella* spp. The major foodborne pathogens known or suspected to be transmissible to humans from farm animals or their products are *Salmonella* spp.; *Campylobacter jejuni* enterohemorrhagic strains of *Escherichia coli* (EHEC), especially serotype 0157:H7; and *Yersinia enterocolitica*. The Centers for Disease Control reported on 151 outbreaks of foodborne bacterial disease in 1982;[8,9] of this number, salmonellae caused 55 outbreaks with 2,056 cases and 8 deaths. By contrast, each of the other three species caused only two outbreaks, with between 31 and 188 cases for each species, and no deaths. However, these data are highly selected and are incomplete.

Although recent surveys[3,4,5,14,15,19,26,27,28] suggest that *Campylobacter* spp. may cause at least as much illness as does salmonellosis, data on infections caused by nine named or proposed named campylobacter species are not available. Laboratory-based national surveillance of campylobacter

infections in the U.S. began in 1982 with a panel of 11 states with additions in 1983 bringing the total to 31 states.[10] The committee has not critically looked at these data. Also, antimicrobial resistance is not recognized as a major issue in treatment, and no data are available to determine whether the administration of antibiotics to farm animals has contributed to antimicrobial resistance in these species.

Similarly, there are no nationwide data available to estimate the impact of infection caused by Y. enterocolitica or EHEC. However, infection caused by Y. enterocolitica is rarely recognized in the United States. Although infections may be grossly underdiagnosed, more data would be needed to establish yersiniosis as an important clinical problem in this country. Most isolates of Y. enterocolitica are susceptible to tetracycline, but ampicillin resistance is common.[6] Strains of EHEC are almost always susceptible to commonly used antimicrobial agents.[22a,23,29a]

There are over 1500 serotypes of salmonellae.[12] However, more than 70% of the infections are caused by 10 serotypes and four are dominant: S. typhimurium causes about one-third of reported infections, S. enteritidis about 10%, S. heidelberg about 10% and S. newport about 5%. The frequency of isolation of S. typhi, which causes typhoid fever, has diminished sharply since the beginning of this century; currently, there are about 500 isolations of S. typhi per year, as opposed to more than 40,000 isolations of other species of salmonellae. S. typhi is not known to infect animals or to have an animal reservoir and is thought to be spread from person to person, so it is not considered in this analysis. The committee concluded that Salmonella is the only genus for which sufficient data are available to estimate the national impact on mortality from infections caused by drug-resistant organisms transmitted from farm animals or their products to humans. The remainder of the quantitative analysis in this report pertains to infection caused by nontyphoidal Salmonella spp. Morbidity was not included in the risk calculations.

MODEL UNCERTAINTY

A second limitation is that the model itself may be incorrect. While the steps outlined above are logically appealing, other chains of critical events could be developed, such as steps in the chain of transmission or pathogenesis, and these might produce materially different estimates.

Numerous difficulties, conceptual and practical, impede the estimation of the mortality rates attributable to salmonella infections, and the committee recommends a

substantial increase in the investigation and development of conceptual models of this matter that include morbidity.

INDEPENDENCE OF ESTIMATES

Third, our use of the model requires that the parameter estimates be conditionally independent; that is, the statistical distribution of one estimate, given others that precede it in the model, depends on those values in a way that is fully specified by the model. For example, the death rate among cases with drug-resistant salmonellosis (see step 3 in Table VIII-1) refers to reported cases in the U.S.; it is presumed that there is a much larger number of unreported cases (see below). The death rate is likely to be lower for the unreported cases than for reported cases. However, it is not generally possible to validate this assumption of conditioned independence from available data for salmonella risks. Further, limitations in the data have required the use of some estimates that are not conditional, or that are less completely conditional, than the model theoretically requires. Both the numerators and the denominators needed to calculate the rates of illness and death are subject to considerable uncertainty. Further, these uncertainties are closely linked, so that the numerator and denominator must be developed together and the resulting (death) rate should be applied to other settings only insofar as the major uncertainties are appropriately correlated.

For example, the death rate of salmonella infections depends very much on whether it is for cases such as are reported to the Centers for Disease Control or for the whole of symptomatic infections in humans. Thus, the seven deaths reported among 503 patients in a 1979-1980 CDC survey yield a death rate of 1.4%.[12] Since the denominator was 503 cases reported in approximately routine fashion to the CDC, one might, with caution, estimate that about 1.4% of all such reported cases of salmonellosis might have been fatal. Because about 50,000 cases of salmonellosis are reported to the CDC annually, one could make an estimate that there are about 700 deaths in 50,000 reported cases per year. In fact, it is reasonable to assume there are many more unreported cases; however, the death rate estimate of 1.4% does not necessarily need to be changed, because, unreported cases might be less likely to be severe or fatal and to go unreported for that reason.

Similar considerations apply to other aspects of salmonella infection, such as the rate of hospitalization, the proportion of patients with "serious" rather than mild disease, and a medical decision to culture stools or other materials.

LIMITATION TO CDC-REPORTED CASES OF SALMONELLOSIS

The committee recognizes that the numbers of cases of salmonellosis reported to CDC per year, ranging from about 40,000 to 65,000, surely is an underestimate of the number of cases in the U.S.; nevertheless, the committee decided to use this range of numbers for the first step in the risk assessment. This decision was made because: 1) several other critical estimates in the risk model apply to this same population of CDC-reported case, and 2) it may be that unreported cases are milder and of lesser consequence, although this has not been shown to be the case. However, investigation of epidemics by CDC of reported cases necessarily underestimates the scope of the problem.

LIMITATION TO ESTIMATES OF MORTALITY

A fifth limitation is that the model deals only with lethal infections. Salmonellae also cause considerable, albeit temporary, personal distress (morbidity), as well as a large economic burden. The clinical manifestations range from asymptomatic colonization through mild or sometimes severe diarrhea, to disseminated and sometimes lethal illnesses, such as meningitis or osteomyelitis. However, statistical data regarding the incidence of various symptoms are minimal or lacking even for severe cases, and there is difficulty in applying such data to the U.S. as a whole.

Fatalities due to salmonella infections are clustered in the very young and in the elderly; Table VII-1 shows the age distribution of salmonella deaths reported to the National Center for Health Statistics (NCHS) for the years 1968-1985. For each end point, the group at risk must be unambiguously defined, essentially all instances in the group must be identified, and the calculated rate must be applied to other groups only when they are likely to have about the same distribution of severity of illness and only when adequate margins of error are attached to the calculated rate. These margins of error will ordinarily be substantially wider than statistical confidence limits.

The hospitalization rate for salmonellosis in the 1979-1980 survey of selected communities was 45% and the rate in the 1984-1985 survey was reportedly similar.[12] However, in a review of recent outbreaks of *S. enteritidis* infections occurring in the northeastern U.S., the hospitalization rate was estimated to be only 12%.[30] Thus, a range of 12% to 45% may be entertained as the estimate for the rate of hospitalizations of patients with salmonellosis. When these rates are applied to the 50,000 reported cases of salmonellosis per year, the number of patients hospitalized for this infection ranges from 6,000 to 22,500 per year.

TABLE VII-1

FREQUENCY AND PERCENTAGE OF DEATHS DUE TO SALMONELLOSIS
(By Age, for 1968-1985)

Age	Deaths	
	Number	Percent Per Year of Age
Under 1 day	1	0.1
1 - 6 days	8	0.6
7 - 27 days	30	2.1
28 - 364 days	165	11.6
1 - 4 years	42	3.0
5 - 9 years	12	0.8
10 - 14 years	11	0.8
15 - 24 years	14	1.0
25 - 34 years	30	2.1
35 - 44 years	42	3.0
45 - 54 years	104	7.3
55 - 64 years	176	12.4
65 - 74 years	314	22.1
75 - 84 years	296	20.8
85 + years	174	12.2
Unknown	2	0.1
All Ages	1,421	100.0

Source: National Center for Health Statistics.[22]

LIMITATIONS IN THE DÁTA

Sixth, the model is limited by the range and quality of data available for the estimates required, as summarized above. The committee has developed three estimates for each quantitative parameter: a mid-range estimate, a high estimate and a low estimate. The mid-range estimate expresses the committee's best judgment about the value that is equally likely to be too large or too small--a median, of sorts, of the committee's collective objective and subjective judgment. The high and low estimates express the committee's best judgments about the range of figures that most other experts would find plausible. These limits are not presented as statistical confidence limits (even subjectively), nor as outside bounds on possibility. For example, if three figures for some parameter were 1 and 10, an estimate outside the range 1, 3, and 10 would not be credible, the committee believes, to most other experts. We have not attempted to attach probability values to these low and high estimates, because we have no direct evidence about what limits other experts would be willing to accept as plausible.

The committee discussed at some length how these three estimates should be derived. The basic point of discussion was the extent to which our collective subjective judgment should be used to modify specific values obtained from the literature. As an example, the fraction of strains resistant to two or more antibiotics has been rising, in contrast to published results that necessarily refer to infections detected in the past. Thus, the published range of rates of resistance to multiple antibiotics will tend to be too low, but by an unknown amount. How much should the committee's judgment about this trend over time be integrated into rates obtained from the literature?

Other problems arise because of the need to use estimates that are statistically conditioned on preceding estimates, though appropriate data may not exist. For example, the fraction of infections due to multiresistant strains should refer specifically to the kinds of cases, with specific details, reported to CDC; however, not all sources meet this requirement. In the end, the committee tended to give its subjective judgment considerable weight. We have not attempted to attach probability values to these low and high estimates, because we have no direct evidence about what limits other experts would be willing to accept as plausible.

The remainder of this chapter outlines the basis for the estimates that the committee used in its risk model to assess the contribution of subtherapeutic use of antibiotics in animal feed to the presence of drug-resistant salmonellae in humans in the United States.

NUMBER OF CASES PER YEAR

The number of cases of salmonella infections per year is large, but not precisely determined. Many observers believe that it is probably 10-100 times larger than the number of confirmed cases reported to the CDC. This is because many patients with salmonellosis do not seek medical attention; when they do, stools or other specimens may not be cultured; when cultures are attempted, they may be unsuccessful in isolating the infecting bacteria, or positive results may not be reported to CDC. Still, most of the data relating to morbidity and mortality from salmonellosis in the U.S. are derived from the CDC. The CDC in turn relies on several sources for its information, including the following:

o A Salmonella Surveillance System, maintained by CDC since 1963, when several large outbreaks of salmonellosis were traced to commercial egg products. The purpose of the surveillance system is to accumulate epidemiologic data such as the age, sex, and county of residence of patients from whom Salmonella isolates are submitted to state health departments for serotyping. Data are also kept on isolates from food and animals.

o Investigations of outbreaks by state, local, and federal agencies.

o Special epidemiologic and laboratory surveillance in selected counties.

In 1979-1980 and 1984-1985, the health authorities in a stratified sample of urban and rural counties were asked to submit all salmonella isolates, together with detailed epidemiologic information, for all patients from whom isolates were obtained.[12] The communities were chosen to provide about 5% of the expected number of reported isolates.[24] Strains from known outbreaks were excluded. The isolates collected in this way were obtained through the usual CDC reporting channels with no specific efforts at case-finding (R. Tauxe, CDC, 1988, personal communication). The isolates were tested for susceptibility to antimicrobial agents and sometimes for their plasmid DNA content. These findings have been used to provide information on the rates of antibiotic resistance, hospitalization for illness, and mortality from salmonellosis.

The salmonella surveillance system conducted by CDC has shown a fairly constant rise in the annual number and rate of reported cases of nontyphoidal salmonellosis at least since 1955 (Figure VII-2). The reasons for this rise are not clear. However, the belief that the rise was not simply the result of better case finding is supported by the observation

that there was no appreciable change in the reported rate of shigellosis over the same period. In the years from 1982 through 1986, the number of cases of salmonellosis reported per year ranged from 40,861 to 65,347.[8] Over 90% of the reported isolates were from symptomatic individuals (P. Blake and R. Tauxe, CDC, 1988, personal communication).

There is substantial underreporting of salmonellosis.[11] Indeed, it has been estimated that in several outbreaks as few as 1% of cases were reported.[2] In a telephone survey conducted recently during a massive epidemic of salmonellosis, it was found that only about 10% of symptomatic infections were reported.[25] Attempts to determine the number of cases more precisely run into the problem of defining exactly what is a case. Should one include as cases only symptomatic patients with infections? Does passage of one or two loose stools qualify? Must symptoms be severe enough to interrupt normal activities for at least 24 hours? Must symptoms be severe enough to require medical attention? The numbers might vary by one or two orders of magnitude, and no answer is inherently correct. Each investigator in the field must develop a conceptual definition that is meaningful and useful for a specific study. This must then be translated into operational terms: How can one collect and interpret data so as to estimate both the number of cases by this definition and the degree of error likely to attend the estimate?

An extensive analysis using three independent methods to derive these estimates for the annual incidence of salmonellosis produced estimates ranging from 800,000 to 3,700,000 infections.[11] A mean estimate of 1.9 million infections in 1984 would imply that about 2.5% of infections (about 50,000 cases) had been reported to the CDC.[11] Therefore, an annual incidence of 50,000 reported cases of nontyphoidal salmonellosis in the United States is a highly conservative estimate. A more probable figure is on the order of 800,000 cases per year, and the upper limit could be as high as 3,700,000 cases per year.[11]

Because many of the estimates considered critical for use in the model were based on data derived by the CDC from cases reported to that agency, the committee used the number of isolates reported as the starting point for the model (see "Limitations in the Data," above). In the estimation of risk, the committee used the following figures for low, mid-range, and high-estimates for the number of reported cases of salmonellosis per year (U.S. only): 40,000, 50,000 and 65,000, respectively.

ANTIBIOTIC RESISTANCE OF SALMONELLA

The proportion of salmonella isolates from humans with

resistance to at least one antimicrobial agent was 16% in the 1979-1980 CDC survey and 24% in the 1984-1985 survey.[20] The proportion with resistance to two or more drugs, i.e., multiresistant strains, increased from 12% to 15% during these same years.[20] These surveys avoided the counting of multiple isolates from the same outbreak or the same patient.

Changes in drug-resistance rates vary among the different salmonella serotypes. In the 1979-1980 study, the rate of resistance was high for S. heidelberg, but low for S. typhimurium; in the 1984-1985 study, the rate of resistance of S. heidelberg decreased, and that of S. typhimurium increased.[20]

There were also changes in resistance to different antibiotics. The rate of resistance to ampicillin rose from 8% to 9% between the two study periods, and the rate of resistance to tetracycline rose from 8.6% to 13%; by contrast, the rates of resistance to chloramphenicol and trimethoprim-sulfamethoxazole were each 2% or less for both study periods.[20] At the request of this committee, the CDC provided additional information which allowed the committee to calculate that 19 of 485 strains (3.9%) were resistant to both ampicillin and tetracycline, whether or not they were also resistant to other agents. In a collection of 2,826 strains isolated from humans from Massachusetts during 1979-1980 the prevalence of resistances was as follows: ampicillin, 5.1%; tetracycline, 8.7%; tetracycline and ampicillin, 3.3%; tetracycline or ampicillin, 10.3%. The overall prevalence of resistant strains in the Massachusetts collection (see Table V-1) was low, compared to other sources, perhaps because of the high proportion of generally susceptible S. enteritidis (31%).

For the estimation of risk, the committee chose the rates for occurrence of antibiotic resistance shown in Table VII-2. The high estimates for resistance were chosen to account for the apparent increase in resistance rates over time, because the reported rates may underestimate the current prevalence of resistance.

MORTALITY RATE FOR INFECTION BY RESISTANT STRAINS OF SALMONELLA

In concept, the number of deaths from salmonella infections should be only the number of infected persons who died, and who would not have died in the absence of these infections.

This concept encounters serious problems in application, because the causes of some deaths are difficult to determine in ordinary conditions of medical practice, and the asserted cause of death on a death certificate often is unproved. Some diseases or conditions act jointly to cause death,

TABLE VII-2

RESISTANCE OF SALMONELLAE TO ANTIMICROBIALS

Rate of resistance of salmonellae to:	Low Estimate	Mid-Range Estimate	High Estimate
At least one antimicrobial	16%	24%	31%
At least penicillin/ampicillin or tetracycline	10%	15%	20%

Source: Adapted by the committee, from data in Table V-3.

although neither alone has resulted in death; in such a case, what is the underlying, "cause of death"? In some instances, a severe infectious disease is almost incidental to a severe underlying terminal condition; shall we count such a death if the salmonella infection only advances the time of death by a month, a day, or an hour? A decision is needed about whether to count as a salmonella death the death of a person who was severely debilitated from other causes but whose uncontrolled salmonella infection contributed to the death.

The committee recognizes that the U.S. National Center for Health Statistics, like other offices of vital statistics, has consistent and well-developed rules for deciding how to report these types of deaths. The committee understands the need for well-defined and consistent statistical data, especially for identifying differences among populations and changes over time. However, the committee emphasizes that these rules deal with the underlying conceptual problems in consistent reporting without solving them in a way that is useful here.

Table VII-3 summarizes recent data on reported death rates of patients with salmonellosis. The CDC study of 1979-1980 identified seven deaths among 503 patients with nontyphoidal salmonellosis, for a death rate of 1.4%; there was a similar rate in the 1984-1985 survey.[12] However, these were all deaths among patients reported to have salmonellosis, regardless of specific causes of death. The committee asked for additional information regarding the role of salmonellosis in causing death. CDC was not able to provide such data for patients who died in the 1979-1980 study, but did provide additional information on the 8 deaths among about 600 patients included in the 1984-1985 survey. According to the committee's interpretation of those data, salmonellosis played an unknown role in three of the deaths and no role in four deaths, and it clearly contributed to the

TABLE VII-3

RECENT MORTALITY RATES FROM NON-TYPHOIDAL SALMONELLOSIS

Prospective CDC Surveillance Data[12]	Community-Acquired Infection			Nosocomial Infection			Specified/or Combined Community Nosocimial Source of Infection			Comments
	Suscep	Multi-Res	Not Spec	Suscep	Multi-Res	Not Spec	Suscep	Multi-Res	Not Spec	
1979-1980									1.4% (7/503)	Role of Salmonellosis in causing deaths not specified.
1984-1985									1.4% (8/600)	Only one death clearly attributable to Salmonellosis (see text).
1984-1985 (recalculated)									0.2% (1/600)	
Outbreaks										
US outbreaks 1971-1983[18]							0.2% (4/1912)	4.2% (13/312)		
US Outbreaks 1971-1980[17]	0.2% (3/1321)	3.4% (7/205)		1.0% (2/202)	11.7% (30/256)					Most of the data base was the same as for 1971-1983.[18]
N.E. USA[30]	0.5% (11/2119)									*S. enteridis*, presumable drug-susceptible (not stated) via grade A eggs in Northeastern USA.
United Kingdom									0.3% (40/12,000)	See Chapter 6 for discussion of cases.
Midwest USA[25]		0.1% (14/12,624)								14 deaths probably or possibly related to Salmonellosis; *S. typhimurium* in pasteurized milk resistant to ampicillin, the tetracyclines, carbenicillin, and sulfisoxazale.

Source: Adapted by the committee, from data by Cohen and Tauxe,[12] Holmberg,[17,18] Ryan,[25] and St. Louis.[30] U.S. Outbreaks 1971-1980[17] includes Puerto Rico.

death of only one patient, in whom the organism was isolated from blood (P. Blake and R. Tauxe, CDC, 1988, personal communication). Accordingly, in only 1 patient of about 600 (0.2%) did salmonellosis clearly contribute to the death of the patient, although the rate may have been as high as 4 in 600 (0.7%).

In a CDC review of outbreaks of salmonellosis by Holmberg et al.,[17] encompassing the period 1971-1983, the overall death rate from drug-susceptible strains was 0.2% and from drug-resistant strains was 4.2%. Of the 13 deaths caused by multiresistant strains, 8 were in elderly persons in the community, and 5 occurred among 18 infants in a single hospital nursery.[18] The basis on which it was determined that salmonella infection caused or contributed to death was not stated.

More recently, Holmberg et al.[17] reviewed both nosocomial and community-based outbreaks that were investigated by CDC and that occurred in the United States between 1971 and 1980. These outbreaks were caused by various species of bacteria. All but one of the outbreaks of salmonellosis reported in this review had already been reported in the earlier paper by this group.[18] In 10 of the community-based outbreaks of salmonellosis that were identified as being caused by drug-susceptible strains, salmonellae caused the death of three persons among 1,321 persons infected, or 0.2%. By contrast, the death rate in four community-based outbreaks caused by multiresistant strains was 7 of 205, or 3.4%.[8] The death rate in seven of the nosocomial outbreaks that were caused by drug-susceptible salmonellae was 1.0% (2 of 202 patients) but in nine of the nosocomial outbreaks caused by multiple-drug-resistant salmonellae, the death rate was 11.7% (30 of 256 patients).

In both the community acquired nosocomial outbreaks just cited[17,18] the data do not allow comparison of the ages of the individuals with salmonellosis. This comparison might be important in view of the greater number of deaths due to salmonella infections reported at the two extremes of age (see Table VII-1). Among the nosocomial outbreaks reported[17], 11 of the 30 deaths due to multiresistant salmonellae occurred in patients in neonatal intensive care units, whereas the 2 deaths due to antimicrobial-susceptible strains occurred in general hospital wards in patients whose ages were not specified. The committee consulted the CDC for additional details on this issue but no further information was available (S. D. Holmberg, 1988, personal communication). Thus, if more outbreaks due to antimicrobial-resistant strains had involved infants and the elderly than outbreaks due to susceptible strains, the higher mortality associated with resistant strains might reflect such a difference in the population at risk.

A review of 65 outbreaks of S. enteritidis infection that occurred between January 1985 and May 1987 in the northeastern United States showed a death rate of 0.5% (11 deaths among at least 2,119 cases).[30] Ten of these deaths occurred among 130 residents in nursing homes. Although antimicrobial susceptibility data were not given, strains of S. enteritidis nearly always have been found to be susceptible to commonly used antibiotics. Grade A eggs or foods containing eggs were implicated in 77% of the outbreaks in which a source of the infecting bacteria could be found.[30]

The two papers on CDC's surveys of outbreaks are summarized by Holmberg et al.[17,18] (Table VII-3) and show a higher death rate from infection due to multiple-drug-resistant strains than from infection due to susceptible strains of salmonellae. The more recent paper summarized data from outbreaks of infections that included bacteria other than salmonellae and reported nosocomial and community-acquired infections separately and more clearly than the earlier summary. The authors concluded that for both community-acquired and nosocomial infections, the mortality rate, the likelihood of hospitalization, and the length of a hospital stay were usually at least twice as great for patients infected with drug-resistant strains as for those infected with susceptible strains of the same species.[17] A higher mortality rate due to infection with drug-resistant strains of salmonellae could result from: (a) a greater virulence of the resistant strains, (b) a propensity for the drug-resistant strains to infect patients with diminished host defenses (see "etiologic fraction" below), or (c) the inefficacy of treatment with drugs to which the bacteria are resistant. Salmonella deaths are also reported by the U.S. National Center for Health Statistics (NCHS) in the National Death Index (NDI), which collects data from the total U.S. and tabulates causes of death using the International Classification of Disease (ICD) categories on the death certificates. The data summarized in the NDI do not provide any information on the individual cases, their location, the infecting bacterial serotypes (except that nontyphoidal salmonella infections are identified as such), serotypes or their drug susceptibilities. Therefore, these data do not have the same application for the analysis of risk as the CDC data. It is useful to compare the salmonella deaths reported by both sources as a check on the accuracy of salmonella deaths reported by each.

The NDI data are based on information given on the death certificates completed by physicians, coroners, or medical examiners. Summaries of deaths from salmonellosis are given in Table VII-4 for the years 1980 to 1985. During this period, "other (nontyphoidal) salmonellosis" (ICD-9, code 003) was reported as the underlying cause of death in 82-117 deaths per year. In addition, during this same period,

TABLE VII-4

NUMBER OF DEATHS DUE TO SALMONELLOSIS (ICD-9, NO. 003) TABULATED IN THE NATIONAL DEATH INDEX

Year	UC*	EA**
1985	117	218
1984	89	164
1983	82	154
1982	87	176
1981	104	-
1980	88	175

Source: National Center for Health Statistics.[22]

* UC = Underlying cause of death (i.e.) disease was listed as initiating cause of death on death certificate).

** EA = Entity axis (i.e., disease was listed as contributing cause of death).

salmonellosis, whether it was only contributory or was thedirect cause of death, was mentioned on the death certificate in 154-218 deaths per year.[22] These numbers of deaths, derived from sources and methods that are different from those used by CDC, are within the range of death rates cited by the CDC. For example, if salmonellosis was the underlying cause of death in 100 deaths per year (intermediate between 82 and 117 deaths per year) and these deaths are considered to occur among the 50,000 reported cases of salmonellosis per year, the death rate would be 0.2%. For the approximately 200 cases per year in which salmonellosis was at least a contributing factor, the death rate would be 0.4%.

The NDI data may underestimate the number of deaths from salmonellosis for several reasons: (1) a death certificate may be filled out by a physician or other authorized person who is not fully familiar with the illness of the deceased and may not recognize the contribution of the infection to "cardiac arrest" or some similar non-infectious process; (2) a salmonella infection may be recognized and reported as septicemia, meningitis, or other infection without identification of the causative organism; or (3) failure to take a bacterial culture from a patient with salmonellosis as a complication of a terminal illness might lead to failure to identify the organism.

The data on case studies in Table VII-3 suggest that the death rate may range from 0.2% to 0.5% in community-based salmonella infections caused by drug-susceptible strains, but could be as high as 1.4% if the CDC surveillance data were for susceptible strains, a fact which cannot be determined from the reports. The death rate may range from 0.1% to 3.4% or 4.2% in community-based outbreaks caused by multiple-drug-resistant strains; however, the two higher values for death rates from resistant strains are derived from on overlapping body of data and are not independent. The death rate was 1% in nonsociomal outbreaks caused by susceptible strains and was as high as 11.7% in nosocomial outbreaks caused by multiple-drug-resistant strains.

The death rates used in the risk assessment model are summarized in Table VII-5. The committee considered whether the low estimate for strains resistant to at least one drug should be set at 0.1% in accord with the report by Ryan et al.[25] on a very large outbreak of milk-borne salmonellosis. The committee concluded that the reported death rate in that outbreak was unusually low, perhaps because extensive publicity led to substantially above-average reporting of marginal cases (the denominator of the reported death rate). Each outbreak is thus considered a sample of one and not weighted according to the number of persons affected. No death rates were available from the literature for strains resistant specifically to at least penicillin/ampicillin or the tetracyclines, although resistant strains isolated in epidemics were frequently resistant to at least one of these drugs. Thus, the committee elected to use the same death rate of 0.2% for strains resistant to penicillin/ampicillin or the tetracyclines as to any other drug (see Table VII-5).

For the mid-range and high estimates of the death rate from strains with no resistance, the committee elected to use estimates that spanned the range of values shown in Table VII-5 for strains of this kind, whether the infections were community-acquired or nosocomial.

For strains with drug resistance, the committee believed that the plausible mid-range or high death rates were appreciably higher than those for strains with no resistance, for three reasons: (a) some patients, although probably few, would receive the "wrong" drug intentionally or inadvertently; (b) drug-resistant strains would tend to cause some infections in the "etiologic fraction" category (discussed below) in patients who might be debilitated and more susceptible to the consequences of infection; (c) the data of Holmberg et al. summarized in Table VII-3 indicate a higher death rate from resistant than from susceptible strains. As discussed earlier, there are theoretical reasons why drug-resistant strains might be more virulent than drug-susceptible strains, including the possibility that the resistance plasmids have acquired virulence genes (or that

TABLE VII-5

RANGE OF DEATH RATES (AS A PERCENTAGE) FOR SALMONELLOSIS FROM SUSCEPTIBLE AND DRUG-RESISTANT STRAINS

	Estimates		
	Low	Mid-Range	High
Susceptible	0.2	0.5	1.0
Resistant to at least one drug	0.2	1.0	4.0
Resistant to at least penicillin/ampicillin or tetracycline	0.2	1.0	4.0

Source: Prepared by the committee (see Table VIII-1).

virulence plasmids have acquired resistance genes). It might be argued that the death rate for strains resistant to atleast penicillin/ampicillin or the tetracyclines should be higher than for strains resistant to any drug, because the former would be more likely than the latter to lead to problems with the "wrong" choice of drug, or with the "etiologic fraction." However, lacking any specific data on this point, the committee chose to use the same mid-range and high estimates for these two kinds of strains. In any event, it seems unlikely that the results from the risk model will be much influenced by the use of similar values for these two kinds of strains. The reported death rates for infection by resistant strains in Table VII-3 are for multiresistant strains, whereas the designation in Table VII-5 is for strains resistant to at least one agent; the committee considers this distinction to be of minor consequence for the present analysis.

FRACTION ASSOCIATED WITH FARM ORIGIN

A critical step in the risk estimate is the determination of the source of resistant strains of salmonellae that cause infection in humans. In fact, the true "origin" of a strain of salmonellae that causes infection in humans--i.e., whether it arose from a food product, contact with another person or a pet, or some other source--is almost never known, except in outbreaks that are investigated. However, there is a common belief that for most strains of nontyphoidal salmonellae, the proximate

source is usually food animals or food products derived from animals. This belief is supported by the findings that carriage of nontyphoidal salmonellae in humans is generally brief and that a wide variety of commonly consumed foods are often contaminated by strains of salmonellae and by the evidence (summarized below) that, in most outbreaks in which the source could be traced, the source has been food products originating on the farm.

Reliance on the results derived from the analysis of outbreaks is problematical, because salmonella infections in humans are usually sporadic. Of course, if adequately detailed investigation could be done, it is likely that many "sporadic" cases would turn out to be small epidemics. There is no evidence that salmonella strains isolated from outbreaks are distinct from strains isolated from sporadic cases; the epidemiologic effects of a given strain are presumably related to the number of organisms in the infecting inoculum and the number of people exposed to this inoculum. Because of these considerations, the committee concluded that the data from outbreaks are usable in the present context. In CDC's review of 52 outbreaks of salmonellosis, food animals or their food products were implicated in 11 of 16 outbreaks (69%) caused by drug-resistant salmonellae, 6 of 16 (38%) outbreaks caused by drug-susceptible salmonellae, and in 1 of 9 outbreaks (11%) caused by salmonellae of unknown susceptibility.[18] By consensus, the committee concluded that the low estimate for the likely percentage of resistant strains that originated in farm animals or their products was 50%, and the upper limit of this estimate might be as high as 100%. For the mid-range estimate, 70% was used--a value similar to that found in the CDC's review of outbreaks cited above.

FRACTION OF ANTIBIOTIC-RESISTANT STRAINS CAUSED BY SUBTHERAPEUTIC USE OF ANTIBIOTICS

Because of the paucity of data, the most uncertain aspect of the committee's risk analysis is the estimation of the portion of drug resistance in salmonellae of farm origin that is attributable to the subtherapeutic use of antibiotics. Farmers use antimicrobials in subtherapeutic dosages in feed for two purposes: (a) growth promotion, and (b) prophylaxis (such as the prevention of atrophic rhinitis in swine or "shipping fever complex" in cattle). FDA's definition of subtherapeutic use includes use for both growth promotion and prophylaxis. The committee's objective is to develop data for this combined use of antimicrobials, as well as for use in growth promotion alone.

The committee could find no data that bear directly on the relative contributions to the development of drug

resistance caused by any of the three major dosage regimens for antimicrobials--for therapy, for growth promotion, or for prophylaxis. Indeed, the data are limited and inconclusive regarding the relative contribution of chronic low-dose administration vs. intermittent high-dose administration of antimicrobial agents in fostering drug resistance (see Chapter III). Overall, the limited data available to the committee suggest that chronic exposure to low concentrations of antimicrobials is at least as likely to foster resistance as intermittent exposure to high concentrations. Given the substantial uncertainties about the causal relationship between the type of antimicrobial dosage regimens and development of resistance, the committee adopted as its mid-range estimate of the relative contributions of the three major farm uses of antibiotics the approximate proportions (percentages by weight) of drugs administered nationwide for each of these purposes to animals (see Chapter IV).

Even this seemingly straightforward approach proved difficult, because of the problems in obtaining reliable estimates of the amounts of the various antibiotics used for each of the three main purposes (see Chapter IV). As a starting point, it appears that, overall, about 12% of antibiotics sold for veterinary use is used for therapeutic purposes and about 88% is used in subtherapeutic dosage regimens (see Table IV-9 for the source of these percentages).

Based on the tonnage ratios shown in Table IV-9, some two-thirds of the drugs used for subtherapeutic purposes is given for prophylaxis and one-third growth promotion; this would result in a partition of the 88% into about 60% for prophylaxis and 28% for growth promotion. However, in the judgment of committee members the fraction used for prophylaxis is probably about three-fourths. This would result in a partition of the 88% used subtherapeutically into 66% for prophylaxis and 22% for growth promotion. The arithmetic means of these estimated percentages are 63% (60-66%) for prophylaxis and 25% (22-28%) for growth promotion, as shown in Table VII-6. Accordingly, on the simple assumption that the contribution of any drug used on the farm to the development of drug resistance would be in linear proportion to the amount used for each of the three purposes, the committee chose a mid-range estimate of 12% for the contribution of <u>any drugs</u> used for therapeutic purposes and a mid-range estimate of 88% for the contribution of <u>any drugs</u> used subtherapeutically (63% for prophylaxis and 25% for growth promotion).

The committee recognizes that there may not be a linear relationship between selection of antibiotic resistance and the total amounts of antibiotics used in farm animals. By consensus, the committee chose the plausible low estimate for

TABLE VII-6

ESTIMATED PERCENTAGE OF ANTIBIOTIC RESISTANCE IN STRAINS OF FARM ORIGIN CAUSED BY SUBTHERAPEUTIC USE OF ANTIBIOTICS IN ANIMAL FEED

Use	Proportion of Tonnage for Specified Use	Contribution of growth-promotion use to resistance: Low Estimate		Mid-Range Estimate		High Estimate	
Any Resistance Caused by Any Drug							
Therapeutic	12	15		12		8	
Subtherapeutic							
Prophylaxis	60-66 (63)	80	85	63	88	42	92
Growth promotion	22-28 (25)	5		25		50	
	100	100		100		100	
Estimated Percentage of Penicillin/Ampicillin Or Tetracycline Resistance Caused by Administration of Penicillin/Ampicillin or the Tetracyclines							
Therapeutic	10	14		10		6	
Subtherapeutic							
Prophylaxis	60	81	86	60	90	34	94
Growth promotion	30	5		30		60	
	100	100		100		100	

Source: Table prepared by the committee.

any drug given for growth promotion of 5% and the high estimate of 50%. These estimates were chosen bearing in mindthat the most plausible figure for the actual tonnage (mid-range estimate) of drugs used for growth promotion was 25%. The remaining percentages of any drugs used therapeutically or prophylactically were partitioned with the same ratio as for the mid-range estimates--i.e., about 1:5--to fill out the data base for the low and high estimates.

The lower half of Table VII-6 presents the committee's estimates of the contribution of penicillin/ampicillin or the tetracyclines to the development of drug resistance when used in the feed of farm animals for one of the three majortreatment purposes. These were derived in an analogous manner. However, for these antibiotics the committee assumed that a higher percentage was used for growth promotion than shown for any drug in the upper half of Table VII-6. In particular, it was considered that the addition of the tetracyclines to swine feed was predominantly for the purpose of growth promotion; therefore, the mid-range estimate of 25% for the contribution of drugs given for growth promotion was raised to 30% for strains resistant to penicillin/ampicillin or the tetracyclines. The other two values in the mid-range estimate for penicillin/ampicillin or the tetracyclines were reduced as follows: for prophylaxis, 63% to 60%; and for therapy, 12% to 10%. The ratio of therapeutic to prophylactic use thus became 1:6, which corresponds to the proportional amounts given to animals for these purposes. Thus, by committee consensus, 5% was chosen for the low estimate and 60% for the high estimate of the proportional use of antibiotics for growth promotion. These estimates are somewhat higher than those for resistance to any drug in light of the extensive use of the tetracyclines for growth promotion. The remaining estimates for therapeutic and prophylactic use were partitioned in a ratio of about 1:6.

Thus, all estimates have been adjusted slightly so that the total for each of the three types of use in each column is 100%. This assumes that essentially all the antibiotic resistance found in salmonellae encountered on the farm is related to the amount of antimicrobial drug in the aggregate, used for each of the three major types of application in the aggregate.

PREVENTABLE CASES OF SALMONELLOSIS: "ETIOLOGIC FRACTION"

The approach used in the risk model has been to estimate the number of persons who die each year as a result of infection with drug-resistant strains of salmonellae that originated on the farm (i.e., salmonellae isolates from meat or animal food products, eggs, or milk) and for which the drug resistance was selected by the subtherapeutic doses of

antibiotic drug in animal feed. However, at the beginning of this section the committee acknowledged that it cannot estimate the total number of cases of salmonellosis, the profile of drug susceptibilities, or the source of the bacterial strain that would likely occur in the United States if subtherapeutic doses of any antibiotics, or of penicillin/ampicillin or the tetracyclines, were not administered to farm animals.

It might be argued that, if all subtherapeutic use of penicillin/ampicillin or the tetracyclines were stopped, deaths due to infection by strains of drug-resistant salmonellae would be replaced by a like number of deaths caused by drug-susceptible salmonellae. However, there are at least three ways in which drug resistance itself might contribute to a higher total number of deaths from salmonella infection: (a) by leading to a "wrong" choice of drug for treatment (presumably an uncommon event), (b) by being intrinsically more virulent and hence lethal (a possibility for which there is some evidence, but which might in turn relate to wrong choice of drug or to etiologic fraction), or (c) by causing some infections that would not have occurred but for the resistance of the infecting bacterial strain to the antimicrobials administered. Infections caused in the third way have been called the "etiologic fraction," and the resulting cases of illness are termed "excess cases." The concept of the "etiologic fraction" arose from two observations: first, the ingestion by salmonella carriers of drugs to which the salmonellae were resistant in occasional instances appeared to provoke the development of clinical salmonellosis;[12,23] second, in one outbreak of salmonellosis, the association between ingestion of penicillin or other antimicrobials and the development of infection was so striking that it led to the initial suspicion that the drug was contaminated with salmonellae.[16] Subsequently, controlled studies have documented repeatedly that antimicrobial ingestion does enhance the likelihood of infection by drug-resistant salmonellae in epidemic situations. The hypothesis, for which supporting evidence exists in animals[5a,5b,20a,20b,20c] is that the antimicrobial drug suppresses the drug susceptible competing fecal flora, and enhances the opportunity for the pathogen to become implanted or, in carriers, to proliferate and cause disease. The effect is to lower the size of the inoculum needed to cause infection. For purposes of calculation, the etiologic fraction is determined by multiplying the relative strength of association between the recent ingestion of an antimicrobial agent and the likelihood of development of salmonellosis--i.e., the "odds ratio"--by the proportion of patients with that risk factor. In a recent review by Cohen and Tauxe[12] of various outbreaks, the proportion of cases in the "etiologic fraction" ranged from 16 to 64%. Whether

patients in the "etiologic fraction" are more susceptible to infection with salmonellae and whether they are more likely to die of this infection is not known, i.e., the relative death rates in the "etiologic fraction" are not known. As the proportion of bacterial strains that are drug-resistant increases, cases belonging to the "etiologic fraction" should constitute a larger and larger proportion of all infections. Table VII-7 summarizes quantitative evidence from six recent studies about the increased risk of drug-resistant salmonellosis among individuals taking antimicrobial agents.

As an illustration, the information from Adler et al.[1] is summarized in Table VII-8 where the figures in parentheses are obtained by difference from the four figures in the top line of Table VII-7. The odds ratio is then calculated from the cross products of the four "internal" cells of Table VII-8 as follows:

$$OR = (28x19) / (21x8) = 3.2$$

Other lines in Table VII-8 are to be interpreted similarly.

The odds ratios, which are close estimates of relative risks, are presented without statistical confidence limits, because confidence limits express only the uncertainty due to random error, and each of these sources is subject to considerable additional nonrandom uncertainty in any generalization to a broader population. For example, a study of a single strain (as in an outbreak) violates the basic assumption of statistical independence; controls drawn from patients on a pediatric ward may be both more vulnerable and more exposed to resistant strains than infants in general (or than the population as a whole); and household contacts of patients may tend to share patterns of antibiotic use with the patients. In addition, the sources of subjects were not always well characterized, antibiotic resistance was determined in different ways, and methods of ascertaining drug use varied. Nevertheless, the odds ratios considered in this analysis are thought to be applicable to patients who ingest penicillin/ampicillin or the tetracyclines and to salmonellae that are resistant to those drugs, in that they were derived primarily from studies in which those were the drugs involved in producing the "etiologic fraction."

Given these different kinds of uncertainty, the consistency in the elevation of odds ratios is impressive. However, because these odds ratios refer to different sorts of subjects and diseases (ill children, outbreaks vs. sporadic cases, etc.) and because one may expect such differences to affect relative risks, none of these figures alone is entirely suitable for estimating the odds ratio for the public at large or for estimating the part of the total national burden attributable to the overgrowth of resistant strains when the normal flora is suppressed by antibiotic

TABLE VII-7

SOME STUDIES OF THE FREQUENCY OF ANTIBACTERIAL THERAPY IN INDIVIDUALS INFECTED WITH ANTIMICROBIAL-RESISTANT SALMONELLA STRAINS

Senior Author	Total Subjects	Infected with Resistant Salmonellae	Recent Drug Use	Both Infected and Recent Drug Use	Odds Ratio
Adler 1970[1]	76 patients on a pediatric ward	36 multi-resistant (single strain)	49 took semisynthetic penicillin or ampicillin, time not stated	28	3.2
Riley 1984[24]	(a) 504 cases, geographically dispersed	66 resistant to 2 or >2 anti-microbials	43 took 1 or >1 antimicrobials within 4 weeks	13	3.3
	(b) 43 patients receiving antimicrobials	13 resistant strains*	25 took ampicillin, amoxicillin, or penicillin	12	15.7
Holmberg 1984[16]	(a) 21 patients with S. newport infection	10 resistant (single strain)	7 took amoxicillin or penicillin within 1 week	7	Infinite
	(b) Same 10 drug-resistant cases + 29 household contacts	Same	Same	7	Infinite
	(c) Same 10 drug-resistant cases + 27 non-S. newport	Same	Same plus ophaloridine	7	Infinite
Mac Donald 1987[20]	485 isolates, geographically dispersed	117 resistant to at least one antibiotic	63 took anti-microbials within 4 weeks	23	2.0
Spika 1987[29]	45 cases, 88 matched controls	45 epidemic multi-resistant S. newport	13 took penicillin or tetracycline within 1 month	11	13.9
Ryan 1987[25]	50 cases, 50 matched controls	50 epidemic multi-resistant S. typhimurium	Not stated	Not stated	5.5

Source: Adapted by the committee from Adler et al.,[1] Riley,[24] Holmberg,[17] MacDonald,[20] Spika,[29] and Ryan.[25]

* Not clear whether resistant to any antibiotic, to 2 or more antibiotics, or to penicillins (with or without other antibiotics).

TABLE VII-8

NUMBERS OF PATIENTS WITH RESISTANT SALMONELLA USING ANTIMICROBIALS

Resistant Salmonella	Recent Drug Use		All Subjects
	Yes	No	
Yes	28	(8)	36
No	(21)	(19)	(40)
Totals	49	(27)	76

Source: Adapted from Adler et al.[1]

use. Those studies that come closest to ideal for estimating a population-wide odds ratio are those of Holmberg et al.,[16,18] Spika et al.,[29] and Ryan et al.[25] The three estimates are: infinite (based on a small sample), 13.9, and 5.5. The committee is inclined to believe that the true population-wide odds ratio, for cases similar to those regularly reported to CDC, for oral intake of any antimicrobial in common use among humans and for salmonellae resistant to that drug (and perhaps others), may be about 5 and is probably between 2 and 20. Clearly, these estimatesare uncertain, and additional research is needed to improve them.

A population-wide estimate of the proportion of salmonella infections or deaths (the "etiologic fraction") can be derived from an odds ratio combined with an estimate of the proportion of the population taking antibiotics as:

$$EF = (OR-1)\ P/\ [1+ (OR-1)\ P],$$

where OR is the odds ratio and P is the proportion exposed to the risk factor (here, antibiotic use). The definition of "taking antibiotics" must be close to that used in estimating the odds ratio, so the committee reviewed the studies in Table VII-7 to see whether any could be used for this purpose. There are uncertainties in each of the studies, but the closest seem to be those of Holmberg (none of 29 household contacts had taken antibiotics within one week, giving a rate of use of 0.0%) and Spika (2 of 88 matched controls had taken antibiotics within one month, or 2.3%). Clearly, the population-wide figure is larger than Holmberg's 0.0%, and it may be close to Spika's figure, scaled down from

2.3% for use in the past month to, perhaps, 0.5% per week. Thus, our best estimate here is 0.5% use within the past week, to which we attach a high estimate of 1% and a low estimate of 0.2%.

Using the low, mid-range, and high estimates of both OR and P, the committee produced nine estimates of the proportion of human salmonella infections due to this mechanism alone (the etiologic fraction):

	ODDS RATIO		
Antibiotic Use (Past Week)	2	5	20
0.2%	0.2%	0.8%	4%
0.5%	0.5%	2.0%	9%
1.0%	1.0%	4.0%	16%

Thus, the committee's best estimate is that 2% of salmonella infections of the sort reported to CDC are a direct result of the use of antibiotics by persons who harbor salmonellae resistant to those antibiotics. We think it unlikely that the high estimates of both OR and P hold or that both low estimates hold (the upper left and lower right corners of the table). The range of the estimates excluding those two possibilities is 0.5% to 9%. The committee fully recognizes the great uncertainty of the estimates here. Nevertheless, we believe that the estimates are worth presenting, partly because they embody our best judgment about the matter, and partly because they point in a clear manner to a need for additional research. The uncertainty is inherent in the limitations of available data, and other methods of analysis and presentation would simply hide the uncertainty, rather than reduce it.

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VIII

THE ESTIMATION OF RISK

RISK ASSESSMENT AND UNCERTAINTY

As noted in earlier chapters of this report, there is no direct evidence that subtherapeutic uses of antibiotics in animal feed create an excess risk of disease or death in humans consuming products from treated animals. This is not unexpected, at the present state of knowledge it would be unreasonable to expect direct evidence, even if the risk were relatively large. Attempts to collect such evidence are beset with serious methodologic difficulties; opportunities for collecting direct evidence on the magnitude of this risk are rare; and when they exist, are not likely to produce unambiguous results. There are questions about what agency has the mandate, funding, or manpower to obtain the information needed to evaluate the effectiveness of any regulatory action regarding the feed additives under consideration here. The cost of the data collection will certainly be high. There are unanswered questions about whether FDA now has the legal authority to demand the collection and submission of the types of data that would be required to show safety, in its broadest sense for human health.

The tools of risk assessment are typically applied in situations of this type where there is a need to acquire some sense of the probable size of a potential public health problem, some relevant data are available, but no means exists to obtain a direct measure of risk.[1]

Because risk estimates produced by such means are based on assumptions and limited data, they should be interpreted and used with caution. Such estimates are best seen as scientific hypotheses about the possible extent of a problem. This does not mean they are "hypothetical" in the weak sense that they are based on speculation. Rather, they are hypotheses that are consistent with all available information and scientific understanding, but that have not been verified by traditional scientific methods. All the estimates presented in this report should be viewed in this perspective.

An essential part of any risk assessment is the characterization of the associated uncertainties. In most cases, including the present one, risks are presented as numerical estimates (for example, as deaths per year) or as

ranges. We caution that such numerical estimates are incomplete descriptions of risk and should not be used without citation of the associated uncertainties, many of which can not be expressed quantitatively.

METHOD OF RISK CALCULATION

Chapter VII explained the risk model, provided a view of the uncertainties associated with each parameters used by the committee and presented the committee's low, mid-range, and high estimates of these parameters required by the model. The estimates of risk presented in this chapter are limited to infections with antibiotic-resistant strains (due to subtherapeutic uses of antibiotics in animals) of Salmonella (other infectious bacteria have been mentioned, but risks are not calculated for them) and further limited to annual numbers of deaths from these infections (risk due to morbidity and from non-lethal cases were not calculated).

Annual numbers of deaths are estimated by a straightforward multiplication of combinations of parameter estimates, as illustrated for the mid-range estimates alone at the opening of Chapter VII. The committee could not select a single "best estimate" of any of the parameters for use in describing risk. All possible combinations of the parameters were thus used to produce a range of possible risks. The various combinations selected for estimating different risks are described in the text to follow. In each case the complete set of possible risks for each combination was calculated, and the results were converted to percentiles and plotted on graphs as cumulative distributions (Figures VIII-1 through VIII-12).

The committee attaches some importance to the 5th, 50th (median) and 95th percentiles as descriptions of the whole set of estimates. However, the 5th and 95th percentiles are not to be interpreted as confidence limits, because they do not reflect any underlying probability distribution. A given percentile simply describes the fraction of risk estimates that fall below it. Thus, for example, if a specific risk is based on multiplication of five different parameters, and there are three different estimates of each parameter (low, mid, and high-range), then there are 243 different possible estimates of risk (3^5). The lowest five percent of these 243 estimates falls below the 5th percentile, etc. The committee believes that it is unlikely that all (or nearly all) of the low or high estimates hold simultaneously. Thus, combinations of parameters that are all (or nearly all) low, or high, are highly implausible. This is based, in part, on the improbability of being consistently wrong in the same direction, and in part on the committee's attempt to set the low and high estimates at the bounds of general plausibility.

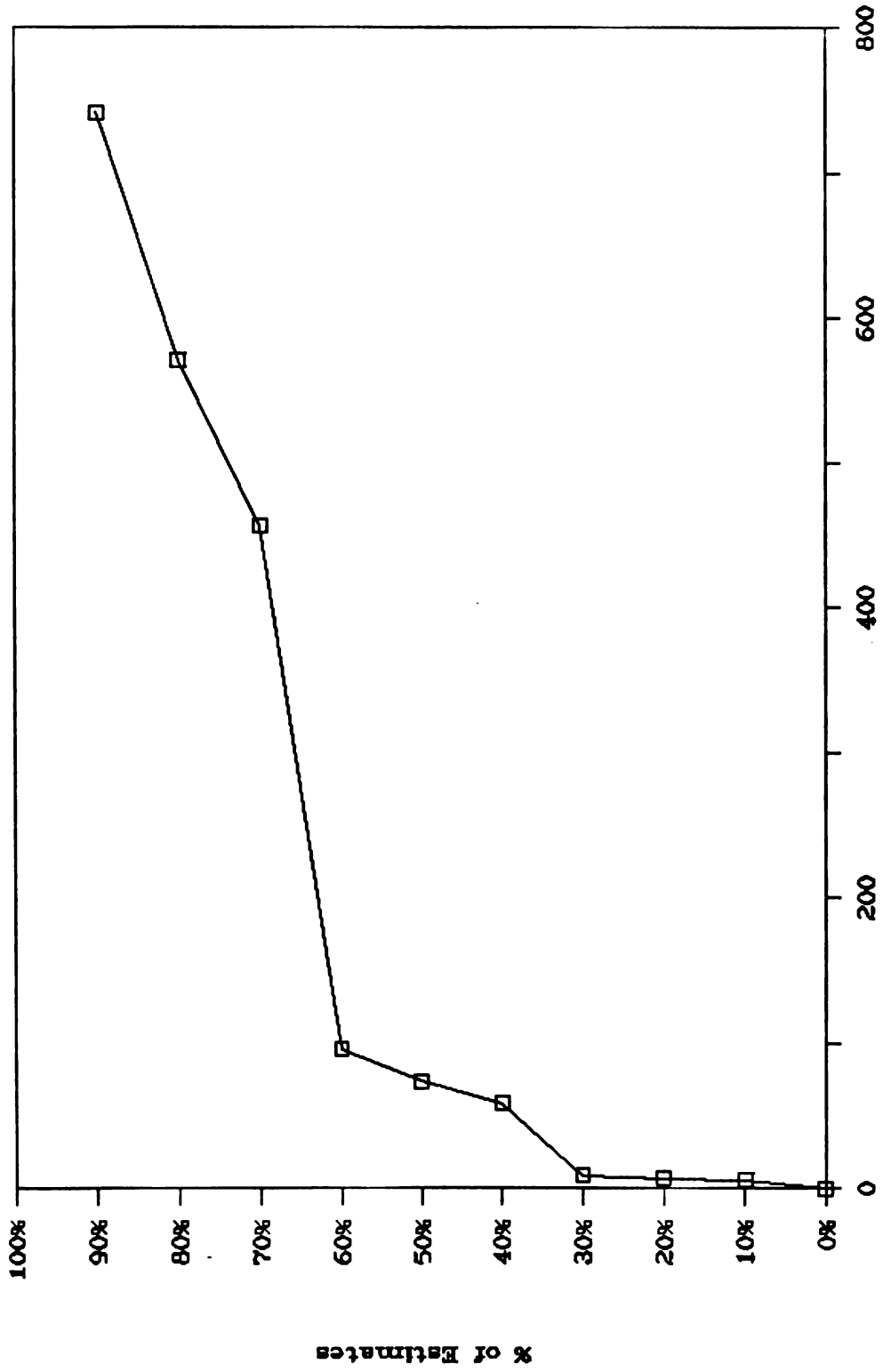


Figure VIII-1. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 2^a, 3^b, 4, 5^a of Table VIII-1).

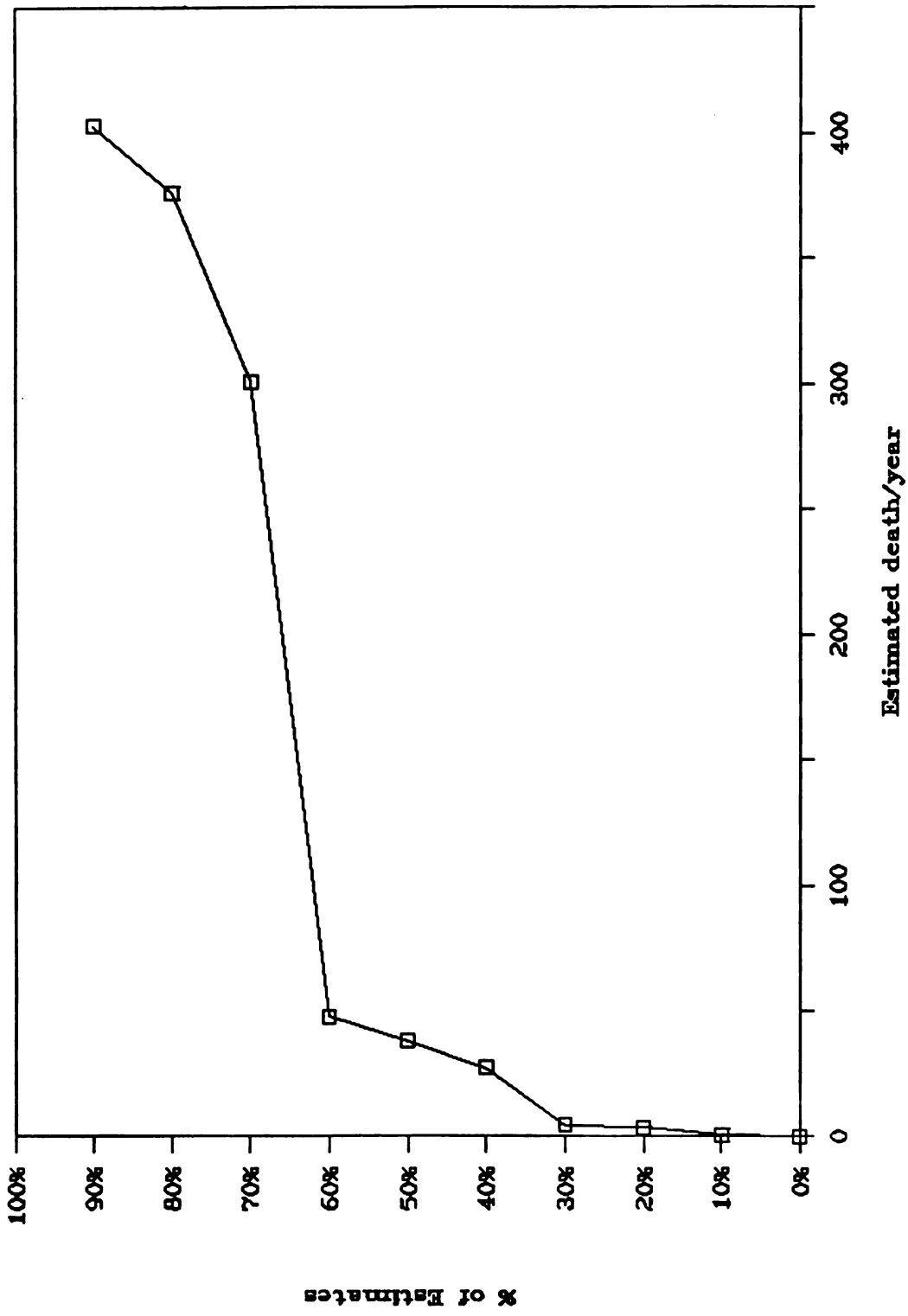


Figure VIII-2. Estimates of annual numbers of deaths from subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2[b], 3[c], 4, 6[a] of Table VIII-1).

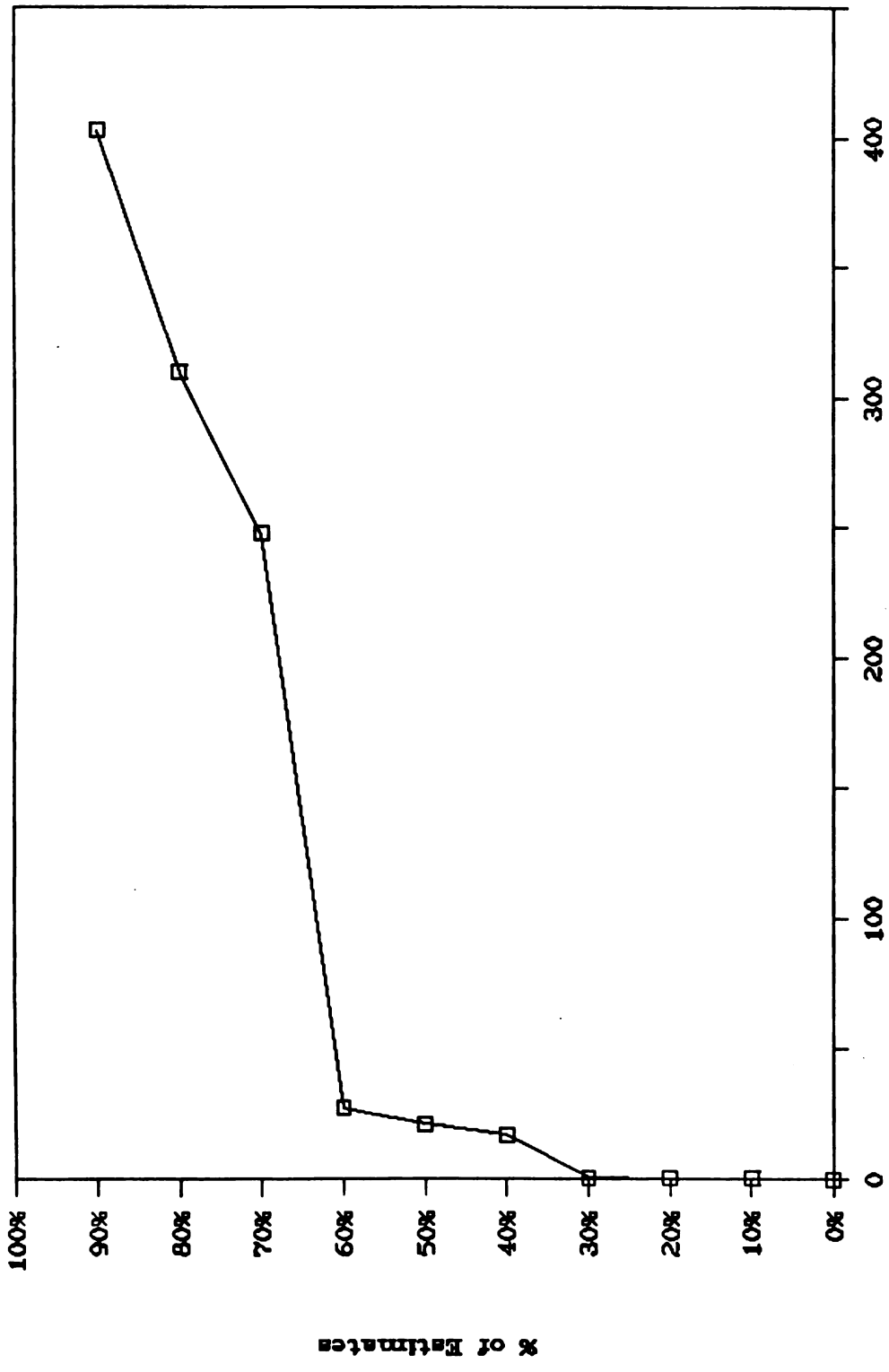


Figure VIII-3. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, 3^b, 4, 5^b of Table VIII-1).

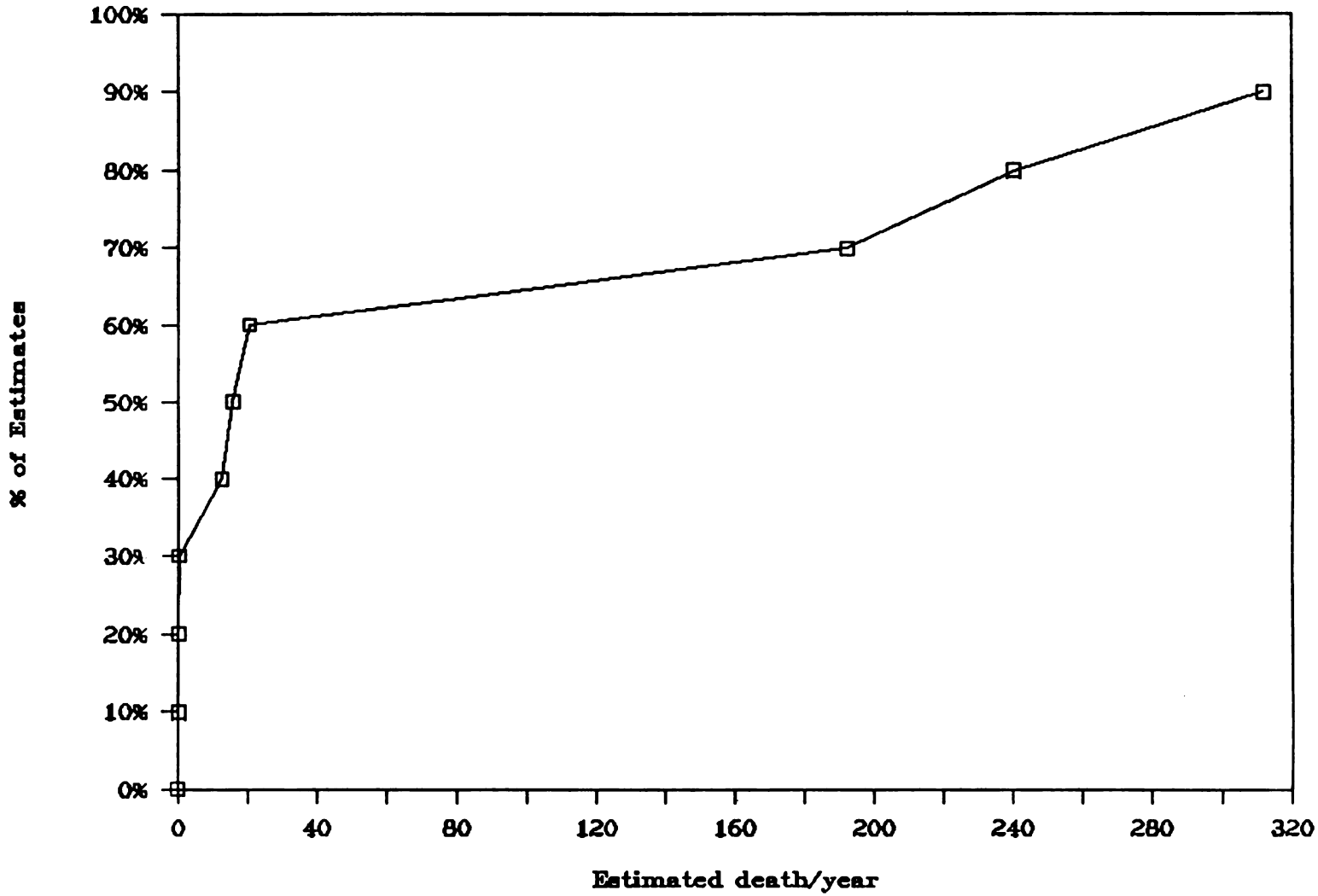


Figure VIII-4. Estimates of annual numbers of deaths from subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2[b], 3[c], 4, 6[b] of Table VIII-1).

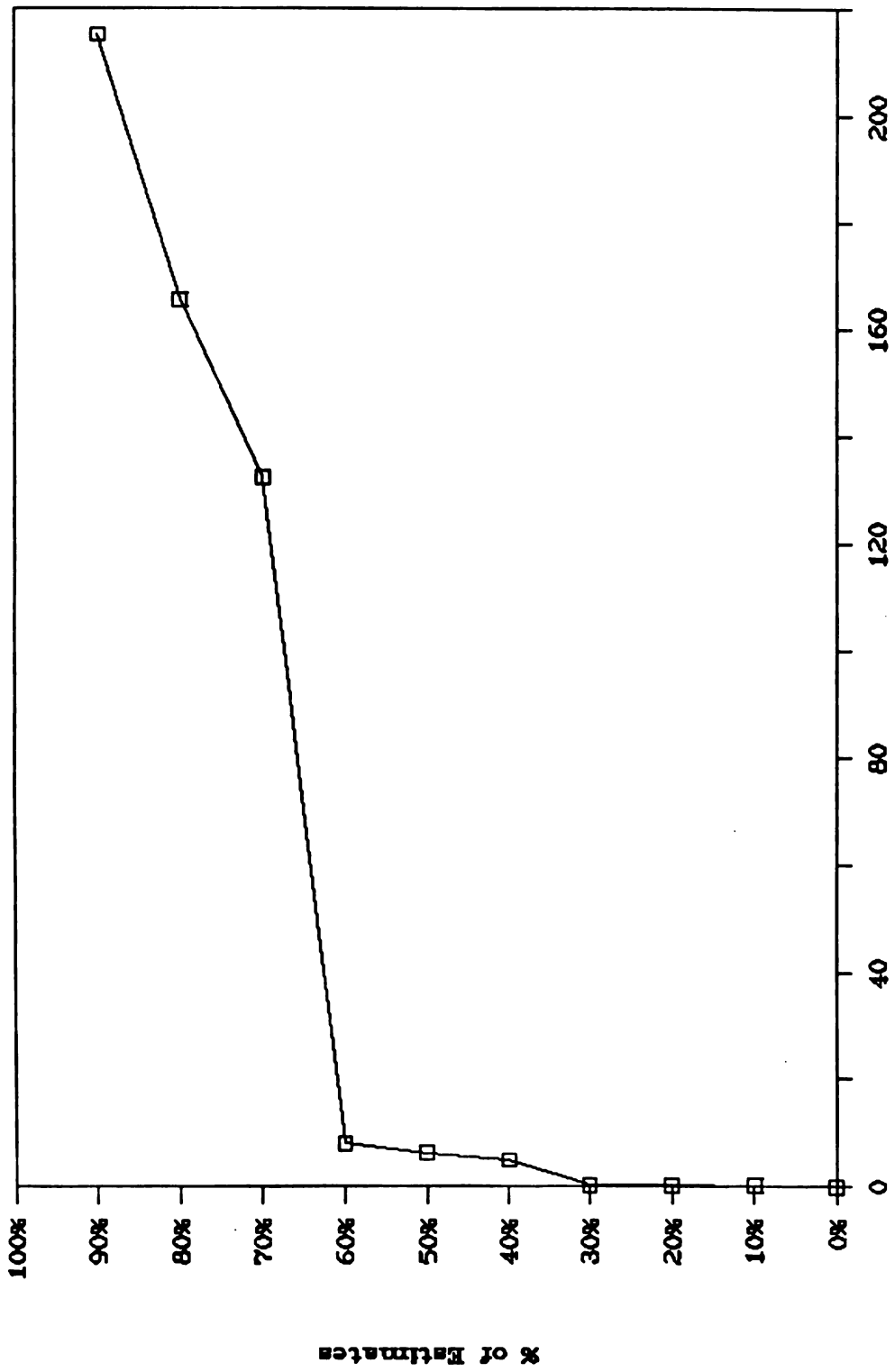


Figure VIII-5. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic use of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3[b], 4, 5[a] of Table VIII-1).

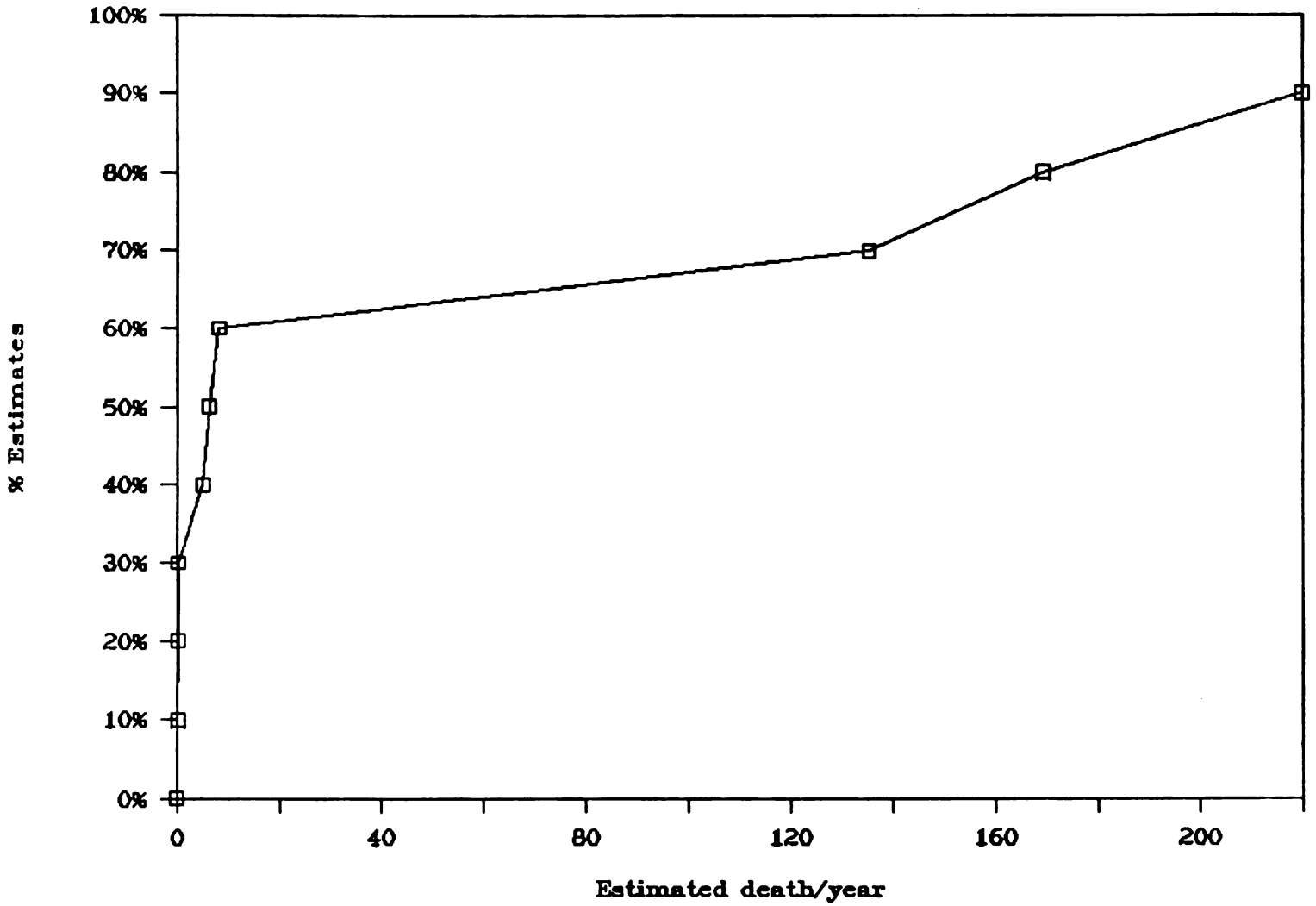


Figure VIII-6. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of penicillin /tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^{c}, 4, 6^{a} of Table VIII-1).

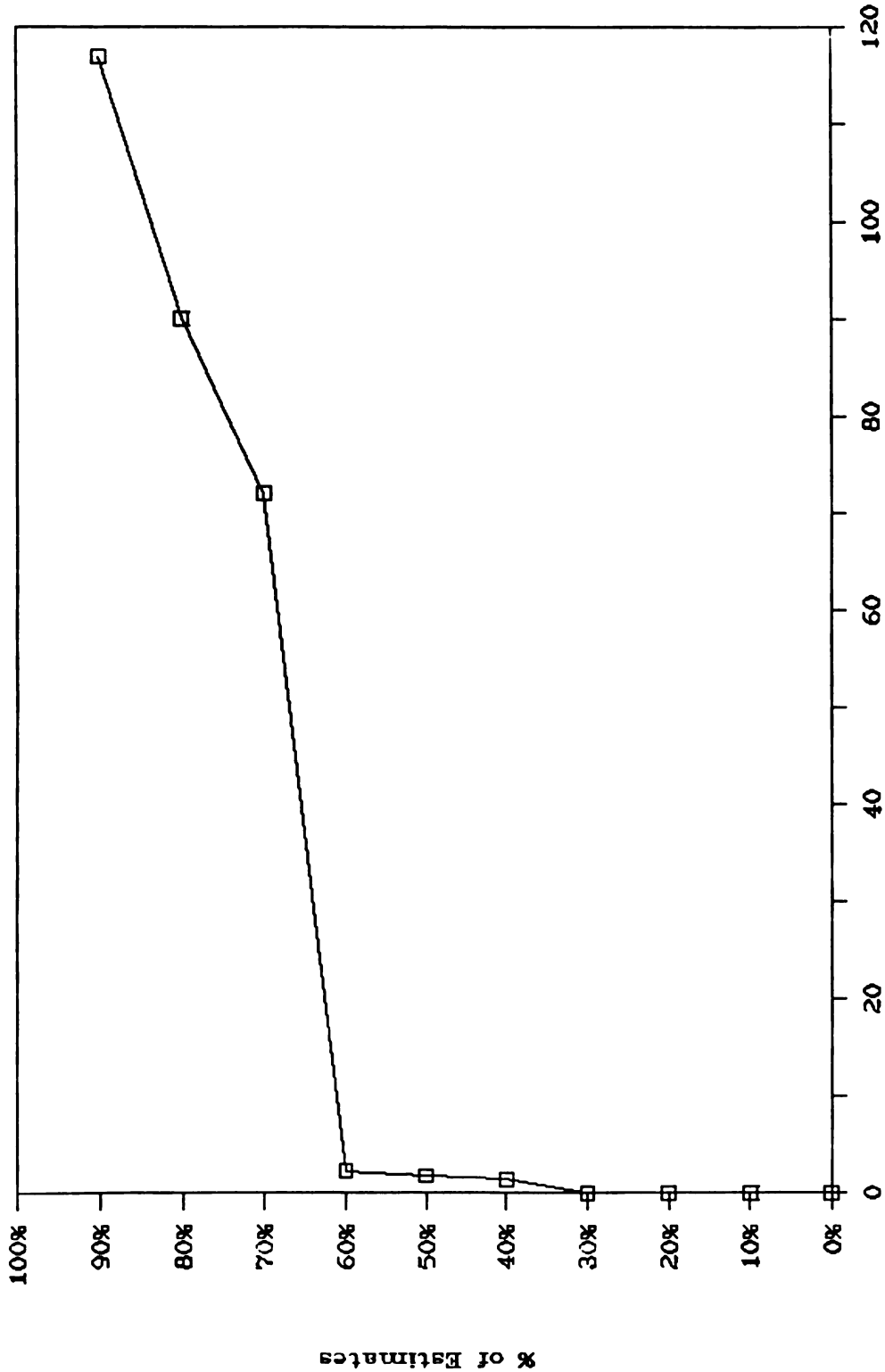


Figure VIII-7. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 7, 3[b], 4, 5[b] of Table VIII-1).

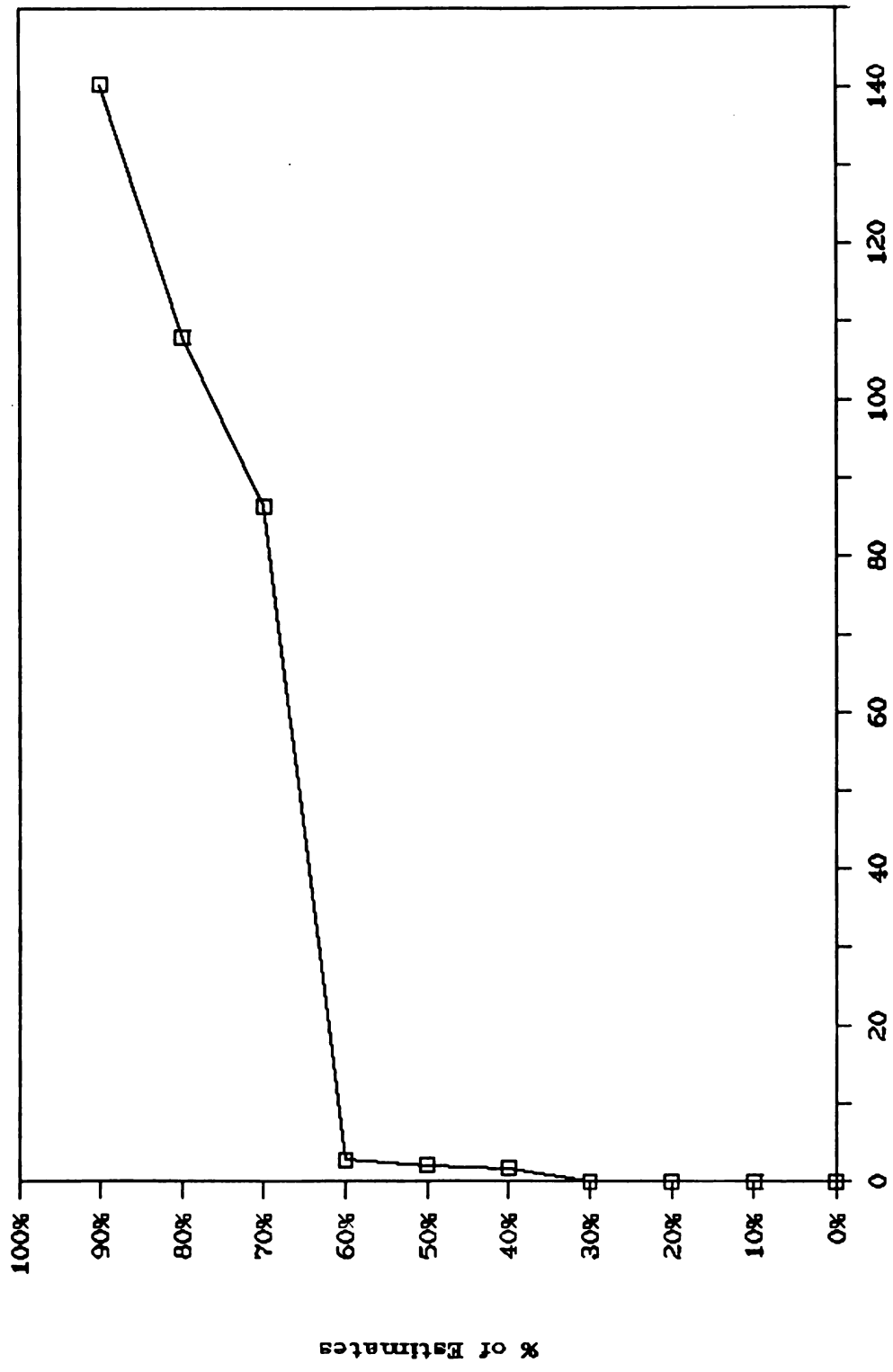


Figure VIII-8. Estimates of annual numbers of deaths in the etiologic fraction attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 7, 3[C], 4, 6[b] of Table VII-1).

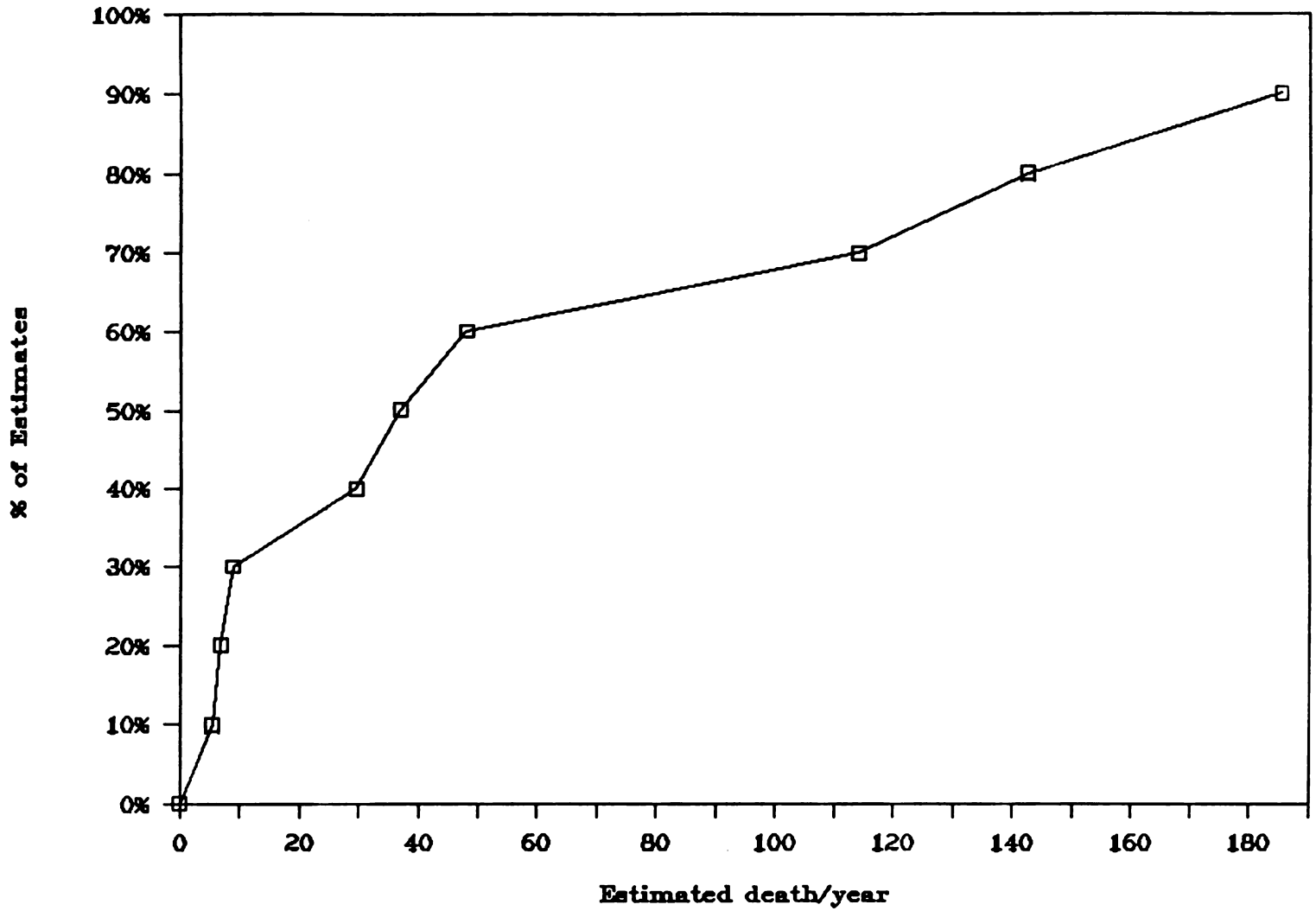


Figure VIII-9. Estimates of annual numbers of deaths arising because of higher death rate and increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of times 1, 2^{a}, (3^{b}-3^{a}), 4, 5^{a} of Table VIII-1).

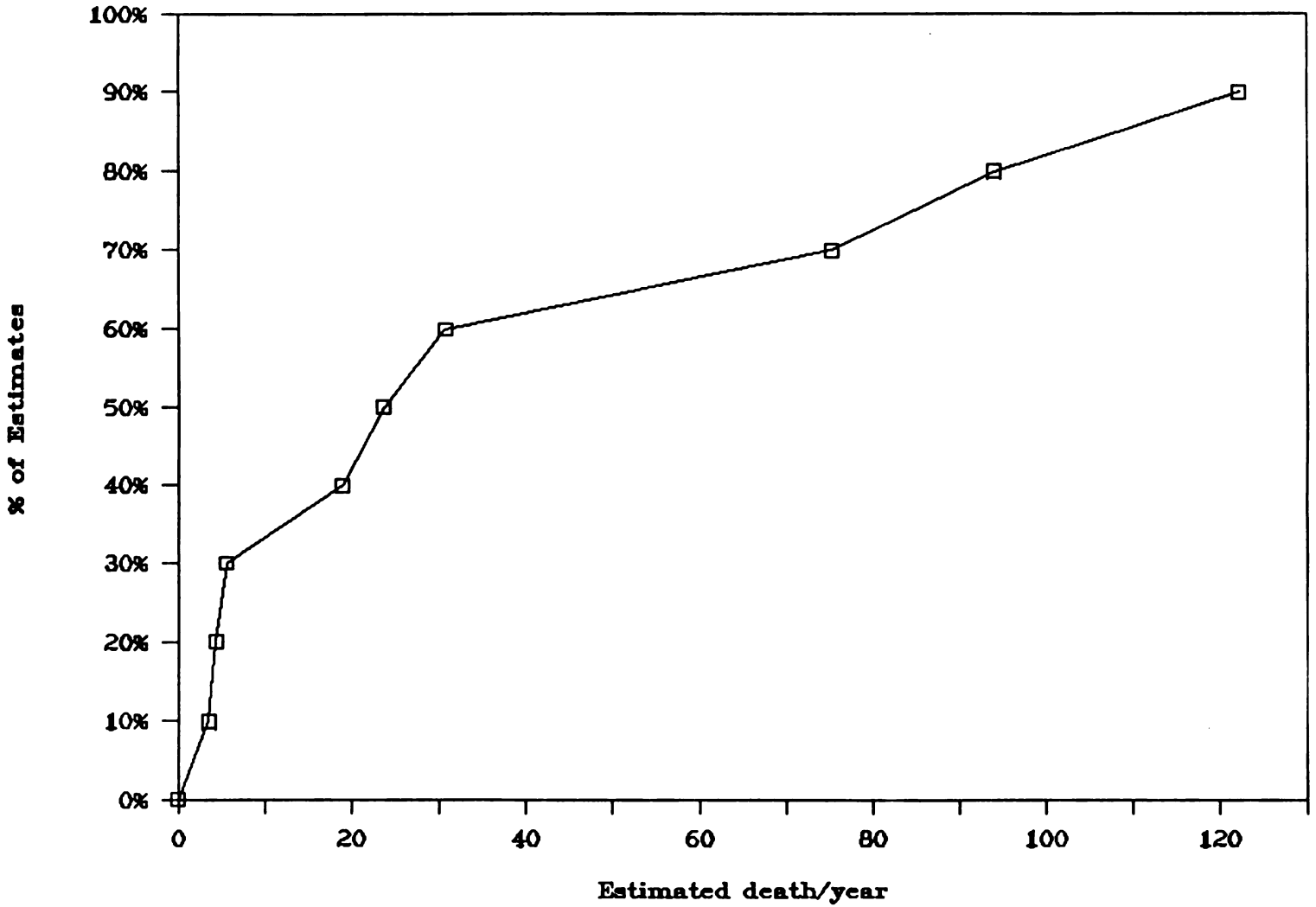


Figure VIII-10. Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^{b}, (3^{c}-3^{a}), 4, 6^{a} of Table VIII-1).

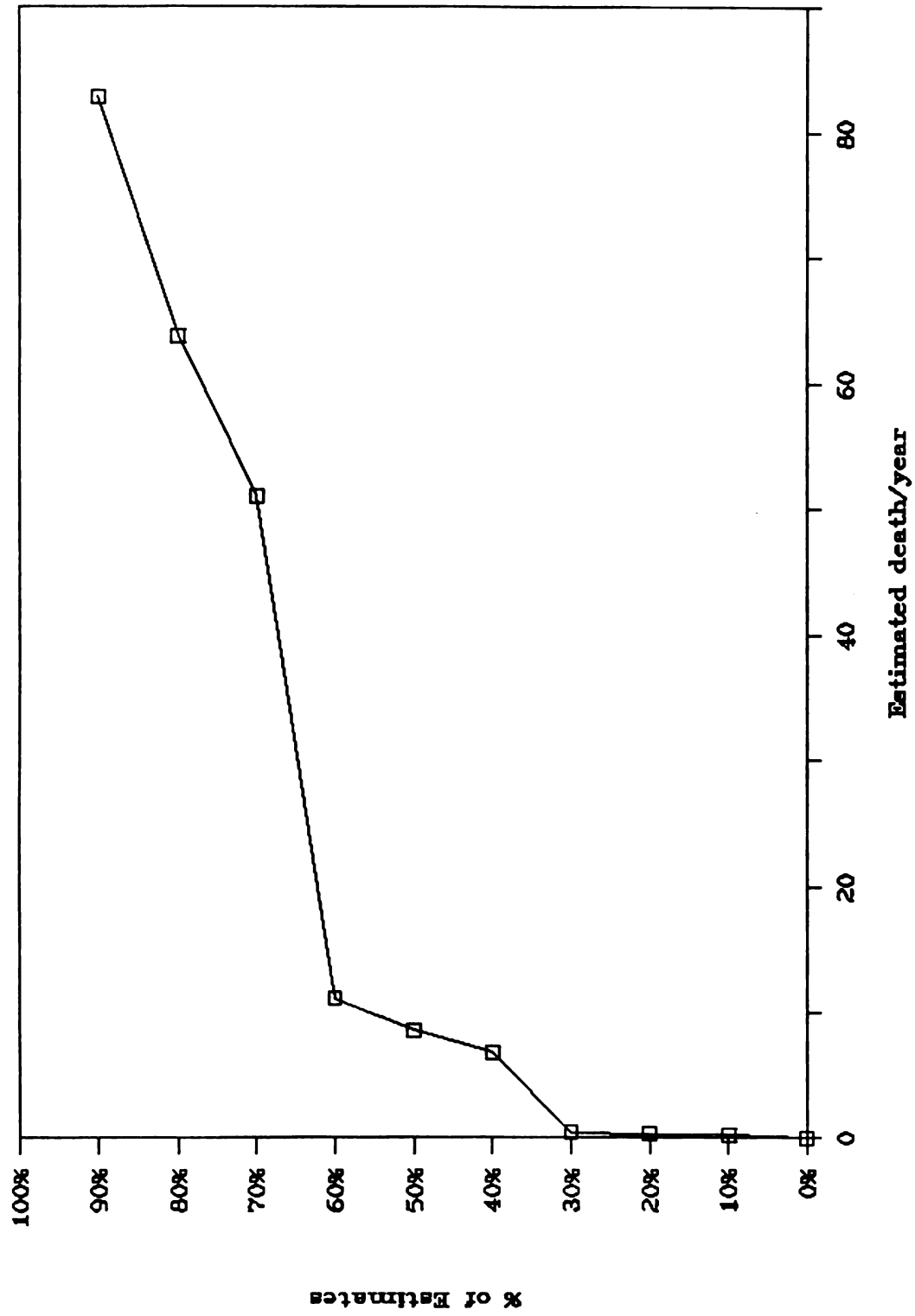


Figure VIII-11. Estimates of annual members of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, (3^b-3^a), 4, 5^b of Table VIII-1).

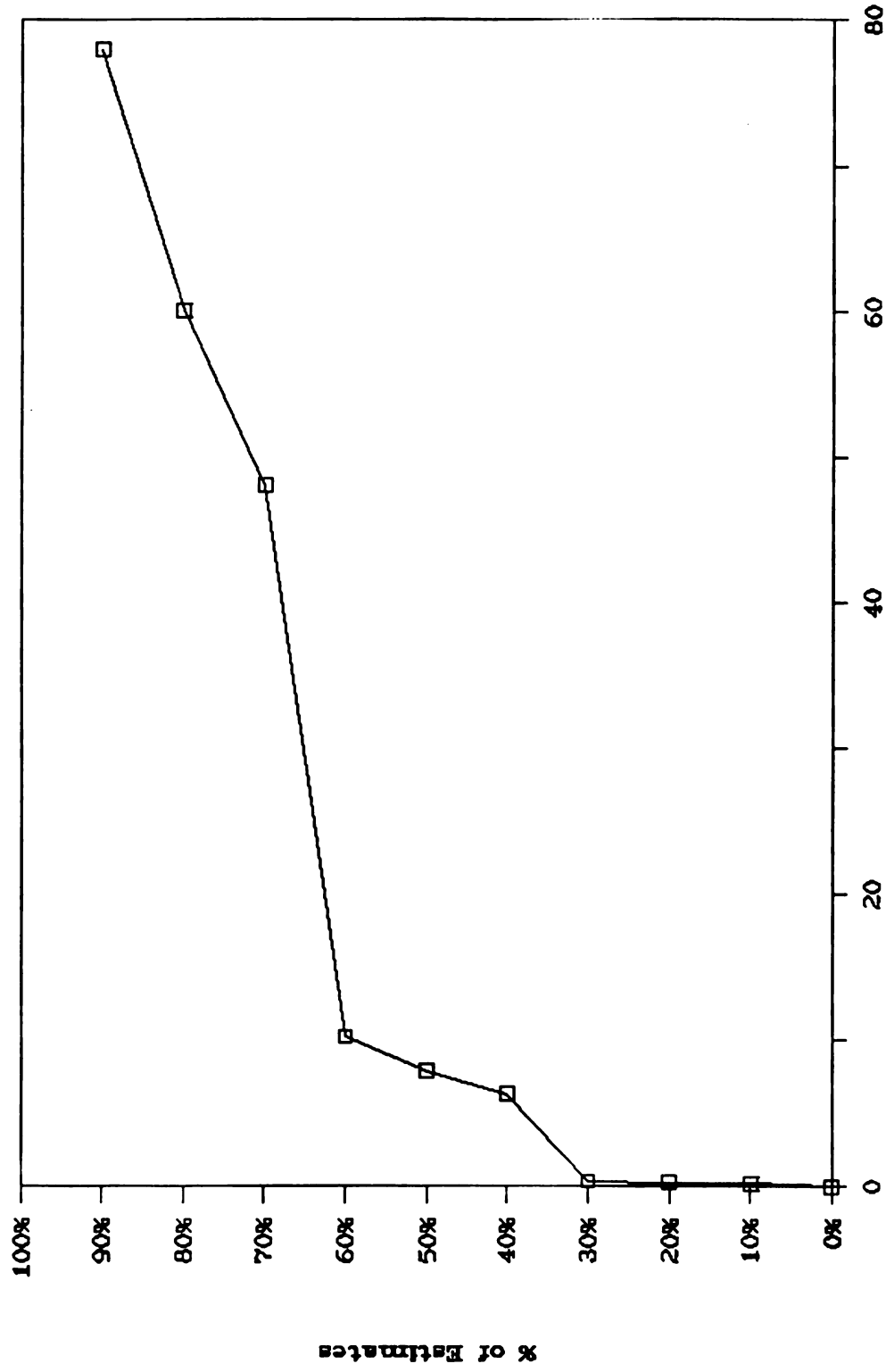


Figure VIII-12. Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^b, (3^{c-3^a}), 4, 6^b of Table VIII-1).

The committee thus tends to place greatest reliance on estimates near the median value.

ESTIMATION OF RISKS

Chapter VII, "The Risk Model", provides the data on which the Committee has based its estimates of human health risk that may be associated with the subtherapeutic use of antibiotics in animal feed. Table VIII-1 gives the parameter estimates used in assessing the whole of the problem of excess human salmonellosis deaths that might be attributable to any low-level farm use of antibiotics. These estimates are taken directly from the text and tables in Chapter VII. Various combination's of these parameters are used to estimate different types of risk. Twelve different sets of risk estimates were produced from these data; these estimates are presented graphically in Figures VIII-1 through VIII-12, and are summarized in Table VIII-2. The basis and meaning of the twelve different sets of estimates that are shown in Figures VIII-1 through VIII-12 are described in the following text.

MAXIMUM POSSIBLE NUMBERS OF EXCESS DEATHS

Figure VIII-1 shows the cumulative distribution of the 243 estimates of annual deaths generated for drug resistance of any type (Table VIII-1, multiplication of parameters from lines 1, 2^a, 3^b, 4, 5^a). Figure VIII-2 shows the corresponding distributions for resistance to penicillin and/or tetracycline specifically (Table VIII-1, multiplication of parameters from lines 1, 2^b, 3^c, 4, 6^a). Estimates presented in Figures VIII-1 and VIII-2 represent subtherapeutic use of antibiotics for both growth promotion and prophylaxis.

Figures VIII-3 and VIII-4 are similar to Figures VIII-1 and VIII-2 except that the estimates are for farm use of antibiotics for growth promotion only, rather than for all subtherapeutic uses on the farm. Figure VIII-3 describes risks for use of any antibiotic and is based on multiplication of lines 1, 2^a, 3^b, 4, and 5^b of Table VIII-1. Figure VIII-4 describes risks for use of penicillin and tetracycline only and is based multiplication of parameters from lines 1, 2^b, 3^c, 4, and 6^b in Table VIII-1.

Readers are cautioned that these are not necessarily "excess deaths" in the sense that the total number is increased by the quantity indicated; they are rather estimates of the annual numbers of deaths attributable to salmonellosis of the specified origin. These may, to some extent, overlap or replace deaths (in these patients or

TABLE VIII-1

DATA USED TO ESTIMATE ANNUAL DEATHS FROM SUBTHERAPEUTIC USES OF ANTIBIOTICS IN ANIMAL FEED

	Estimates		
	Low	Mid-Range	High
1) Reported Salmonella per year	40,000	50,000	65,000
2) Resistance of Salmonella to antimicrobials			
a) Resistance to any antibiotic	*0.16	0.24	0.31
b) Resistance to penicillin/ tetracycline	0.10	0.15	0.20
3) Death Rate -- infection by strains			
a) Not resistant to any antibiotic	0.002	0.005	0.01
**b) Resistant to any antibiotic	0.002	0.01	0.04
**c) Resistant to penicillin/ tetracycline	0.002	0.01	0.04
4) Fraction of those deaths associated with strains of farm origin	0.5	0.7	1.0
5) Fraction of antibiotic resistance of farm origin caused by subtherapeutic use of any antibiotic in animal feed			
a) Prophylaxis and growth promotion	0.85	0.88	0.92
b) Growth promotion only	0.05	0.25	0.50
6) Fraction of antibiotic resistance caused by subtherapeutic use of penicillin/tetracycline in animal feed			
a) Prophylaxis and growth promotion	0.86	0.90	0.94
b) Growth promotion only	0.05	0.30	0.60
7) Etiologic Fraction	0.005	0.02	0.09

Source: Table prepared by the committee. The bases for all estimates are provided in Chapter VII. Note: penicillin/ tetracycline = penicillin, ampicillin or tetracyclines.

* Decimal fraction or proportion.

** The committee could not find evidence sufficient to justify the use of different death rates for strains resistant to one drug rather than another, or for multiresistant strains vs. strains resistant to only one drug.

TABLE VIII-2

SUMMARY OF THE VARIOUS ESTIMATES OF ANNUAL NUMBERS OF DEATHS FROM SUBTHERAPEUTIC USES OF ANTIBIOTICS

Source of Estimates	Best Estimate[a]	Range[b] (median)
Figure VIII-1[c]	70	5-700
Figure VIII-2[d]	40	1-400
Figure VIII-3[e]	20	1-400
Figure VIII-4[f]	15	1-300
Figure VIII-5[g]	06	1-200
Figure VIII-6[h]	06	1-200
Figure VIII-7[i]	02	0-100
Figure VIII-8[j]	02	0-100
Figure VIII-9[k]	40	3-200
Figure VIII-10[l]	20	2-100
Figure VIII-11[m]	08	1-100
Figure VIII-12[n]	08	1-100

Source: Adapted by the committee from Table VIII-1 and Figures VIII-1 through VIII-12.

[a] 50% of estimates fall below this figure (rounded to one significant figure).

[b] 5% of estimates fall below lower end of the range; 95% of estimates fall below upper end.

Estimates of annual numbers of deaths:

[c] from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 2^a, 3^b, 4, 5^a of Table VIII-1).

[d] from subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^b, 3^c, 4, 6^a of Table VIII-1).

[e] from subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, 3^b, 4, 5^b of Table VIII-1).

[f] from subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^b, 3^c, 4, 6^b of Table VIII-1).

[g] in the etiologic fraction attributable to subtherapeutic use of any antibiotic for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^b, 4, 5^a of Table VIII-1).

[h] in the etiologic fraction attributable to subtherapeutic uses of penicillin /tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 7, 3^c, 4, 6^a of Table VIII-1).

[i] in the etiologic fraction attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 7, 3^b, 4, 5^b of Table VIII-1).

[j] in the etiologic fraction attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 7, 3^c, 4, 6^b of Table VIII-1).

[k] arising because of higher death rate and increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion (multiplication of times 1, 2^a, $(3^b\text{-}3^a)$, 4, 5^a of Table VIII-1).

[l] arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for both prophylaxis and growth promotion (multiplication of lines 1, 2^b, $(3^c\text{-}3^a)$, 4, 6^a of Table VIII-1).

[m] arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of any antibiotic for growth promotion only (multiplication of lines 1, 2^a, $(3^b\text{-}3^a)$, 4, 5^b of Table VIII-1).

[n] Estimates of annual numbers of deaths arising because of increased difficulty of disease treatment attributable to subtherapeutic uses of penicillin/tetracycline for growth promotion only (multiplication of lines 1, 2^b, $(3^c\text{-}3^a)$, 4, 6^b of Table VIII-1).

others) from salmonellosis that would have occurred anyway with some other strain. Conversely, it is possible that these estimates underestimate the real number of excess deaths if, for example, resistant strains tend to be more virulent than drug-susceptible strains, or if the estimates in successive lines of Table VIII-1 are not independent, (see Chapter VII regarding independence).

ESTIMATES OF DEATH BASED ON ETIOLOGIC FRACTION

As explained above, the estimates for deaths from all Salmonella strains with drug resistance attributable to low-level farm uses of antibiotics are not necessarily estimates of the excess number of salmonellosis deaths from such use. A fraction of the excess can, however, be estimated--the "etiologic fraction" discussed in this section and the death of farm origin "harder-to-treat fraction discussion in the following section. These two fractions may overlap (e.g., figures for the etiologic fraction may reflect some increase in the difficulty in providing effective treatment) and, further, these two fractions do not necessarily account for the whole effect of farm use of subtherapeutic levels of antibiotics (e.g. there may be a difference in virulence).

Estimates for deaths attributable to the etiologic fraction--that is, cases of salmonellosis that would simply not have occurred in the absence of resistance--require some modification in approach. Parameter estimates are given in Table VIII-1. The odds ratios in Table VII-7 are calculated for the whole population of exposed persons; of these, some proportion harbor resistant strains. The estimated odds ratios would be larger--perhaps substantially larger--if they were calculated to express the risk in persons who harbor such resistant strains. Use of the odds ratios in Table VII-7, therefore, already incorporate a reducation factor to express the risk in the population as a whole. Furthermore, this automatically reflects the actual proportion of persons who have resistant strains (perhaps in addition to susceptible strains) and does not depend on the kind of estimate in line 2 of Table VIII-1, which deals with proportions of strains rather than with the whole set of resistant strains that may inhabit one person. This approach ignores the likelihood that persons within families, within hospital wards, or otherwise in proximity may tend to carry the same strains of salmonellae, but no data on this seems to be available for use here. Because of the frequency distribution of resistant strains already incorporated into the odds ratios (line 7), no further adjustment for resistance (line 2) is needed or appropriate.

Perhaps future research studies can estimate odds ratios for the "etiologic fraction" of cases among persons who are hosts to one or more resistant strains. The odds ratios are likely to be substantially higher, but will be reduced by the (then appropriate) inclusion of such factors as those in line 2. Until this kind of additional information is available, we believe that our present analytic approach to the etiologic fraction is correct. In addition, the committee is concerned that death rates in the "etiologic fraction": (see line 3) may be above average, because some persons who receive antibiotics do so because of conditions related to immunosuppression, general debility, or other illnesses that may damage normal body defenses. In the absence of data, however, the committee has chosen to apply the death rates in line 3 of Table VIII-1 to the "etiologic fraction".

Two sets of estimates are presented for the "etiologic fraction" component of salmonellosis. Figure VIII-5 presents the 243 estimates for deaths in the etiologic fraction attributable to subtherapeutic farm use of any antibiotic for both prophylaxis and growth promotion (multiplication of parameter in lines 1, 7, 3^b, 4, 5^a, of Table VIII-1), and Figure VIII-6 presents similar estimates for penicillin/ampicillin and/or tetracyclines uses only (multiplication of parameters in lines 1, 7, 3^c, 4, and 5^b of Table VIII-1). Figures VIII-7 and VIII-8 present similar estimates for growth promotion alone; Figure VIII-7 concerns farm use of any antibiotic and Figure VIII-8 concerns uses of penicillin/ampicillin and/or tetracyclines only.

EXCESS DEATHS DUE TO INCREASED DIFFICULTY OF TREATMENT

While few or no strains of salmonellae are resistant to all clinically useful antimicrobials in the modern therapeutic armamentarium, some individual drugs are potentially toxic, have unwanted effects in particular groups of patients, may require parental administration, and some are very expensive. Further, critical time is required to determine patterns of resistance of bacterial isolates in specific infections. Thus, it would be medically inappropriate, to treat each suspected case of salmonellosis with the whole combination of antimicrobials that could conceivably be effective. More selective therapy is medically appropriate, but it has the unfortunate effect in some cases of delaying or replacing treatment by the optimum drug or drug combination, and as a result death rates may be higher in salmonellosis with resistant strains than with susceptible strains.

Whatever the reason(s), it has been commonly observed that infections with resistant strains of salmonellae more often end in death than infections with susceptible strains,

suggested by lines 3^a, 3^b, and 3^c of Table VIII-1. The difference between these lines can be interpreted as an index of the increased difficulty of providing effective therapy in cases of resistant salmonellosis. Because the estimates in lines 3^a, and 3^b and 3^c are so closely linked, the committee simply worked with the three differences (at low, mid-range, and high levels) rather than the 9 possible combinations.

Estimates of the size of this effect for all subtherapeutic uses of any antibiotic are presented in Figure VIII-9 (multiplication and parameters in lines 1, 2^a, (3^b-3^a), 4 and 5^a, Table VIII-1) and similarly in Figure VIII-10 for resistance to penicillin/ampicillin and/or tetracycline antibiotics (lines 1, 2^b (3^c-3^a), 4, 6^a).

Similar figures, but limited to drug use for growth promotion, are given in Figure VIII-11 (lines 1, 2^a,(3^b-3^a), 4, 5^b) for any drug resistance and Figure VIII-12 (lines 1, 2^b, (3^c-3^a),4, 6^b) for penicillin or tetracycline resistance.

SUMMARY OF NUMERIC RESULTS

Each of the figures in this chapter presents a range of risks, reported as annual numbers of deaths. This procedure was used because the committee had no basis for selecting any single "best" estimate. This procedure produces, for the data in each figure, a total of 243 estimates. The committee believes that the best single estimator is the median of the 243 estimates, and that the range from the 5th to 95th percentile is quite likely to contain the unknown true value. Because of the way these estimates were developed they do not provide ordinary statistical confidence limits (as explained above), but they should in practice provide even greater certainty than, say, 90% or 95% confidence limits. The committee believes that the minimum and maximum estimates presented in the figures are <u>not</u> scientifically plausible because they would require that the mid-range estimates for the parameters (Table VIII-1) all be consistently or nearly consistently wrong by a large margin and all be in the same direction.

Figures are presented to one decimal to emphasize that they are estimates, not counts. Data from the twelve figures are summarized in Table VIII-2. The following is an illustration of how the figures and data in Table VIII-1 are to be read:

<u>Figure VIII-1</u>. Estimates of annual numbers of deaths from subtherapeutic uses of any antibiotic for both prophylaxis and growth promotion. Figure VIII-1 is read as follows:

(i) Five percent of the estimates fall below 5 to 6 deaths per year, and 95% fall below 700 deaths per year. Thus, the committee believes that the true number is very likely to be between 5 and 700 deaths per year.

(ii) The likeliest estimate is 70 deaths per year. This is the committee's best single estimate for mortality in this category.

The estimates in Table VIII-2 are derived from Figures VIII-1 through VIII-12, and each range is based on different assumption's regarding uses (e.g., any antibiotic vs. penicillin/ampicillin and, or tetracyclines only, on prophylaxis and growth promotion uses vs. growth promotion only). The ranges also differ with regard to other assumptions (e.g., inclusion of "etiologic fraction", consideration of increased difficulty of treatment). The specific meaning of each set of estimates is indicated by the Figure headings, that are reproduce at the foot of this table.

INTERPRETATION OF RESULTS

The various estimates of risk presented in Table VIII-2 are based on somewhat different assumptions and have different meanings, as indicated in the foregoing text and as summarized in the Figure headings. For each set of estimates the committee places greatest reliance upon the 50th percentile figure, which has been termed the "likeliest estimate" in Table VIII-2. The range shown in Table VIII-2 almost certainly encompasses the true figures.

The committee is not able to assign a numerical probability to the likelihood that the estimates shown are correct. As noted earlier, none of these estimates has been verified by traditional scientific methodologies (i.e., experimental or well-controlled field studies), and thus should be interpreted as scientific hypotheses about the possible extent of the problem that are consistent with all available scientific information. The committee knows of no direct evidence to support these estimates. They should be considered as having scientific support roughly comparable to that available for estimates of low dose carcinogenic risk associated with chemical carcinogens subject to regulation.

The estimates of death presented in Table VIII-1 can be placed in the context of other types of risk estimates. FDA, for example, generally holds that, for carcinogenic drugs used in animals that leave toxic food residues, lifetime risks of cancer (presumed to be equivalent to lifetime risks of death), of around 10^{-6} or less are of insignificant public

health consequence. Using this yardstick, and assuming the entire population of the United States to be potentially exposed to such residues, the numbers of excess annual cancers (i.e., deaths), assuming the risk to be accurately known, can be estimated for one such drug as follows:

$$240 \times 10^6 \text{ persons} \times 10^{-6} = 240 \text{ lifetime deaths, or}$$
$$= 3\text{-}4 \text{ deaths per year.}$$

The total number of deaths due to carcinogenic residues depends on the number of such drugs in use.

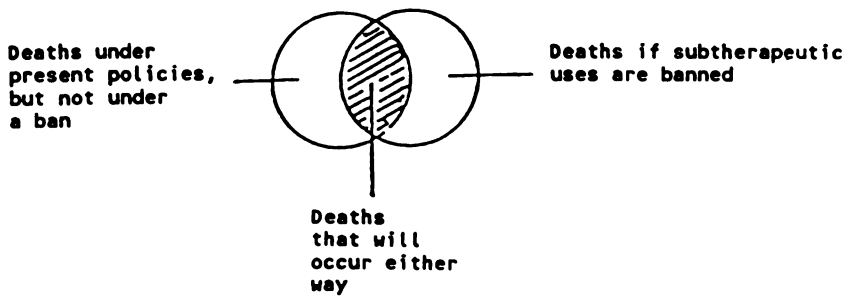
Actual numbers of deaths are probably much lower than these figures indicate, because actual residue levels rarely approach the maximum allowable and because it is unlikely that most of the population is exposed to these drugs on a continuing basis. Moreover, the risk estimation method used for carcinogens is designed to overstate risk. That is, the procedures used to estimate excess cancer risk include adoption of upper 95% confidence limits on the dose-response curve and several other assumptions about interspecies and high-to-low dose extrapolation that almost guarantee that the actual numbers of deaths will be less than those shown above. In fact, actual risk may be zero. The above figures are helpful nonetheless, because they reflect the hypothetical number of excess deaths that might be considered of negligible public health consequences.

The estimates of annual numbers of excess deaths presented in Table VIII-1 are derived by a method that is not strictly comparable to that used by FDA for carcinogenic drug residues, so care must be taken in comparing these two sources of risk. However, no better basis for comparison is known to the committee, and, with the appropriate qualifications, the drug-residue cancer risks, apparently considered acceptable by FDA, do provide a moderately useful yardstick against which the risks in Figure VIII-1 can be measured. Moreover, the committee does not mean to suggest that the risks considered acceptable by FDA for carcinogenic animal drug residues are necessarily applicable to the determination of acceptability of the risks that are the subject of this report. Whether the risks presented in Table VIII-1 are to be considered acceptable or unacceptable depends on many factors that fall outside the scope of the committee's charge. Such determinations of acceptability are risk-management decisions and thus are properly left to FDA.

EFFECTS OF DISCONTINUATION

Will the number of deaths from salmonellosis and its complications be reduced or otherwise altered by the discontinuation of the use of the subtherapeutic doses of antibiotics in farm animals, or by discontinuation of use specifically for growth promotion? The committee, in the discussion that follow below, is inclined to think that the total number of deaths due to salmonellosis would decline. However, these matters are not at present subject to scientific proof.

The committee did not deal with the conversion of drug-susceptible bacterial organisms to drug-resistant clones (by plasmid transfer) in situ, but with the reduction in numbers of bacteria of drug-susceptible strains and the subsequent overgrowth with the more drug-resistant strains to fill the vacated ecologic niche. In this context, it may be useful to consider a simple diagram with two circles, one with deaths at some future time if no discontinuation of antibiotics is instituted, and one if discontinuation has been put in place as follows:



A ban then, would remove deaths in the left-hand lunule in this figure and replace them with by deaths in the right-hand lunule. The committee might pose the question about whether this shift is worth making. The committee has not attempted any risk-management policy analysis (it was not part of the committee's mandate), but believes that the following comments are within its mandate. The left-hand lunule alone is approximated, for various facets of the problem, by Figures VIII-1 through VIII-6, and certain aspects of the net difference of the left-hand minus the right-hand lunule (the best benefits of discontinuation of antibiotics) should be approximated by Figures VIII-7 through VIII-12. The committee believes that overall there would be a net benefit in reduced mortality (thus, the right lunule might be smaller than the left), but this cannot be proved with mathematical certainty, nor can the size of the net benefit be estimated with precision.

Critical to consideration of a ban is the likelihood of a long-term reduction in the proportion of salmonella strains

with resistance to antibiotics. The genie is out of the bottle; will it return? Resistant strains appear to have no survival advantage in the absence of challenge by antibiotics (otherwise they would have driven out susceptible strains long before the modern era), but there is little evidence that they have a survival disadvantage either. Further, other uses of antibiotics will continue including therapy of infections in both humans and animals, and compliance with a ban on subtherapeutic uses might be incomplete. Thus, it may be that a ban would retard the increase in proportion of resistant strains, but not stop or reverse the increase.

REFERENCES

1. National Research Council, Committee on the Institutional Means for Assessment of Risks to Public Health. Risk Assessment in the Federal Government: Managing the Process. Washington, D.C.: National Academy Press, 1983.

IX

DISCUSSION

The use of tetracycline and penicillin in subtherapeutic concentrations in animal and poultry feeds has aroused concerns about the possibility of a risk to human health. There are good reasons for concern: the known properties of transferable resistance plasmids and transposons among bacteria, the powerful action of antimicrobial drugs in selecting for antimicrobial-resistant bacteria, and the high levels of antimicrobial resistance found in *E. coli* and salmonella isolates from farm animals and humans. In addition, it is now possible to detect clonally salmonella strains from various sources in the food production chain (from farm to consumer) and so establish linkages between isolates from humans and from farm animals (or animal food products). This report deals mainly with the magnitude of the human health hazard and with whether sufficient data are available to assess the risk.

There is no direct evidence to quantify the human health hazard from antibiotic-resistant pathogenic bacteria created by the use of subtherapeutic amounts of penicillin or the tetracyclines in animal feed. Using the available indirect evidence shows these antibiotics in subtherapeutic concentrations do present a hazard to human health and may contribute to a percentage (see Figure VII-2) of the approximately 500 deaths annually in the United States from salmonellosis. Although the focus in the analysis of risk has been only on deaths attributed to salmonellosis, there are the same concerns about risk due to *E. coli* (and other *Enterobacteriaceae*) and to other pathogens (both gram-negative and gram-positive) known to be drug resistant that might infect both animals and humans. Human exposure to enteric organisms (pathogens and commensals) of animal origin is extensive. In food-animal processing plants, the incidence of bacterial contamination has been reported as high as 34% for chickens, 74% for beef, and 84% for pork.[10] Figures reported for comparable *E. coli* contamination range from 73% for beef carcasses, 81% for chicken and 97% for pig carcasses. The *E. coli* contamination presumably is from fecal sources. In studies from Great Britain,[7,8] 38% of *E. coli* in calf feces were resistant to one or more antimicrobials, and other studies showed values of 49% for pigs and 83% for poultry. In the state of Washington in surveillance for enteric pathogens in a poultry processing

plant, 47% of poultry were contaminated with Campylobacter jejuni and 4.7% were contaminated with Salmonella species.[13] Contamination with Campylobacter spp. was found in 22% of poultry from retail sources and 3.5% with Salmonella spp. Contamination with C. jejuni was observed in 0.4% of beef samples, but no salmonella contamination was found. Salmonella contamination was 2.7% in pork products. In this study, 30% of the salmonella isolates from retail poultry were resistant to tetracycline. These findings show that E. coli, Salmonella spp., and C. jejuni commonly are found on meat and poultry products. Human ingestion of these bacteria might result from contamination of hands during food preparation or consumption of inadequately cooked animal food products. Colonization of the human intestine by antibiotic-resistant E. coli, in the absence of antibiotic use, has occurred following handling of commercially prepared chicken carcasses in the kitchen.

The ability of particular E. coli strains to colonize the intestinal tract both of humans and various species of animals depends on the presence of colonization factors and specific cell surface characteristics common to both, because many of the O-serotypes of E. coli found in poultry, pigs, and calves also have been found in humans.[7,8] Therefore, it is likely these E. coli are from a common pool.

It has been shown that E. coli strains from the alimentary tract of humans and chickens are identical by O, H, K serotyping, by antimicrobial resistance patterns, and by plasmid restriction endonuclease profiles.[7,8] Also, serotype identity among E. coli strains of one specific serotype (02:K1) have been identified commonly in human urinary tract infections and neonatal meningitis and in animal disease (bovine mastitis, and chicken septicemia).

Recently, a group of such strains of both human and animal origin was submitted to clonal analysis by comparison of outer membrane protein (OMP) patterns, lipopolysacehharide patterns, electrophoretic mobilities of enzymes, biotyping, etc.[1] Human isolates were found to fit into two clonal groups, poultry isolates belonging to one and bovine isolates to the other. Human isolates of one clonal group were distinguishable from poultry isolates of the same group by their plasmid content; human isolates of the second clonal group were distinguishable from bovine isolates of that group by a minor alteration in the OMP pattern and by their plasmid pattern. Whether these differences in plasmid pattern (or in the OMP pattern, in the case of bovine isolates) indicate that the populations of human and animal isolates are not overlapping, even though very similar, is unclear.

In view of the exchange of E. coli and Salmonella spp. that can occur between food animals and humans, movement of antimicrobial-resistance genes from the intestinal flora of animals to the flora of humans may occur by carriage of

plasmids and transposons. Such movement of antimicrobial-resistance genes may follow movement to and persistence in the human alimentary tract of the foodborne enteric bacteria or from subsequent conjugative transfer of the plasmid to a resident constituent of the human intestinal flora. Conjugative transfer of R plasmids can be detected in the human intestinal tract in the presence of an antimicrobial that allows an increase in the number of R^+ donor cells and other cells that have received the R plasmid.[2] However, such transfer might not occur commonly in the absence of antimicrobial selection[12] in humans, although it occurs quite efficiently in the rumen of sheep after 24 hours of starvation.[14]

The foregoing suggests that the populations of enteric bacteria of animal and human origin might be considered as a common pool of antimicrobial-resistance genes (transposons, R plasmids, and chromosomal genes) capable of being amplified through antimicrobial exposure and subsequent selection.

DISSEMINATION OF RESISTANCE GENES AND GENOMES

Use of each new antimicrobial agent introduced over the past half-century has caused the emergence and global dissemination of bacterial genes encoding resistance to the agent. Growing prevalence of genes that encode resistance to older agents has prompted development and use of new ones, which have caused succeeding rounds of emergence and spread of new resistance genes. Dissemination of a resistance gene incurs different kinds of costs as it proceeds. When the resistance is not recognized or when optimal medical skills, laboratory services, or newer antimicrobial agents are not promptly available, the health burden is treatment failure with prolonged morbidity or death. When optimal support is available, which is rare everywhere at first and seldom in poorer regions, costs shift towards the expense of the support and the costly new agents and the toxicity of some of the agents.

Recognition of the emergence and spread of resistance and of its costs initially raised fears that the activities encoded by the emerging and spreading resistance genes would exceed our ability to develop new agents. However, nearly all of the target sites in bacteria that are exploited by existing agents were exploited in the first-quarter century of the antimicrobial era. The finding of few new target sites in the second-quarter century suggested that these sites were an unreplenishable resource--one increasingly endangered by proliferating resistance genes that prevented intact antimicrobial molecules from reaching the target sites.

The fear that we would run out of effective antimicrobial agents altogether was greatly diminished in the past decade by the introduction of many new agents that evaded the effects of existing resistance gene products and reached and inhibited the old target sites. Within the past year, however, a number of new resistance genes have been detected in different parts of the world that inactivate many of the largest class of the newer agents, the third-generation cephalosporins.

It was recognized early that use of antimicrobial agents was the major force driving the emergence and dissemination of resistance genes, and that such use should therefore be reduced to its essential minimum. What was not clear earlier, however, was the interrelatedness of what might be called a global system of antimicrobial resistance and the consequent effect of use in one area upon resistance in another. New evidence for this has been developing from both molecular and epidemiological work.

The molecular studies show that resistance genes and the plasmids that carry them constitute intricate assemblages of multifunctional modular components with the size and complexity but not the packaging of viruses. For such a genome to have arisen de novo in a patient or his neighbors in an intensive care unit would be the equivalent of spontaneous generation. Each must have had a lengthy evolutionary history. Studies in molecular biology and molecular genetics are beginning to suggest some of that evolutionary history. Individual resistance genes evolve from ancestral genes, are moved to other genomes or transposons or by site-specific recombination, acquire promoters, become linked to genes under different selection, are transferred on conjugative plasmids to other strains occupying other niches in bacterial ecosystems, and are carried in bacteria to other bacterial habitats. Each such event in the evolution of a resistance gene or its plasmids may initiate a new stage in its dissemination by extending its range or persistence. And the chance of occurrence of each such event would be greatly enhanced by antimicrobial use, which amplifies at every step the prevalence of the gene and its genome and hence the chance that something new will happen to them. Besides showing that resistance genes and their plasmids must have extended lineages, the molecular work is now also beginning to trace some of those lineages. Genetic maps of the large transposon Tn 21 suggest that it carried mercury resistance as well as resistance to several of the early antimicrobials before being included in the first recognized resistance plasmids (in shigellosis in Japan in the late fifties). This transposon has subsequently turned up in plasmids in many parts of the world, including most of the varied plasmids that first brought gentamicin

resistance to German medical centers and plasmids carrying a variety of different β-lactamase genes.

A corollary of the extended lineages of resistance genes and plasmids is that the resistance observed in the bacterial populations of one patient or one medical center is a consequence of prior use of antimicrobials, not just there but in other bacterial populations as well, including others that may have been remote in time and place.

This growing understanding of the interrelatedness of the resistance observed in the world's interconnecting bacterial populations intensifies earlier concerns about antimicrobial agents as animal feed additives. Animals get nearly half of the antimicrobials used in the United States, and the pool of resistance genes and genomes in their flora may be much greater than that in humans. Bacteria of animal origin are not a remote and separate population, moreover, but enter most households continually on slaughtered animal carcasses. If use of antimicrobials in one bacterial population affects prevalence of resistance in other bacterial populations more than slightly, then antimicrobial additives in animal feeds would contribute significantly to resistance in human flora.

Epidemiologic observations in the last few years has added to these examples of specific resistance plasmids that are found in isolates of bacteria from both animals and humans in the United States, and has in some cases reconstructed a path of spread from animal to human. These examples have thus far been observed in isolates of Salmonella spp., thus their elaborate serotyping by a network of medical and veterinary laboratories made them peculiarly traceable and particularly suited at this stage to risk assessment modeling. It should be emphasized, however, that salmonellae are a small part of the aerobic flora in the gastrointestinal tract of animals, and an even smaller part of that of humans; and these salmonellae represent less than 1% of the bacteria against which therapy in humans is directed and in which resistance may be a problem. The concerns outlined above, although now best exemplified by salmonellae, apply to all bacteria that infect humans.

PAUCITY OF DIRECT EVIDENCE

There is little evidence directly linking subtherapeutic use of penicillin and tetracyclines in animal feeds to human infections with pathogenic bacteria. As summarized in Chapter V, there is good evidence from only two studies that non-salmonella enteric organisms in which antimicrobial resistance was induced by the subtherapeutic administration of antimicrobial agents might be spread from animals to humans.[5,6] Two other studies[4,10] failed to show that

multiple-drug-resistant strains of animal origin cause infection in humans exposed to these strains, but it is possible that the power of these studies to detect such infections was too low to make a negative result meaningful.

A number of studies have attempted to demonstrate that outbreaks of drug-resistant salmonellosis in humans may be attributed to the administration of antimicrobial agents in subtherapeutic dosages to farm animals; however, in all but one instance there has been some defect in the proof of the chain of transmission (see Chapter V). A convincing case was an outbreak of infection due to chloramphenicol-resistant S. newport in which infections in humans were attributed to the ingestion of ground beef from animals medicated with chloramphenicol (a drug not approved by regulation for use in beef animals);[15] evidence for identity of the strains through the chain of transmission was based both on the unusual pattern of antimicrobial resistance and on plasmid analysis.

In summary, whereas the theoretical basis for concern that the subtherapeutic administration of antimicrobial agents to animals may foster infections by drug-resistant pathogens in humans is immense, the direct evidence of such an effect is sparse and generally indirect. There are many possible reasons why such an effect might be difficult to detect:

- The degree to which antimicrobial agents are administered for subtherapeutic purposes is generally unknown, varies from farm to farm and from time to time, and is not generally monitored; likewise, the proportion of drugs given for subtherapeutic, as opposed to therapeutic, purposes is not well defined.

- The relative influence of subtherapeutic use (growth-promotional and prophylactic administration) and therapeutic use on the prevalence of drug-resistant strains is not known with certainty and may differ among drugs.

- The prevalence of salmonellae, both susceptible and resistant, in various food products is not routinely monitored, except in special circumstances, such as outbreaks; even in special circumstances, it is generally assessed only retrospectively, when the situation may be quite different from that at the start of the outbreak (when samples of suspect food products are not available).

- Most cases of salmonellosis are unidentified, and in only rare outbreaks is an effort made to identify the source of the infecting organism; no effort to identify a source is made in sporadic cases.

o There are many sporadic cases of salmonellosis which create considerable "background noise" for the investigator attempting to define the chain of transmission of a specific salmonella strain; precise, rapid, and efficient molecular techniques for the identification of unique isolates (i.e., to prove the clonal identity of isolates) have only recently become available.

There is no direct evidence that establishes the proportion of human multiple-drug-resistant salmonellae that is of animal origin or the proportion due to person-to-person transmission. Only a small proportion of multiple-drug-resistant salmonellae in humans occurs as part of a recognized outbreak or epidemic. When such outbreaks have been detected and investigated, CDC surveillance data have indicated that foods of animal origin are implicated in almost 70%.[3]

It is reasonable to speculate that sporadic cases of human salmonellosis caused by multiple drug-resistant salmonellae may occur as part of undetected outbreaks, and still others are undoubtedly of indirect animal origin, resulting from person-to-person spread. There is not an extensive body of data bearing on this issue. Some investigators believe that the number of cases of person-to-person spread of salmonellosis has been underestimated, and that contamination of food products by human carriers, as well as animal sources, of multiple-drug-resistant salmonellae must be considered in the estimates of cases. Some multiple-drug-resistant salmonellae, notably Salmonella wein, (uncommon in the U.S.) have no apparent animal source.

Nevertheless, there is a general parallelism between the prevalence of multiple-drug-resistant salmonellae in animals and in humans, and numerous investigators in the United States believe, therefore, that the majority of human multiple-drug-resistant salmonellae are, directly or indirectly, ultimately of animal origin.

It must be emphasized again, however, that food processing techniques are designed to prevent contamination and transmission of animal pathogens to humans via the food chain. In the majority of outbreaks of multiple-drug-resistant salmonellae in humans, it has been possible to demonstrate flaws or defects in food processing techniques that allowed the contamination with salmonellae. Such defects have nothing to do with whether the salmonellae are fully susceptible to antibiotics or are multiple-drug-resistant. Ultimately, therefore, the farm animal-to-human chain of transmission, of all salmonellae will be interrupted more reliably by careful attention to accepted techniques of food processing and preparation than by any other public measure that could be contemplated.

EFFECT OF DRUG RESISTANCE ON HUMANS

The committee has been asked whether drug resistance of salmonellae caused by subtherapeutic administration of antibiotics in feed causes an increase in the number of cases of salmonellosis in humans or complicates treatment of these cases. These questions are difficult to answer, although they are obviously fundamental to the assessment of risk. Drug-resistant Salmonella spp. infectious to both humans and animals could cause an increase in morbidity in humans in four ways:

o By increasing the overall prevalence of these pathogens (both resistant and susceptible strains) in animals or their food products, could increase the potential for exposure of humans to salmonellosis. Whether or not this increase occurs is unknown. The prevalence of resistant strains in animals might be increased by the subtherapeutic administration of antimicrobial agents and the subsequent suppression of the normal gut flora of the animals; that would be analogous to the "etiologic fraction" in humans. However, the prevalence of susceptible strains might be reduced concomitantly, with an overall effect that is difficult to predict.

o By increasing the virulence of drug-resistant pathogens (see Chapter III). It is unclear whether virulence is increased; some evidence suggests that virulence may be increased, other evidence, possibly less convincing, suggests that the opposite result may occur. To the extent that the epidemiologic behavior of other resistant species is a guide to the effect of the widespread use of antimicrobial agents on the prevalence of pathogens in the environment, it cannot be determined that such resistance will decrease the prevalence or virulence of the resistant species. Overall, the incidence of reported cases of salmonellosis in the United States has continued to rise, slowly but steadily, over recent decades concomitantly with evidence of increasing prevalence of drug resistance in the isolates. Whether or not the increase in reported cases of salmonellosis is related to the subtherapeutic use of antibiotics in animal feed is not clear, of course; but certainly it cannot be said that the incidence of this infection in humans has been decreasing while the subtherapeutic administration of antimicrobials to animals has been steadily increasing. However, many other confounding factors make it difficult to determine the cause-and-effect relation between the subtherapeutic administration of antimicrobials to animals and the increasing number of cases of salmonellosis caused by both susceptible and resistant isolates. Among these

confounding factors is the increasing prevalence of "fast food" in the American diet; these foods are prepared often in large batches wherein a small amount of contaminating bacteria may have a magnified impact.

o By evoking the effect of the "etiologic fraction." Evidence clearly indicates that in some individuals infected with drug-resistant strains of salmonellae the infection is sustained because, prior to infection, they were ingesting antimicrobial agents to which the bacterial strains were resistant. It is believed that these individuals would not have been infected had the strains been susceptible.

o By interfering with the efficacy of antimicrobial treatment. There are some patients infected by drug-resistant strains of nontyphoidal salmonellae for whom antimicrobial treatment is ineffective because the pathogens are resistant. The committee believes that such instances are rare at the present time.

SUBTHERAPEUTIC VS. THERAPEUTIC DOSES

The presence of antimicrobial agents in the environment obviously causes the selection of microorganisms that are resistant to those agents. The clearest example of this phenomenon is seen in the *in vitro* determinations of antimicrobial resistance of isolates of bacteria, which are performed daily in clinical microbiology laboratories. With the rare exception of chromosomally mediated drug resistance, most such resistance is due to transferable resistance factors, or R plasmids. By definition, the minimal inhibitory concentration (MIC) of an antimicrobial agent for a given bacterium is the lowest concentration that inhibits completely the growth of the organism. At sub-MIC concentrations there can still be measurable, dose-dependent growth inhibition that is not complete.

In determining the relative effects on drug resistance of subtherapeutic vs. therapeutic concentrations of antimicrobial agents, several considerations must be evaluated: (1) how often and for how long does the concentration of the drug reach or exceed the MIC? (2) how quickly do the resistant organisms grow during this period? (That is related to evaluating the relative growth advantage of the resistant vs. the susceptible organisms.) (3) at concentrations of drugs below the MIC, is there a dose-related effect on the efficiency of R-plasmid transfer?

Virtually all work to date on drug resistance involves the study of clonally pure single strains of bacteria. Thus, the important issue of spread of drug resistance via R plasmids from resistant to susceptible strains, particularly

of different species, has not been fully explored at a range of drug concentrations that would shed light on the differential efficiency of drug-resistance selection.

It is nevertheless possible to speculate on the effects. Assume the simple case of two strains in the environment at equal inocula; one strain possesses a transferable R plasmid and is drug-resistant, the other strain lacks an R plasmid and is drug-susceptible, but can acquire drug resistance on acquisition of the R-plasmid by conjugative transfer. Consider the effects on the environment of several different concentrations of drugs. At high, super-MIC, drug levels, only the resistant strain survives. There is a net increase in drug resistance, as a consequence of continued growth of the resistant strain, but there is no spread of resistance. All resistance increase is from clonal expansion. At low enough drug levels (i.e., sub-MIC) there is no selective effect of the antibiotic. At MIC (bacteriostatic, but not bactericidal), there is selection and expansion not only of the original R-plasmid-containing strain, but also of the relatively rare R-plasmid conjugative recipient. Under this condition, the diversity of drug-resistant bacteria (i.e., two different strains), as well as the extent of resistance, is increasing.

Although these conditions have been neither adequately modeled for potential analysis nor appropriately tested in an *in vitro* situation that would reflect actual forces *in vivo*, the theoretical considerations raise concern that subtherapeutic concentrations of drugs may be doing as much harm as therapeutic concentrations, if not more, particularly in view of their more continuous use. Veterinary studies discussed above lend credence to this concern.

A complete chain of direct evidence linking human disease caused by multiple-drug-resistant organisms to subtherapeutic use of penicillin and the tetracyclines in animal feeds does not exist. Such evidence as does exist is limited to outbreaks of multiple-drug-resistant salmonellosis. Conclusive direct evidence of such a linkage would include full characterization of the infecting salmonellae based on available techniques of plasmid analysis; epidemiologic evidence implicating a particular food; isolation of the infecting organism from the implicated food and proof of its clonal identity; epidemiologic evidence linking the contaminated food with a particular farm service; isolation of the infecting organism from the implicated animals or poultry with proof of its clonal identity; and documentation of the subtherapeutic use of penicillins or tetracyclines in feeds consumed by the implicated animals or poultry. It might still be argued that multiple-drug-resistant salmonellae were present before subtherapeutic use of penicillins or tetracyclines was initiated, but most scientists would accept the outlined chain of epidemiologic

and microbiologic evidence as providing direct and conclusive proof of a cause-and-effect association.

In only one outbreak, reported by Spika et al.,[15] was this chain of transmission fully documented; the antibiotic used in this instance was not penicillin or tetracycline, but rather chloramphenicol. The use of chloramphenicol as a feed additive has never been approved by FDA in the United States, although in this instance it was used therapeutically. All other reported outbreaks that implicated multiple-drug-resistant salmonellae from an animal or farm source fall short in their provision of evidence that conclusively links the source of the drug-resistant organism with subtherapeutic use of antibiotics in animal feeds. Those reports did not document fully the chain of transmission, prove identity of the infectious salmonellae with those from the implicated farm source, and document the type or amount of antibiotic used in the animal feeds. The last has been particularly difficult to ascertain in most of the disease outbreaks, i.e., to establish retrospectively the precise antibiotics or the amount used in feed.

Thus, the studies of outbreaks of multiple-drug-resistant salmonellosis in humans, although they are the best evidence available, have not provided direct evidence of the human health risks due to subtherapeutic use of penicillin or the tetracyclines in animal feeds.

LOSS OF DRUG RESISTANCE

Upon cessation of drug use, there should be a measurable and continuous decline in the concentration of drug in the environment. At a point at which this concentration is significantly below the MIC of the susceptible strains, those strains should manifest a growth advantage over otherwise identical bacteria that in addition possess R plasmids. This advantage should be in direct relationship to the amount of diverted energy and raw materials the cell needs to keep the R plasmid on board (i.e., new DNA, RNA, and protein synthesis) and may be subtle. In sharp contrast to the drug-loaded environment, where the effect of the drug on the population of susceptible bacteria is seen within hours or days (because of the enormous growth advantage of the resistant bacteria), the effect of antimicrobial removal may take months or even years to be manifested fully. The more subtle the growth advantage of susceptible bacteria in the drug-free environment, the longer the period before the outgrowth of susceptible (i.e., R-plasmid-free) bacteria can be seen. For these reasons any analysis of the effects of drug removal from the environment must be extended past the immediate postwithdrawal period.

The prospective CDC studies of salmonellosis in selected urban and rural counties showed that the overall frequency of resistance to one or more antimicrobials had increased from 16% to 24% between 1979 and 1984.[3,9] However, in one serotype, S. heidelberg, the frequency of resistance declined from 67% to 35% during the 5-year period. Poultry was a common reservoir of S. heidelberg; from 1979 to 1983, CDC reported 69% of the nonhuman isolates of S. heidelberg from this source. By the late 1970s, most poultry producers had stopped using penicillin and tetracylines as growth-enhancers; for 1979-1982, only 4% of broiler-chicken producers were reported as using low doses of the tetracylines in feed. The decline of resistance in this salmonella serotype associated temporally with the decrease in use of penicillin and the tetracylines as growth-enhancers suggests that decreased antimicrobial resistance might follow reduced use of these drugs in subtherapeutic dosages. However, whether the use of penicillin and the tetracylines for disease prevention also decreased during 1979-1982 is unclear. The number of isolates of S. heidelberg studied was small; a much larger group of isolates should be examined to establish the validity of this interesting preliminary observation.

In practice, the indications for the subtherapeutic use of antimicrobials for disease prevention appears to the committee to be interpreted broadly. The goal of such use might be to halt the spread of overt disease that has appeared in a few members of a herd. It appears to be used at certain periods in the rearing of farm animals when they are considered to be particularly vulnerable to various infections (e.g., shipping-fever complex when cattle are moved into feedlots, and respiratory diseases in pigs). Often, subtherapeutic dosages are employed in feed for long periods without clear indications. In the case of swine, they are used regularly at specific stages of production: starter, grower, lactation, breeding, and gestation. Some farmers may be using antimicrobial-containing feeds without being aware of it. Mixing procedures may be such that the concentrations achieved may exceed those targeted.

Although distinctions have been made between use of subtherapeutic doses of antimicrobials in feed for growth promotion and for disease prevention the value of distinguishing between these two uses is rendered uncertain by many aspects of current practice. It seems most reasonable, therefore, to continue to categorize both uses as subtherapeutic, as they are currently viewed by the FDA. Better defined guidelines for use of subtherapeutic concentrations of antimicrobials for disease prevention would be of benefit.

FOREIGN EXPERIENCE IN BANNING ANTIBIOTICS AS FEED ADDITIVES

The Swann committee report of 1969 in England addressed the issue of feed antibiotics (subtherapeutic use) and their effects on the selection of strains of bacteria resistant to antimicrobial drugs.[13] It recommended that all antimicrobials used in humans be prohibited from use for growth promotion in animals. It also stated that antimicrobial drugs used for humans could be used in treating animals for disease or prophylactic indications when prescribed by a veterinarian.

In subsequent years, the central veterinary laboratory regularly conducted antibiotic-susceptibility testing on strains of salmonellae submitted to it. Its intention was to determine if the Swann committee mandates influenced the antimicrobial susceptibility patterns. It collected data on trends of drug-resistance patterns over the years 1972, 1974, 1976, 1977, 1984, 1985, and 1986. Two major observations were based on these data. First, resistance patterns persisted throughout the period; rarely was there any decrease. Second, one group of related phage types of *S. typhimurium* (204C was the predominant type) appeared in calves in 1979. These strains are multiple-drug-resistant and are responsible for the increase in resistance patterns detected during this period. In 1985, 204C constituted 62% of salmonella strains isolated from cattle. Almost all strains (more than 89%) were resistant to tetracyclines, ampicillin (and related penicillins), trimethoprim, and chloramphenicol. Resistance to gentamicin has gradually increased. *S. dublin* strains during these surveys were the second most common isolates after *S. typhimurium*. Most *S. dublin* were isolated from cattle.

In 1985, less than 1% were resistant to tetracycline, ampicillin, trimethoprim-sulfonamide, and chloramphenicol. Streptomycin and sulfonamide resistance was more common--66.7% and 28.6%, respectively. Most other salmonella serotypes isolated from cattle were susceptible to these antibiotics.

In poultry, selected antimicrobial drugs demonstrated failure to inhibit growth of salmonellae; 24% of strains were resistant to streptomycin, 8% to tetracycline, 72.8% to sulfonamide at 50 μg, and 11% at 500 μg sulfonamide, and 0.8% to chloramphenicol.

It is clear that the phage type 204C of *S. typhimurium* is an example of an unusual strain that can periodically cause epizootics. In 1964-1965, an outbreak of *S. typhimurium* phage type 29 occurred in cattle. By 1969, this epidemic was largely over. That strain may have been selected through antibiotic pressure. The persistence of it and phage type 204C appears to be related to biologic

properties that permit intestinal colonization and ability to induce disease. Strain 204C has the propensity to acquire plasmids. It probably became a problem in calves because of multiple exposures associated with the many times when these animals were transported from broker to broker. The great mobility of calves among brokers was different from the situation in the years before the Swann committee recommendations.

The use of antibiotics in animal husbandry in England has not decreased, but rather has continued to increase. This increase is due to prophylactic and therapeutic uses. Penicillin and the tetracyclines continue to be the most widely used drugs. This fact suggests that they have not lost their effectiveness for treating animal diseases. Although nonprescription uses of antimicrobials have been documented by the British Veterinary Association, the higher concentrations of antimicrobials in prescription-authorized therapeutic and prophylactic uses are blamed for selecting resistant strains of salmonellae and other bacteria in animals. The short life span of the food animals and the apparent rapid decline in the number of resistant strains once the antimicrobial drugs are withdrawn are thought to be relatively effective barriers to a more widespread dissemination of these potential pathogens.

The incidence of salmonellosis in humans in England has shown a persistent yearly increase from 1970 to 1980. In the subsequent four years, the incidence appeared to increase rapidly presumably because of increased numbers of S. typhimurium cases. During these years other serotypes appeared, increased to a peak, and then usually subsided to low numbers. Reasons for these variations are unknown. The isolates of S. typhimurium phage type 204C from humans have not had the same high incidence of resistance to antimicrobial drugs as those from animals. In 1985, 207 human isolates were tested and more than 92% were susceptible; in the same laboratory, of 1,050 bovine isolates, only about 20% were susceptible. The 207 human isolates represented 4% of all S. typhimurium isolated in 1985, while 50% of the bovine strains were of phage type 204C. Strain 204C may be in the food chain, but it has not evolved in the same fashion as have the bovine strains; it is less common and has not developed the same high incidence of resistance to antimicrobial drugs.

The data indicate that the Swann committee recommendations have not had a significant effect on the number of resistant strains of salmonellae. This may be an unfair assessment, because there is no organized data base from before the recommendations with which to compare data collected later. Furthermore, some changes in agricultural practices have occurred which have enhanced the spread of salmonellae.

Although annual mortality rates in humans associated with salmonellosis in England were not available to the committee, there were reports of deaths in various outbreaks, but details were not obtained. However, there is no evidence of an increasing mortality rate, as might be anticipated with an increasing incidence of infections.

THE RISK MODEL

The committee learned that a similar risk model had been used by the National Resources Defense Council (NRDC) in its petition dated 20 November 1984 to the Secretary of Health and Human Services asking for suspension of the approval of the new animal drug applications for subtherapeutic use of penicillin and the tetracyclines in animal feeds.[16] The NRDC alleged that the use of these drugs presented an imminent hazard to the public health. The committee's risk model and the parameter estimates used in it are summarized in Table VIII-1. The NRDC used "best estimates," while the committee used three estimates: low, mid-range, and high and applied these to five distinct parameters in the risk model. In comparison, the NRDC estimate of the number of deaths per year due to salmonellosis associated with subtherapeutic use of penicillin and the tetracyclines was 116 deaths, that corresponds to this committee's mid-range estimates in Table IX-1 of 30 deaths--a rather similar result in the face of so much uncertainty. The second NRDC estimate, 264 deaths per year, is based on a different method that starts from an estimated 1,000 deaths per year due to salmonellosis, a figure we believe to be too high.

The Food and Drug Administration (FDA), a constituent agency of the Department of Health and Human Services (DHHS), carefully analyzed the NRDC petition and recommended that the Secretary of DHHS deny the petition on the grounds that an "imminent hazard" had not been demonstrated.[17] The petition was in fact denied. The FDA's analysis concluded that the NRDC had not shown in its petition that antibiotic resistance caused by the subtherapeutic use of penicillin or the tetracyclines in animal feed had a significant impact on the outcome of a significant number of cases of salmonellosis and thus, that no "imminent hazard" had been demonstrated.

The FDA's analysis first discussed the difficulty of treating infections by resistant salmonellae. It did not accept arguments about increased difficulty in treatment, because most infections with salmonellae are uncomplicated and resolve without treatment (so "antibiotic treatment is not recommended in patients with the uncomplicated diarrheal type of salmonellosis," and for those cases occurring outside the intestine the drug of choice is chloramphenicol, to which

only about 0.7% of salmonellae are resistant and for which alternative drug therapies exist).

The FDA also concluded that the data then available did not demonstrate any alteration in virulence and contended that some salmonella deaths (such as from heart attacks caused by dehydration and stress due to salmonellosis) are unrelated to antibiotic therapy.

The FDA then considered what we call the "etiologic fraction," as well as inappropriate therapy for infections not at first recognized as salmonellosis, and concluded that neither had been shown to present a major problem.

Finally, the FDA commented on the NRDC parameters (see Table IX-1) and took special issue with the estimated death rate of 4.2%, on the grounds that it was subject to a number of potential biases and limitations, including lack of documentation that salmonellosis was the primary cause of the reported deaths. (The largest difference between our mid-range estimate and the NRDC estimates is in the death rate. We queried the CDC, as noted above, and found that some deaths were indeed not due to salmonellosis and that others were questionable; we reduced our mid-range estimate accordingly). The FDA also concluded that the NRDC's estimate of 69% of resistant salmonellae traceable to animal sources was based on a very limited sample and that such deaths caused by subtherapeutic use of penicillin or the tetracyclines (estimated by NRDC as 50%) could not be estimated accurately from the available data. The committee has considered these objections carefully, in light of advances in scientific understanding since 1984 and the whole body of data available at the time that it worked on this matter (the first half of 1988). The committee has not tried to judge the merits of either the NRDC's petition or the FDA's response. The committee believes, however, that some estimates can be made, as shown in Figures VIII-1 through VIII-12. These estimates are still highly uncertain, as indicated in the figures themselves.

EVIDENCE SUGGESTING THE PRESENCE OF HAZARD

The estimates presented here have wide margins of possible error, as reflected in the ranges from the 5th to 95th percentiles (percentile is the scriptor for fraction of estimates falling below it and are not confidence limits) in Figures VIII-1 through VIII-12. This is a direct reflection of the compounding of estimates of component factors that themselves have substantial ranges from the lowest plausible to the highest plausible estimate. If our model is to be adopted for future use, we urge that the responsible authorities promote the appropriate research to produce the data needed to narrow each of the ranges of estimates shown

TABLE IX-1

COMPARISON OF PARAMETERS IN THE PRESENT REPORT WITH THOSE OF THE NRDC REPORT: HUMAN SALMONELLOSIS DEATHS ATTRIBUTABLE TO ANY LOW-LEVEL FARM USE OF PENICILLIN OR THE TETRACYCLINES

	Present Report (Mid-Range Estimates)	NRDC Report (First Model)
Reported cases per year in the U.S.	50,000	40,000
Resistance to penicillin and/or tetracyclines	0.15	0.20
Death rate for salmonellosis from resistant strains	0.008	0.042
Fraction associated with strains of farm origin	0.70	0.69*
Fraction caused by subtherapeutic use of penicillin and/or the tetracyclines	0.50	0.72
Product of these estimates: Deaths per year in the U.S. from salmonellae resistant to penicillin and/or the tetracyclines because of subtherapeutic use of these drugs on the farm.	30**	116

Source: Prepared by the committee using data from Table VIII-1 and from the Natural Resources Defense Council.[16]

* "Traceable to animal sources."

** Differs slightly from Table VIII-2, because the product of the mid-range estimates is not necessarily the median of the 243 products.

in Table VIII-1, and thereby substantially reduce composite ranges of estimates shown in Figures VIII-1 through VIII-12.

The presently available data are an incomplete "patchwork" from a variety of sources; they are not collected systematically for the nation, they are complex, they are frequently of poor quality and require extrapolation for use in risk assessment, and they are not focused on the specific points of direct interest. These characteristics of the available data are inherent in the problem of collecting data and are not the fault of any one government agency or researchers who have studied this problem over the past several years. For example, none of the sources summarized in Table VII-8 was focused on estimation of the "etiologic fraction," none presented an estimate of that fraction, each had very small samples for this use, and each was subject to substantial bias in the identification and recruitment of subjects. Similarly, there have been few opportunities for the accurate and unbiased estimation of population-wide death rates, though countless reports of salmonellae deaths and death rates have been published for use in other contexts.

We regard the model itself as neutral--this is, unbiased with respect to errors or uncertainties in the estimates it produces--though of course it reflects all the errors or uncertainties that are inherent in the parameters it uses (Table VIII-1). Although the model itself is neutral, it can perhaps be improved, especially with respect to the path implied by the column headings of Table IX-1, the number of steps (which we took to be five), and modifications to make better use of available data. We invite and urge others to prepare alternative models, and we hope that funding agencies and sponsors of research in this field will increase their support of efforts to develop improved models.

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X

CONCLUSIONS

The committee has reviewed the extensive and sometimes conflicting literature pertaining to possible human health risks associated with the use of subtherapeutic concentrations of penicillin and the tetracyclines (and other antimicrobials) in animal feed. It evaluated investigations of the molecular nature of plasmids, transposons, and other bacterial antimicrobial-resistance determinants and their transfer; data on the extent of antimicrobial resistance in Salmonella species (and in other enteric pathogens) isolated from humans and farm animals; epidemiologic studies in humans and farm animals; data on reported cases of human illness and deaths due to Salmonella transmitted to humans from farm animals via meat and poultry products; information on the extent of subtherapeutic use of penicillin, the tetracyclines, and other antimicrobials in animal feed; and data from Great Britain on the effects of the restrictions placed some years ago on the use of antimicrobials in animal feeds in those countries.

The committee also reviewed the available published reports dealing with four subjects recommended for further study in the 1980 report of the National Research Council Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds: the effects of subtherapeutic and therapeutic doses of antimicrobials on the prevalence of antimicrobial-resistent enteric bacteria (including salmonellae) in farm animals; the extent of carriage of resistance-factor-containing bacteria in vegetarians and nonvegetarians (to ascertain the extent to which such carriage is associated with meat consumption); the extent of carriage of resistance-factor-containing Enterobacteriaceae in abattoir workers, their families, and neighborhood controls (to assess the association with occupational exposure to bacteria from animal sources); and the prevalence of urinary tract infections (and urinary tract infections due to resistance-plasmid-containing Enterobacteriaceae) in female workers in poultry-processing plants and a control group of women without contact with farm animals or their unprocessed meat products.

We consulted with and heard testimony from the epidemiology staff of the Centers for Disease Control, other medical epidemiologists, veterinarians, representatives of

the Animal Health Institute, microbiologists, and representatives of the pharmaceutical industry.

Using all the resources noted above, we were unable to find a substantial body of direct evidence that established the existence of a definite human health hazard in the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feeds. However, we believe that important--but as yet scant--data indicate the flow of distinct salmonella strains from farm animals, through the food processing chain, to humans in whom they cause clinical salmonellosis. In the one compelling instance of such a clear link, the multiple-antibiotic-resistant S. newport originated in farm animals exposed to chloramphenicol, a drug not approved by the Food and Drug Administration for use in feed. The committee believes that the molecular fingerprinting techniques used in this study can provide (when unique markers are present) the direct evidence needed to trace the source of antibacterial-resistant bacteria to human infection. If records of amounts of antibiotic use are maintained on farms producing food for human consumption, better evidence can be established for incriminating subtherapeutic/therapeutic doses in disease outbreaks.

The committee believes that there is indirect evidence implicating subtherapeutic use of antimicrobials in producing resistance in infectious bacteria that causes a potential human health hazard. The evidence is of several kinds:

- There are extensive experimental data on the properties of R plasmids and their capacity for transfer of antimicrobial-resistance determinants, both in the test tube and in the intestinal tract, particularly in the presence of antimicrobial selective pressure.

- There is evidence of widespread use of subtherapeutic concentrations of penicillin and the tetracyclines (and other antimicrobials) on farms and feedlots.

- There is ample evidence of high levels of antimicrobial resistance among animal isolates of salmonellae.

- Animal and poultry carcasses in meat-processing plants are often contaminated with Escherichia coli and other enteric pathogens. Few data are available on the frequency of antimicrobial resistance among such isolates. If the prevalence of antimicrobial resistance among reported isolates from diagnostic laboratories is a true representation of antimicrobial resistance in farm animals going to slaughter, the frequency of resistance among enteric pathogens in animal and poultry carcasses would be expected to be high. However, if the salmonella isolates reported

from diagnostic laboratories are principally from animals that are ill and have received antimicrobials, the figures would clearly overestimate the frequency of resistant isolates from meat and poultry carcasses.

o Handling and ingestion of improperly cooked, packaged frozen or refrigerated meat and poultry contaminated with bacterial pathogens provides exposure to an infecting inoculum.

o Experience with antimicrobial drugs in humans over the last 45 years has revealed the emergence of resistant strains associated with extensive drug use and the need to avoid unnecessary and prolonged use, particularly "prophylactic" use without clear and proven indications.

In addition, the committee has used the results provided by the risk assessment model presented to estimate quantitatively the possible risk of mortality associated with antibiotic-resistant salmonellae due to the subtherapeutic use of penicillin or the tetracyclines in animals. In the 1980 NRC report, the Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds concluded that "the postulated hazards to human health from a subtherapeutic use of antimicrobials in animal feeds were neither proven nor disproven." In other words, the risk of human health as a result of subtherapeutic use of antimicrobials in feed was not estimated.

We found the available data base on some aspects of the problem to be limited in quality and quantity; indeed, the data had not been gathered prospectively for the purpose of this type of analysis. The committee has used what it considers the best available information, indicating, where appropriate, the inherent weaknesses in the data. Admittedly, in some instances, we used only the best estimates available in the risk assessment. The assessment does indicate the presence of risk. Although it does not provide a distinct numerical "answer" to the question of the magnitude of the human health risk involved, it does provide some indication of the probable size of the risk in terms of numerical estimates or ranges. These are presented below as numbers of deaths per year attributable to the subtherapeutic use of antimicrobials (or penicillin and tetracyclines) in the listing of specific conclusions:

BIOLOGIC IMPACTS

o Use of each new antimicrobial agent over the last half-century has eventually mobilized genes that encode resistance to the agent and disseminated them widely through

the world's interconnecting bacterial populations. Use of the antimicrobial agent disseminates the resistance genes in stages, each of which begins with a rare molecular event that facilitates further dissemination. Although use of antimicrobials in a patient or the patient's neighbors might have triggered overgrowth and clinical manifestation of the resistant strain, the evolution and delivery of its resistance genome was the result of prior use in many, probably distant, bacterial populations.

o Results of surveys of isolates of salmonellae from animals and humans in the United States and restriction-endonuclease fragment patterns of resistance plasmids from selected isolates suggest that clones of resistant salmonellae are endemic in animals and sporadic or occasionally epidemic in humans.

o Herds of farm animals given subtherapeutic amounts of antimicrobial agents have more antimicrobial-resistant intestinal bacteria than herds given no antimicrobials.

o The most important determinant in the selection of antimicrobial-resistant strains in a bacterial population is exposure of that population to antimicrobials. Total duration and concentration of antimicrobial use are important in selection for resistance. Any measure that fails to reduce total use appreciably is unlikely to affect the prevalence of antimicrobial-resistant strains.

o Resistance to antimicrobial drugs among salmonella strains can interfere with the efficacy of antimicrobial therapy of human salmonellosis. (Such resistance is usually R-plasmid-mediated, so it can involve other drugs, such as trimethoprim-sulfamethoxazole, chloramphenicol, and ampicillin.) Although such interference with the efficacy of therapy almost certainly occurs (i.e., patients are treated with an antimicrobial that is ineffective because of drug resistance), it is probably quite uncommon in nontyphoidal salmonellosis.

o The available data are inadequate to conclude that either subtherapeutic or therapeutic concentrations of antimicrobials are more selective of drug-resistant bacteria. On theoretical grounds, it is likely that therapeutic and subtherapeutic dosages exert equal selective pressure for clonal expansion of resistance, but subtherapeutic dosages exert more pressure for conjugative transfer of drug resistance, because of the dosages and the durations of administration.

o Animal and poultry products (including veal, beef, pork, chicken, eggs, and milk) are the principal sources of human nontyphoidal salmonellosis. Also, some E. coli serotypes can also be found in the intestinal flora both of humans and of farm animals. Thus, there could be an interconnecting link between these two large pools of enteric microorganisms, facilitated by the high frequency of contamination of animal and poultry carcasses in slaughterhouses. Such a potential link would provide a means of movement of R plasmids of farm origin to the human alimentary tract. The interconnection, because of its nature, would constitute an almost exclusively one-way passage.

o The overall prevalence of resistance to any of five commonly used antimicrobials is about 4 times as great in collections of salmonella isolates from farm animal and poultry (65%) as those in collections of isolates from humans (15.5%). This difference suggests that the predominant pool of resistant salmonellae is in farm animals. Because ultimately almost all human infections with nontyphoidal salmonellae result from strains originating in farm animals, the antimicrobial resistance observed in human isolates most likely is derived from the animal pool of resistance genes, rather than from selection due to antimicrobial use in humans.

EPIDEMIOLOGIC FINDINGS

o Evidence is sparse that directly links the use of penicillin and tetracycline in subtherapeutic concentrations in animal feeds to human infections. Several studies have yielded reliable evidence of spread, from farm animals and poultry to humans, of E. coli strains in which antimicrobial resistance had been induced by administration of subtherapeutic concentrations of antimicrobials as feed additives. There is evidence from only one study of the direct spread of multiple-antimicrobial-resistant salmonellae from farm animals to humans via meat products. However, the antimicrobial used on the farm was chloramphenicol, a drug not approved by FDA as a feed additive in animals used for food production. It might be difficult, or impossible, to provide a total chain of evidence directly relating the majority of cases of human infection with antimicrobial-resistant salmonellae to a source on the farm or feedlot or to relate the presence of the resistance to the use of specific antimicrobials in subtherapeutic concentrations in feed. By the time a detailed investigation of an outbreak of human salmonellosis occurs, evidence of prior antimicrobial use patterns might not be available.

o It has not been possible to determine whether antimicrobial resistance of salmonellae caused by the administration of subtherapeutic concentrations of antimicrobials in animal feed increases the number of cases of human salmonellosis.

o Whether the presence of antimicrobial resistance in salmonellae increases virulence is uncertain; the available data are limited and conflicting. In special circumstances, as when R plasmids are linked with virulence genes (e.g., those for enterotoxin or hemolysin in E. coli), selection by antimicrobial agents might promote spread of virulent strains; however, such an occurrence has only rarely been reported. It is not clear whether the overall prevalence of salmonellae in food products is increased by virtue of antimicrobial resistance. However, the incidence of human salmonellosis in the United States is increasing, and the increase is unlikely to be an artifact of better reporting. As long as most strains of Salmonella are susceptible to the antimicrobials to which they are exposed, subtherapeutic administration of antimicrobials might reduce the prevalence of salmonellae in meat and poultry products that humans ingest. However, as the prevalence of resistant strains increases because of repeated and prolonged exposure to antimicrobials, subtherapeutic administration might actually favor the increase by suppressing the normal competing flora and promoting R-plasmid spread. Direct proof of this pattern in salmonellae in farm animals is lacking.

o The current frequency of R-plasmid-mediated antimicrobial resistance among isolates of E. coli and salmonellae in the intestinal contents of farm animals and poultry is high--much higher than in human isolates. It would be difficult to predict the period required, after curtailment of the use of subtherapeutic concentrations of penicillin and the tetracyclines in animal feed, for R-plasmid-mediated antimicrobial resistance to decrease in any extent in salmonellae and E. coli strains. Major decreases might occur only after the passage of years, in view of (1) the current degree of resistance, (2) the extensive environmental contamination on farms and feedlots with resistant organisms, (3) the prolonged prior subtherapeutic use of antimicrobials, which has allowed extensive permeation of resistance genes (transposons) throughout the highly colonization-adapted coliform flora of farm animals, and (4) the need to introduce competing, antimicrobial-susceptible coliform bacteria. Results of studies in confined populations of swine indicate that it could take many years for major decreases in levels of resistance to occur.

o Although the extent of antimicrobial resistance among salmonella strains isolated from humans is probably growing, it is still low enough for suitable intervention to forestall possible further increases and eventually to lower the overall extent of antimicrobial resistance.

ANTIBIOTIC USE PATTERNS

o The use of subtherapeutic dosages of penicillin and the tetracyclines in animal feeds is extensive in the United States. Such use is for the purpose of either growth promotion or disease prevention and often continues for a substantial portion of the growth cycle of farm animals. The specific rationale for use in a given herd at a given time is not always clear. Of over 31 million pounds of antimicrobials produced each year in the United States, about 42-48% is designated for addition to animal feeds or other unspecified (minor) uses. The best estimates (they are only estimates) indicate that penicillin and the tetracyclines account for almost 60% of the antimicrobials sold to the feed trade (and presumably ultimately used on farms and in feedlots). Of the total amount of tetracyclines produced in this country, for use in both humans and animals, approximately 70% is sold for use in livestock and poultry feeds. An estimated 88% of all antimicrobial use in livestock and poultry is in subtherapeutic concentrations. Thus, subtherapeutic use of penicillin, the tetracyclines, and other antimicrobials in animal feeds--which accounts for some 40% of antimicrobial production in the United States--constitutes a sizable segment of the total antimicrobial selective pressure (for resistant enteric microorganisms) exerted on the combined human and farm-animal intestinal bacterial populations.

o Interpretation of the results in Great Britain after banning the subtherapeutic use of penicillin and the tetracyclines in animal feed is difficult, in part because <u>total</u> farm use of these antimicrobials might not have decreased because use could have taken the form of therapeutic or prophylactic doses in feed for disease treatment or prevention as prescribed by a veterinarian. The appearance of new epidemic strains of antimicrobial-resistant salmonella serotypes during the period of interdiction of subtherapeutic use further confounds interpretation. It might take years for dilution of antimicrobial-resistant strains of Salmonella and <u>E. coli</u> in the farm animal population before any substantial changes might be observable.

RISK ANALYSIS

o The committee has been unable to find substantial direct evidence that bacterial resistance resulting from the use of subtherapeutic concentrations of penicillin or the tetracyclines in animal feed causes an excess risk to human health as a result of consumption of food products derived from the treated animals, as a result of contact with such animals, or as a result of exposure to an environment contaminated by resistant enteric bacteria from such animals. Lacking this direct evidence, the committee turned to the tools of risk assessment to develop some quantitative estimate of the probable risk to human health associated with this form of the subtherapeutic use of these antimicrobials.

o Use of penicillin and the tetracyclines in subtherapeutic concentrations in animal feed has led to increased antimicrobial resistance in foodborne commensals and pathogens. The risk analysis in this report focused only on human infection with salmonella serotypes, because available data on other species were insufficient. The committee has not assessed the potential risk to human health associated with drug resistance in other gram-negative bacillary species (Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli) of animal origin, because the data on human cases are too limited and because antimicrobial susceptibility data on those bacteria are not routinely obtained.

o Because the committee's risk assessments are based on estimates using sparse data, these estimates should be interpreted and used with caution. Such estimates are best seen as scientific hypotheses about the possible extent of a problem. This does not mean that they are "hypothetical" in the weak sense of being speculative. Rather, they are hypotheses that are consistent with all available information and scientific understanding, but they have not been tested by traditional scientific methods. All the estimates presented in this report should be viewed in that perspective.

o Annual numbers of deaths from salmonellosis attributable to subtherapeutic uses of any antimicrobials for prophylaxis and growth promotion have been estimated. The likeliest estimate is 70 deaths per year.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 40 deaths per year. Caveat--these are not necessarily "excess deaths," but rather estimates of the

yearly mortality attributable to salmonellosis of the indicated origin. The deaths might to some extent replace deaths (in the same patients or others) that occur from infections due to salmonellae susceptible to penicillin/ampicillin and tetracycline if subtherapeutic dosages of these antimicrobials had not been used in animal feed. Estimation of such "replacement" of deaths is not possible with the evidence at hand.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of any antimicrobial for growth promotion only is 20 deaths per year. As in the preceding (and following) estimates, the caveat regarding "excess deaths" applies.

o The likeliest estimate of mortality from salmonellosis attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline only for growth promotion is 15 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of any antimicrobial for prophylaxis and growth promotion is 6 deaths per year. The "etiologic fraction" is the proportion of persons exposed to an antimicrobial-resistant salmonella strain who are at increased risk of illness by virtue of recent use of antimicrobial drugs for whatever reason. Therefore, such deaths can be considered as "excess deaths"; i.e., they would not occur if the infecting salmonella strain were not antimicrobial-resistant and if its multiplication were not promoted, presumably, by suppression of growth of the competing normal antimicrobial-susceptible normal flora. In the same way, the number of foodborne pathogens (inoculum size) needed to precipitate disease might have been decreased. Whether a similar effect can be produced by prior antimicrobial use in persons infected with antimicrobial-susceptible salmonellae (due to possible differential antimicrobial susceptibility between susceptible salmonellae and normal components of the intestinal flora) is unknown, and the committee has not been able to find data bearing on this question.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 6 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic faction" attributable to

subtherapeutic uses of any antimicrobial only for growth promotion is 2 deaths per year.

o The likeliest estimate of mortality from salmonellosis in the "etiologic fraction" attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline only for growth promotion is 2 deaths per year.

o Infections with antimicrobial-resistant strains of Salmonella are more often fatal than infections with susceptible Salmonella. Therefore, the increased difficulty of providing effective therapy for human disease can be estimated. The increased difficulty in providing effective treatment may be due to increased virulence of antimicrobial-resistant strains, to the presence of resistance to one of the antimicrobials ordinarily used to treat such infections when they are severe or when they occur in particularly vulnerable persons, or to some other factor. The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of any antimicrobial for prophylaxis and growth promotion is 40 deaths per year.

o The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of penicillin/ampicillin and/or tetracycline for prophylaxis and growth promotion is 20 deaths per year.

o The likeliest estimate of mortality from salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of any antimicrobial only for growth promotion is 8 deaths per year.

o The likeliest estimate of mortality form salmonellosis arising because of increased difficulty of treatment attributable to subtherapeutic uses of penicillin/ampicillin and/or the tetracyclines only for growth promotion is 8 deaths per year.

o Evaluation of the foregoing estimates of mortality from salmonellosis attributable to subtherapeutic uses of antimicrobials in animal feed requires consideration in a broader context. What possible benefits accrue from such subtherapeutic use of antimicrobials in food production? Would human deaths from salmonellosis be reduced by the discontinuation of subtherapeutic use of penicillin/ ampicillin and/or the tetracyclines? The committee's thesis is that, although some deaths due to antimicrobial-resistant strains might be "replaced" by deaths due to susceptible strains, the total number of deaths would decrease, however,

this cannot now be proved. The committee offers no recommendations regarding policy-making because that was not part of its mandate.

XI

RECOMMENDATIONS FOR FUTURE RESEARCH

The committee offers no recommendations of possible solutions to risk management of the overall problem under consideration. It has directed its attention mostly to its charge to review the human health consequences and the risk associated with the use of penicillin and the tetracyclines at subtherapeutic concentrations in animal feed. Recommendation of any action would be appropriate only after regulatory agency review and weighing of both the benefits and risks of use of these antibiotics in subtherapeutic dosages.

The committee does, however, offer recommendations concerning further investigations that would be helpful in resolving the issue, which has been intensely debated for some 15-20 years. Many of the recommendations for study would remain appropriate whether current policies regarding subtherapeutic antimicrobial use remain in effect or are changed by a regulatory agency. In the former instance, the data obtained would serve to strengthen the informational underpinning of risk estimation. In the latter instance, they would make it possible to compare data on the prevalence of antimicrobial resistance among enteric pathogens and human health risks before and after institution of any change in approved antimicrobial use.

STUDIES TO SUPPLEMENT THE DATA BASE FOR RISK ANALYSIS

The risk assessment performed by this committee used the best available data related to the six essential elements in its risk estimates: resistance of human salmonella isolates to antimicrobials, annual reports of cases of salmonellosis, death rates associated with antimicrobial-susceptible and antimicrobial-resistant strains, fraction of human salmonellosis deaths associated with strains of farm origin, fraction of antibiotic resistance in strains of farm origin caused by subtherapeutic use of antimicrobials in animal feed, and "etiologic fraction." In compiling its risk estimates the committee was limited by the paucity of some types of data and the consequent need to extrapolate from the results of small studies to the global problem, by the fact that in some subjects reliable data were almost totally lacking and had to be substituted for with "best estimates,"

and by the nature of the available relevant data which often had been collected for other purposes. The risk assessment was confined to infections caused by Salmonella species, only a portion of the problem, because basic surveillance data on infections due to other gastrointestinal pathogens of animal origin--such as Campylobacter jejuni, Yersinia enterocolitica, and enterohemorrhagic E. coli--were not available.

The committee hopes that FDA will find the risk assessment performed by this committee to be useful in its decision-making. The committee acknowledges the qualitative and quantitative deficiencies of the primary data and the broad range of estimates used in the assessments of risk. Narrowing the range of these estimates would necessitate refining and enlarging the data base used in the risk analysis. In the committee's view, such an effort appears reasonable.

ANTIMICROBIAL-RESISTANT SALMONELLA STRAINS AND THEIR SOURCES

Improved surveillance of salmonella isolates in the United States is essential for better understanding and control both of bacterial resistance to antimicrobial agents and of disease due to salmonellae in humans and animals. Improved surveillance would be easy to put into place, because it would build on, and actually require only a small increment to, a large existing system.

The Existing System

Each year, hundreds of thousands of physicians, analyzing illnesses of millions of patients, send hundreds of thousands of stool specimens to more than 5,000 microbiology laboratories. This is a time-consuming process by which skilled technologists isolate 40,000 or more salmonella strains and conduct tests for resistance to antibacterials. Most of the salmonella isolates are then forwarded to state reference laboratories, where technologists with special training, skills, and reagents laboriously type them into more than 1,000 possible serotypes. Serotype reports are returned to the referring laboratories, where they only rarely contribute to the management of patients.

Epidemiologists in the separate states use the reports of isolated salmonella serotypes to delineate recognized outbreaks and detect others. The accumulated reports of all the state reference laboratories are collected, tabulated, and published by the Centers for Disease Control (CDC).

In a similar system, state veterinary laboratories, the National Veterinary Reference Laboratory, the Food Safety and

Inspection Service (FSIS) of the U.S. Department of Agriculture, and various university diagnostic laboratories isolate and serotype salmonellae from specimens taken from animals; however, these data are unpublished and have not been included in the data base used by this committee.

Additions to the System

The antimicrobial susceptibilities of each salmonella isolate need to be recorded with its serotype.

Reference laboratories need to enter their results each day into a networked computer system that analyzes all data automatically and comprehensively.

Resistant isolates classified by a computer as epidemiologically important should be forwarded to a laboratory that will catalog their plasmids.

Addition of Susceptibility Test Results

One good reason for adding results of susceptibility tests is to obtain accurate and complete measurements of prevalence of resistance, regional variations, trends over time, etc. All those would have been valuable to this committee, but could only be pieced together crudely from fragmentary reports of limited comparability (see Chapter V).

A second, and probably better, reason is to improve epidemiologic information, which is the only justification for the present elaborate system. Essentially, outbreaks are confirmed or recognized now by virtue of an excess of isolates of one serotype over the expected incidence. The outbreak clone boosts the serotype isolation rate above the threshold of random appearances. However, for the more commonly isolated serotypes (which account for most of the isolates), the threshold for detecting excess is high. Generally, only large outbreaks are investigated, except for outbreaks caused by rare serotypes; in fact, most outbreaks are overlooked. Coupling antibiotype to serotype permits detection of small outbreaks that are due to resistant subclones that belong to common serotypes. Two isolates of a subclone can be recognized to constitute an outbreak; without the antibiotype, dozens might be needed. Recognition of more small outbreaks or of large outbreaks sooner improves the understanding and control of salmonella disease and of the flow of resistance genes in animals and humans. Recognition of more outbreaks also provides more opportunities to trace chains of transmission.

A third reason for recording antibiotypes is that it is the key to the use of plasmid cataloging, as described below, which adds another level of subclone discrimination.

Determining the resistance of all salmonella isolates in the United States would be easy and inexpensive. Four-fifths of the human isolates exhibit no antimicrobial resistance. The ones that do nearly always have resistance at least to either tetracycline, streptomycin, sulfonamide, or ampicillin; dropping four disks on a small plate or part of a plate would screen out the susceptible (nonresistant) four-fifths, leaving 8,000 resistant isolates--an average of less than one per day per state reference laboratory. Each would need a routine disk-susceptibility test plate, which entails several minutes of work and a dollar's worth of supplies. Some 4,000-5,000 animal isolates of salmonellae would need susceptibility testing each year.

An Integrated Computer System

Salmonella outbreaks often appear as a small number of isolates scattered across several states. Accordingly, continuing analysis of all U.S. data in an integrated system is needed to detect these outbreaks early or at all. Such a system would become more important as antibiotyping and plasmid cataloging began to discriminate more salmonella subclones. It would also be helpful in developing or adapting shared software, such as automatic notification whenever any clone or subclone in any state or combination of states exceeded its outbreak threshold. Integration should be considered soon, before individual reference laboratories acquire various incompatible systems.

Plasmid Cataloging

Plasmid cataloging would be a useful diagnostic tool and should begin with a survey of the restriction endonuclease digestion patterns of resistance plasmids from animal and human salmonella isolates representing prevalent antibiotypes for a number of serotypes (see Table V-2). Experience has shown each set of plasmids from isolates of one antibiotype-serotype combination would be expected to have one or more restriction patterns. Wholly different restriction patterns in any set would discriminate subclones presumed to be unrelated. Patterns with small differences undoubtedly represent evolutionary variants of one clone and may be distinguished with microepidemiologic studies. For example, plasmids from six human isolates from different parts of one state over a two month period had identical restriction patterns, whereas those from humans or animals in other states all differed slightly from those six and from one another.

As the catalog of U.S. salmonella plasmids might build, in parallel with a computerized isolate data base, the epidemiology of salmonellae in animals and humans could begin to emerge at a new level of detail and quantitation. The distribution of specific clones and subclones among animal populations could be delineated, and rates of appearance in humans could be established. That would provide better understanding of the epidemiology of salmonellae in animal and human outbreaks, give a basis for each of them at the outset to known plasmid families with known prior distributions, and hence provide early clues to possible chains of spread.

One laboratory could catalog the salmonella plasmids and explore technology for improved cataloging of plasmids; restriction endonuclease profiling, although workable now, is likely to be supplemented (if not replaced) by newer methods that would be faster and provide more critical molecular detail. In particular, as more is learned about the stages of spread of resistance through bacterial populations and the molecular changes that accompany those stages, it will be of great value to find correlates in the data on salmonellae.

Implications of Change in Use of Animal Feed Additives

It can be questioned whether a different program of salmonella surveillance would be needed if use of animal feed additives changed. The program recommended above would probably be a sensitive monitor of such change, as well as providing improved observation of the present situation.

HUMAN MORBIDITY AND MORTALITY DUE TO ENTERIC PATHOGENS OF FARM ANIMAL ORIGIN

"Salmonella deaths" might be construed as deaths due primarily to salmonellosis (with bacteremia and shock, endarteritis, metastatic abscesses, and severe gastroenteritis with dehydration, usually in infants or elderly); those in patients with underlying diseases in whom salmonella gastroenteritis contributed to the death, but was not the primary cause of it; and those in patients from whom salmonellae were isolated, but in whose deaths had other causes. In cases with other causes, hospitalization might have been initiated by salmonella infection that had subsided and their deaths have had unrelated causes. Similarly, salmonella gastroenteritis and asymptomatic carriage can occur as almost incidental matters in patients with other major medical problems to which they succumb.

Often, the above distinctions have not been made in published series of cases and reports of outbreaks, and

information on death certificates is not of sufficient quality to allow such distinctions to be drawn. Such data can probably be developed only with expanded studies of selected counties of the type performed by CDC.[7] However, it will likely require more detailed analysis of hospital records and information (based on chart-directed recall) from attending physicians to categorize "salmonella deaths" more definitively. In addition, definitive classification of those deaths as to contribution of salmonella infection (primary cause, contributing cause, of unknown relevance, or unrelated) should be stratified according to antimicrobial susceptibility pattern of the strain involved (susceptible, resistant to penicillin or tetracycline, resistant to multiple antimicrobials, etc.). In the characterization of strains, primary attention should be on resistances to antimicrobials known to be R-plasmid-mediated.

The selected-counties study of the Salmonella Surveillance System is the only current system of surveillance of infection with Salmonella of which the committee is aware. Additional useful information could be obtained through the system if the following modifications were introduced:

- Expansion of the number of communities to yield a larger data base.

- Continuation of the selected-counties study in the form of annual surveillance, rather than monitoring every 4-5 years.

- Determination of morbidity (days of diarrhea, hospitalization rates, etc.) associated with infection caused by antimicrobial-susceptible and antimicrobial-resistant strains.

- Inclusion of infections with Campylobacter jejuni based on the same kinds of epidemiologic questionnaires (given the increasingly evident impact of campylobacter infection).

QUANTITIES OF ANTIMICROBIAL DRUGS USED IN SUBTHERAPEUTIC CONCENTRATION IN ANIMAL FEEDS

Amounts of antimicrobials in the aggregate (and of individual antibiotics, such as penicillin and the tetracyclines) used in animal feed are not known. The best available information comes from the Animal Health Institute and from industrial sources as estimates that are admittedly rough. Valuable data could be provided by monitoring and surveillance to determine actual subtherapeutic use of

antimicrobials in animal and poultry feeds, perhaps through sampling of farms and feedlots. Sampling should reflect characteristics of a cross section of users of antimicrobial feed additives.

o Characteristics of facilities to be monitored. Sampling should include large and small farms, appropriate geographic areas, the major animal sources of meat products (beef cattle, veal cattle, pigs, and poultry), and (in the case of cattle) widely different methods of animal rearing (confined, high-density herds, and open-range grazing). It should include the spectrum from small local facilities to huge industrial operations and existing data bases, such as APHIS-USDA.

o Rationales for subtherapeutic use of antimicrobials given by user at each administration. Was it for growth promotion, disease prevention, or some other purpose? Did the farmer know, in fact, whether feeds contained an antimicrobial additive and, if so, what it was? For what fraction of the total growth cycle is the regular use of penicillin and tetracyclines (and other antimicrobials) in subtherapeutic concentrations in feed a regular practice?

o Concentrations of penicillin and tetracyclines (and other antimicrobials) achieved in feed on farms and in feed lots. Are the prescribed subtherapeutic concentrations being achieved after mixing on farm and feedlot? Are they being exceeded unwittingly?

o Would routine record-keeping of antimicrobial use on farms and feedlots be feasible? Such records would be of value, after antimicrobial-resistant strains involved in outbreaks of human salmonellosis were traced to the farm source (by plasmid fingerprinting techniques), in determining relationship to prior use of subtherapeutic concentrations of antimicrobials in feed given to the farm animals involved.

ROLE OF PRIOR EXPOSURE TO ANTIMICROBIALS IN INFECTION BY ANTIMICROBIAL-RESISTANT STRAINS OF SALMONELLAE ("ETIOLOGIC FRACTION")

Studies of the role of the "etiologic fraction" in humans should be expanded to provide a larger statistical basis for the estimates of risk to human health. Further data might be gleaned both from outbreaks, as has already been done, and from the Salmonella Surveillance System for selected counties. It would also be of interest to develop data on the morbidity and mortality rate in patients who constitute the etiologic fraction. Do these patients exhibit

more or less severe illness than other patients overall? The selection of matched controls must be carried out with great care, because the patients in the etiologic fraction are likely to differ from the general population at risk of salmonellosis by virtue of their recent intake of antimicrobial drugs, their greater age, their underlying illnesses, and by their receipt of a smaller inoculum.

As far as we are aware, the term "etiologic fraction" has been applied only to infections in humans, but there might also be an etiologic fraction in animals--i.e., decreased colonization and infection of animals by susceptible strains, an effect that might be termed a "negative etiologic fraction." Indeed, evidence supports the latter hypothesis.[3] A negative etiologic fraction for infections caused by susceptible strains will increase the proportion of infections caused by resistant strains.

A more crucial question concerns the net effect of the subtherapeutic administration of antibiotics on the overall prevalence of carriage of salmonellae (resistant or susceptible) in animals. Studies to evaluate that question could be carried out by passive observation of naturally occurring infection during the administration of antibiotics or by deliberate feeding of salmonella strains under controlled conditions. The results would probably be highly influenced by the choice of antibiotics for administration, as well as by the dosage and frequency of administration, the degree of resistance (or susceptibility) of the infecting strains, and the degree of resistance of the normal flora (possibly including both aerobes and anaerobes). Small differences in any those variables would influence the subtle interaction between the suppressive effect of a drug on a bacterial pathogen and on the normal bacterial flora and the interaction between these bacteria.

A negative etiologic fraction might also be hypothesized in humans. Indeed, some have suggested that the inappropriate use of antibiotics for such illnesses as viral pharyngitis, although attended by potential adverse effects, might have a beneficial effect in preventing secondary infection or complications of infection caused by susceptible pathogens. This committee's charge does not deal with the administration of antibiotics to humans, so that subject has not been pursued in this report.

MORBIDITY AND MORTALITY ASSOCIATED WITH UNREPORTED CASES OF SALMONELLOSIS

There is reliable evidence that only about 1-10% of cases of salmonellosis are identified in outbreaks; a comparably small fraction is thought to be reported in sporadic cases. Perhaps more important, it is often commonly

assumed that the unrecognized or unreported cases are generally less severe than the recognized or reported ones. The committee, in estimating risk, had no data on salmonellosis morbidity and of course could use data on salmonellosis deaths only if such deaths were reported.

It might be possible for CDC to conduct telephone surveys during outbreaks of salmonellosis to detect not only the unreported proportion of cases, but also to obtain data on morbidity in those cases; the results for both mortality and morbidity could be compared with data on reported cases. If morbidity were similar in the reported and unreported cases--i.e., chance, rather than severity of illness, would be determining whether an infection is reported--this would suggest that the impact of salmonellosis in our risk assessment, which is based on 40,000-65,000 cases per year, should be scaled sharply upward to take account of unreported cases.

STUDIES TO DETERMINE EXISTENCE OF DIRECT EVIDENCE OF HUMAN HEALTH HAZARD ASSOCIATED WITH SUBTHERAPEUTIC USE OF PENICILLIN AND TETRACYCLINES IN ANIMAL FEEDS

Only CDC is in a position to perform such studies. The use of molecular techniques to characterize salmonella isolates clonally and to identify antimicrobial-resistant strains through the food chain from farm animal to human consumer has proved successful. Do the one or two examples reported constitute clear evidence of a common phenomenon, or are they exceptions? The weak point in the argument is the lack of a direct demonstration of the role of subtherapeutic use of approved antibiotics, such as penicillin or tetracyclines, as the farm origin of the infecting salmonella clone. More salmonella outbreaks should be studied with molecular fingerprinting techniques and with conventional epidemiologic methods, including documentation of the qualitative and quantitative aspects of antimicrobial use on the implicated farms or feedlots.

OTHER STUDIES

ANTIMICROBIAL RESISTANCE AMONG, AND FREQUENCY OF DISEASE DUE TO, OTHER FOODBORNE ANIMAL PATHOGENS THAT AFFECT HUMANS

Recognition over the last decade that other bacterial enteric pathogens, such as Campylobacter jejuni and E. coli 0157:H7, can spread from animals to humans and that Campylobacter might infect humans more often than any other intestinal pathogen, raises the question of whether

monitoring the frequency with which they cause human and animal infections might illuminate the issues faced by this committee.

Information on those pathogens seems sparse, compared with information on salmonellae. Serotyping does not yet subdivide them as elaborately as it does salmonellae and is in any case not yet routinely practiced by a national network of laboratories. Resistance does not seem to impede therapy often or to be as varied as in salmonellae. Further work on these pathogens needs to be supported, but surveillance data on them are not likely to influence antimicrobial use greatly in the near future.

SELECTIVE EFFECTS OF THERAPEUTIC AND SUBTHERAPEUTIC DOSAGES OF ANTIMICROBIAL AGENT

Given the paucity of in vitro data of the kinds required to predict the effects of different dosages of drugs on selection of antibiotic-resistant strains, much more work is recommended. In particular, in a mixed bacterial population, what are the effects of different dosages of antimicrobials on the growth characteristics of resistant and susceptible bacteria, and on the genetic spread of R plasmids, particularly between species? Such studies require careful determination of growth rates and conjugative transfer rates under well-controlled conditions. The resulting information should make it possible to develop appropriate computer models to allow prediction of dosage effects on the emergence of drug-resistant organisms.

EFFECT OF ANTIBIOTIC RESISTANCE ON VIRULENCE OF FOODBORNE PATHOGENS OR SEVERITY OF DISEASE PRODUCED

Recently, some plasmids in salmonellae have been identified as being involved in the expression of virulence.[1,2,8] Plasmids in *S. typhimurium* (90 kilobases), in *S. dublin* (75 kb), and in *S. enteritidis* (54 kb) are necessary for these three serotypes to express virulence in mice. Plasmid-free "cured" strains lose virulence in mice, and reintroduction of the plasmids into the "cured" strain, or into a naturally occurring plasmid-free isolate, restores virulence.

Studies defining the prevalence of virulence plasmids in the common Salmonella serotypes (antimicrobial-susceptible strains) isolated from farm animals (and humans) not exposed to antibiotics will be helpful in providing baseline information concerning possible effects of subtherapeutic administration of antimicrobials in feed. Virulence plasmids might not be present in most strains of antimicrobial-

susceptible salmonellae in farm animals; in that case the possible effect of antimicrobial selection or persistence and expansion of the population of virulence plasmids in farm animals receiving subtherapeutic concentrations of antibiotics warrants investigation. Similarly, direct comparison of human (and animal) isolates of antibiotic-susceptible and -resistant salmonella isolates of specific serotypes in mouse-virulence assays bears directly on the question of whether antibiotic resistance in such a foodborne pathogen can affect its virulence.

BACTERIOLOGIC CONTAMINATION OF ANIMAL FOODSTUFFS WITH ENTERIC PATHOGENS

FSIS has the responsibility for ensuring the safety of meat and poultry products. FSIS has an overall goal of ensuring that meat, poultry, and their products are wholesome, unadulterated, and properly labeled and do not constitute a health hazard to the consumer. In 1983, FSIS asked NRC to evaluate the scientific basis of the current system for inspecting meat, poultry, and meat and poultry products. The 1985 report[4] that resulted from that request offered numerous recommendations, not all of which are directly relevant to the present study. However, one of the major conclusions of the report states that Salmonella and Campylobacter species are major causes of diseases transmissible to humans through the consumption of meat and poultry products and that current postmortem inspection methods are not adequate to detect these organisms. The report recommended that efforts to control and eliminate contamination with microorganisms include evaluation of rapid diagnostic procedures for detecting Salmonella and Campylobacter especially. Postmortem inspection methods have been relatively effective for the detection of unwholesome meats; before these methods are abandoned, FSIS should determine the effectiveness of the methods that would replace them.

At the request of FSIS, another study was done[5] to evaluate the current FSIS poultry inspection programs in the framework of a risk-assessment model incorporating statistical procedures. The report of that study drew several conclusions and offered recommendations that are relevant to the current study. "There is conclusive evidence that microorganisms pathogenic to humans (such as Salmonella and Campylobacter) are present on poultry at the time of slaughter and at retail." The report stated that "the critical control points at which known pathogenic microorganisms such as Salmonella and Campylobacter may be introduced into the poultry system should be identified and monitored, preferably as a part of an HACCP (Hazard Analysis

Critical Control Point) program" and that FSIS begin to lay the groundwork for a more comprehensive program with statistically based sampling that modifies the traditional bird-by-bird inspection.

In 1969, the NRC Committee on Salmonella[6] recognized that there was no way to be absolutely certain that a particular lot of nonsterile food is free of salmonellae. It recommended the development of a sampling plan to provide adequate assurance that the number of salmonellae present, if any, is below a statistically defined limit that reflects minimal hazard to the consumer. A subcommittee of that committee supported the HACCP concept as an effective and rational approach to the assurance of safety.

FSIS is now evaluating the effectiveness of the HACCP procedure for analyzing the slaughter of poultry and the procedures for handling finished products. FSIS plans to determine where the critical control points are and what procedures would be best for controlling bacterial contamination from the time when the animal reaches the processing plant to the time when the finished product goes to market. Today there is no routine microbiologic sampling of meat or poultry. To help in evaluating the critical control points in processing of poultry, an agency pilot plant in Puerto Rico is gathering baseline data at each critical control point in processing. Investigators will look at microbiologic contamination before animals reach the processing plant and during processing.

The present committee recommends that, with respect to microbiologic sampling of meats, consideration be given not only to the identification of pathogenic organisms, but also to their testing for antimicrobial susceptibilities. The resulting microbiologic data should be available to researchers for use in studying the relationship of antibiotic use to drug resistance in pathogens isolated from foodborne outbreaks of human disease.

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