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The Effects on Human Health of Subtherapeutic Use of Antimicrobials in Animal Feeds

Committee to Study the Human Health Effects
of Subtherapeutic Antibiotic Use in Animal Feeds
Division of Medical Sciences
Assembly of Life Sciences
National Research Council

NATIONAL ACADEMY OF SCIENCES
WASHINGTON, D.C. 1980

NOTICE

The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the Councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

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OF SUBTHERAPEUTIC ANTIBIOTIC USE IN ANIMAL FEEDS

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PREFACE

In 1978, the Congress of the United States provided to the Food and Drug Administration (FDA) an appropriation designated for the National Academy of Sciences to evaluate epidemiological approaches to the effects on human health of subtherapeutic[1,2] use of antimicrobials in animal feeds as defined by the FDA.[3]

The task accepted by the committee was:

"1. To study the human health effects of subtherapeutic use of penicillin and tetracycline (chlortetracycline and oxytetracycline) in animal feeds.

"2. To review and analyze published and unpublished epidemiological data and other data as necessary in order to assess the human health consequences of subtherapeutic use of penicillin and tetracycline in animal feeds.

"3. To assess the scientific feasibility of additional epidemiological studies, and, if needed, to make recommendations about what kinds of research should be carried out, the estimated cost and time required to complete such research and the possible mechanism to be used to conduct such studies."

To complete this task, the committee decided that it should:

1. define and evaluate the effects on human health that are associated with bacterial resistance to antimicrobials,

2. measure changes in numbers of pathogens and define changes in their virulence and in the prevalence of antimicrobial resistance resulting from the use of antimicrobials in animals, and

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[1] 200 g or less/ton for 2 weeks or longer.
[2] Throughout this report, use of the word ton denotes the "short" ton (2,000 lb), which is most commonly used in the United States. Exceptions to this are noted in the text.
[3] Including milk replacers, medicated blocks, and liquid feeds, but not as used in water alone. FDA does not have jurisdiction over drinking water.

3. differentiate the effects attributable to subtherapeutic levels of antimicrobials from those due to other uses in animal husbandry and in medical applications to animals and human beings.

Because of insufficient data in certain areas, the committee was not able to accomplish all of the above.

The significance of possible effects on human health due to the use of antimicrobials in animal feeds has been the subject of exhaustive reviews by several groups of distinguished scientists in the United States and Europe. Controversy surrounding the restriction of subtherapeutic uses of antimicrobials in animals can be expected to continue until more definitive epidemiological evidence confirms or refutes the potential hazards to human health postulated by these review groups.

The formulation of policy concerning such restriction requires that both benefits and adverse effects be weighed. This committee, however, was not asked to determine policy. Rather, it was charged with and undertook a more limited responsibility: evaluation of existing evidence and development of recommendations pertaining to the kinds of research needed to provide a clearer view of the effects on human health.

COMMITTEE PROCEDURE

An extensive bibliography of the research reports relating to the subtherapeutic use of antimicrobials in animal feeds has been prepared. Plans are being made to publish the bibliography in the near future.

Selected consultants and a special advisory panel from the National Academy of Sciences Board on Agriculture and Renewable Resources were asked to write papers on certain aspects of the problem for use by the committee. These papers are attached to this report as appendixes.

1. The Clinical Use of Antimicrobials and the Development of Resistance--John P. Utz (Appendix A)

2. Possible Human Health Effects of Subtherapeutic Antimicrobial Use as Pesticides--Robert N. Goodman (Appendix B)

3. Genetics of Antimicrobial Resistance--George A. Jacoby and K. Brooks Low (Appendix C)

4. Impact of Antimicrobials on the Microbial Ecology of the Gut--Dwayne C. Savage (Appendix D)

5. Antimicrobial Residues and Resistant Organisms: Their Occurrence, Significance, and Stability--Stanley E. Katz (Appendix E)

6. Zoonotic Aspects of Subtherapeutic Antimicrobials in Feed--John F. Timoney (Appendix F)

7. Transmission of Food-Borne Diseases--Implications of the Subtherapeutic Use of Antimicrobials --Jackson S. Kiser (Appendix G)

8. Food Contamination--William E. Pace (Appendix H)

9. Infectious Disease: Effect of Antimicrobials on Bacterial Populations--Thomas F. O'Brien (Appendix I)

10. Immunological Consequences of Antimicrobials in Animal Feeds--N. Franklin Adkinson, Jr. (Appendix J)

11. Antibiotics in Animal Feed. A report prepared by the Committee on Animal Health and the Committee on Animal Nutrition, Board on Agriculture and Renewable Resources, Commission on Natural Resources, National Research Council, National Academy of Sciences (Appendix K).

On August 22, 1979, the committee met in Washington, D.C. to hear presentations by its consultants and to exchange information and opinions. On the following day, August 23, 1979, a public meeting was held to receive information from persons and organizations. The members of the committee reviewed and evaluated pertinent epidemiological research and, during the week of September 16-21, 1979, convened to develop proposals for further epidemiological studies.

ACKNOWLEDGMENTS

It is a pleasure to express, on behalf of the entire committee, a special note of thanks to the staff: Dr. Enriqueta C. Bond, Dr. Roy Widdus, Mrs. Frances Peter, and Mrs. Susan Barron, whose informed and tireless efforts ably supported the committee. We are grateful

for the assistance of those consultants who supplied information and critical reviews of a vast literature and for the help of many members of the staff of the Food and Drug Administration, the Department of Agriculture, the Center for Disease Control, and the Office of Technology Assessment, especially Mr. Philip Frappaolo, Dr. Norman Tufts, Dr. Lester Crawford, Dr. Howard Teague, Dr. John Spaulding, Dr. Robert Brown, Dr. John Bennett, and Dr. Roger Feldman. The committee appreciates the cooperation and information provided by American Cyanamid Company, Pfizer Inc., National Pork Producers Council, representatives of the poultry industry, the Animal Health Institute, and other organizations and individuals too numerous to list.

Last, but not least, we thank the members of the public and the international community of scientists who submitted suggestions and information for our consideration.

Reuel A. Stallones
Chairman
Committee to Study the Human
Health Effects of Subtherapeutic Antibiotic Use in
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SUMMARY

Soon after antimicrobials were introduced into medical practice, the selection pressure exerted by their use caused an increase in the prevalence of microorganisms with resistance to antimicrobials, thereby reducing the effectiveness of therapy. The expanding array of antimicrobial drugs has provided alternative agents in most cases, but control of infections is sometimes delayed because resistance may not be recognized initially. Moreover, the alternative drugs may be more toxic, more expensive, or less effective than those that would be used if the infecting organisms were not resistant.

Most clinically significant antimicrobial-resistant bacterial strains are selected during the administration of antimicrobials to humans, but concern has been expressed that the continuous use of antimicrobials in the feed or drinking water of animals is also responsible for the emergence of resistant strains that may endanger human health. Penicillin and the tetracyclines are effective as additives to animal feed, but since they are also particularly effective and widely used in the therapy of human disease, the Food and Drug Administration has proposed to restrict their use in animal feeds at subtherapeutic doses.

Bacterial resistance to antimicrobials is a genetically determined characteristic, and genes for resistance may be carried either on chromosomes or on extrachromosomal elements called resistance (R) plasmids or R factors. Microorganisms possessing plasmid-mediated resistance to antimicrobials are designated R^+. R factors may be transferred from some bacterial species to certain other species, and resistance to several different antimicrobials is often linked on the same plasmid. Consequently, administration of one antimicrobial may result in the appearance of bacteria with resistance not only to the administered drug but also to one or more others.

The use of antimicrobials in animal husbandry for the improvement of growth and efficiency of feed conversion, for prophylaxis, and for the treatment of diseases has steadily increased since 1950, as has animal production. In 1978, approximately 48% of the antibiotics produced were designated for addition to animal feeds or for other (minor) uses. Antimicrobials are perceived as especially beneficial when animals are stressed either by intensive husbandry or shipment (BARR, Appendix K).

Data demonstrate that the use of antimicrobials in animal husbandry increases the prevalence of R^+ enteric organisms in animals. Some of these organisms may be pathogenic for humans. A number of

investigators have asserted that the administration of antimicrobials in subtherapeutic doses to animals raised for human consumption increases the total numbers of R^+ bacteria above that resulting from therapeutic uses of antimicrobials in animals and both therapeutic and prophylactic uses in humans. If this is true and if resistant bacteria are carried through the food processing system to the retail store, animal handlers, meat processors, and consumers would be at increased risk of infection by antimicrobial-resistant pathogens or have an increased likelihood of acquiring a nonpathogenic resistant organism capable of transferring such resistance to pathogens. The committee concluded that not enough information is available on these issues to determine the effects on human health.

Several generalizations can be made:

1. Little is known about the composition of the gastrointestinal flora of humans or animals, especially their anaerobic components (Savage, Appendix D). In those studies of R factors that have been conducted on the enteric flora of animals or humans, investigators have observed changes in the populations of Escherichia coli or those of its close relative Salmonella because these organisms are easy to culture and manipulate and because they are pervasive pathogens of both animals and humans.

2. The subtherapeutic use of antimicrobials does increase the prevalence of resistance among the E. coli and Salmonella of treated animals.

3. Persons in close contact with animals receiving antimicrobials are more likely to harbor antimicrobial-resistant E. coli than are persons not so exposed. However, studies do not usually indicate the type, duration, and dose levels of the antimicrobials received by the animals. Subtherapeutic use is generally not distinguished from therapeutic use.

4. Abattoir workers carry some of the same phage types[1] that occur in the slaughterhouse environment and in slaughtered animals. Because this information was generated by a study of a small number of people, the comparisons are not conclu-

[1]Phage typing is a procedure used by diagnostic laboratories to characterize and identify strains of bacteria according to their pattern of lysis by bacterial viruses (phage).

sive. Furthermore, these workers were exposed to animals that had probably received therapeutic as well as subtherapeutic doses of antimicrobials.

5. There are no data from which to assess the relationship between consumption of meat from animals that received subtherapeutic amounts of antimicrobials and the prevalence of antimicrobial-resistant E. coli in the general human population. Limited observations suggest that vegetarians do not harbor fewer resistant E. coli than do meat eaters.

6. No data exist to establish a relationship between illness caused by antimicrobial-resistant, pathogenic bacteria, and contact with animals or meat from animals given only subtherapeutic amounts of antimicrobials.

7. No data exist to quantitate the frequency of transfer of antimicrobial-resistance factors from the bacterial flora of animals to the flora of humans or of the transfer within the flora of humans.

8. The therapeutic or prophylactic use of antimicrobials in humans results in a greater prevalence of R^+ strains in the bacterial flora of treated people and their immediate contacts.

9. The disadvantages of using an alternative drug where antimicrobial resistance is present depend on the drugs chosen and each clinical situation. Thus, precise quantitation of the threat of potential "compromise of therapy" posed by an increased prevalence of antimicrobial resistance is exceedingly difficult. However, information on nosocomial infections (which are often resistant) does illustrate the magnitude of the problem.

10. Restrictions on the use of antimicrobials in other countries may well have altered the pattern of their use without significant reduction in the total amount used or in the apparent consequences for humans.

After reviewing the evidence, the committee concluded that the postulated hazards to human health from the subtherapeutic use of antimicrobials in animal feeds were neither proven nor disproven. The lack of data linking human illness with this subtherapeutic use must not be equated with proof that the proposed hazards do not

exist. The research necessary to establish and measure a definite risk has not been conducted.

The committee also concluded that it is not possible to conduct a feasible, comprehensive epidemiological study of the effects on human health arising from the subtherapeutic use of antimicrobials in animal feeds, partly because it is impossible to determine the antimicrobial history of the animal from which a particular piece of meat came. However, the committee does present several study possibilities to investigate certain aspects of the problem. These include (1) a study to determine the contribution of subtherapeutic and therapeutic antimicrobial dosing regimens to the prevalence of R^+ enteric organisms in meat animals; (2) a study to measure the extent to which carriage of bacteria having R factors is associated with meat consumption by comparing the enteric flora of vegetarians and meat eaters; (3) a study to measure the extent to which occupational exposure of humans to bacteria from animals in abattoirs is associated with carriage of bacteria having R factors and secondarily to gauge the spread of R factors among close contacts of abattoir workers; (4) a comparison of controls with subjects having urinary tract infections to determine if carriage of antimicrobial-resistant fecal flora is associated with increased morbidity or mortality from the infection.

The committee recommends further research on the mechanisms by which subtherapeutic levels of antimicrobials promote growth of animals. Understanding of this mechanism may lead to the development of other substances or procedures (e.g., immunizations) that provide the same effect, thereby rendering moot the question of possible effects on human health. However, the committee also recommends continued monitoring and occasional review of the possible effects on humans resulting from the subtherapeutic use of antimicrobials in animal feeds.

CHAPTER 1

THE USE OF ANTIMICROBIAL AGENTS[1]

The discovery of the first selective antimicrobial agent approximately four decades ago was a major milestone in the history of medicine and human health. The subsequent development of antimicrobial therapy largely centered on the search for drugs with effectiveness against microbial species that were not susceptible to drugs then in use.

These powerful new drugs have been shown to save lives when used to treat some severe infections and to reduce the burden of illness when used prophylactically in certain clinical situations (Utz, Appendix A). Since antibiotics[1] are isolated from microorganisms, strains of some microbial species have predictably evolved the capacity to inactivate them or become impermeable to them, i.e., these strains have developed resistance to these antibiotics. Resistance to synthetic antimicrobial agents arises from the variation normally displayed by individual microorganisms within species. Thus, the consequence of expanded use of antimicrobial drugs has been an increased prevalence of resistant organisms resulting from the selection process. In certain places, such as hospitals, contact between individuals has facilitated the spread of these resistant bacteria.

Consequently, researchers sought agents that were active against strains in which resistance had become prevalent. The expanding array of antimicrobials, particularly antibiotics, provided alternatives in most cases. But control of infections is sometimes delayed if the resistance of the infecting organism is not recognized immediately, and physicians may need to use drugs that are more toxic, more expensive, or less effective than those that would be selected if the infecting organisms were not resistant (Utz, Appendix A).

Trends in the antimicrobial resistance patterns of a number of clinically important pathogens have been reviewed by Finland (1979) and Stollerman (1978). The prevalence of multiply antimicrobial-resistant Staphylococcus aureus increased until 1960 but subsequently declined in association with a change in phage types. Recently, strains of Streptococcus pneumoniae with multiple resistance have been found in a number of countries. Strains of Haemophilus

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[1]An antibiotic is a chemical substance produced by microorganisms that has the capacity at low concentrations to inhibit the growth of or to destroy bacteria and other microorganisms. An antimicrobial is any agent that destroys microorganisms or suppresses their multiplication or growth.

influenzae producing β-lactamase and occasional strains with resistance to chloramphenicol have also been observed, as have strains of Neisseria gonorrhoeae producing plasmid-mediated β-lactamase. Other changes are noted by Finland (1979).

Differences in resistance encountered in certain pathogens of humans are often observed in separated geographical areas (Finland, 1979), in different hospitals (O'Brien, Appendix I), or in local outbreaks, e.g., of chloramphenicol-resistant strains of Salmonella typhi in Vietnam and Mexico (Finland, 1979). The committee could find no comparable assessments of the trends in resistance to antimicrobials that might have occurred in the major pathogens of food animals over the last three decades.

The prevalence of clinically significant antimicrobial-resistant bacterial strains correlates with the increasing use of antimicrobial agents in the course of clinical practice (Finland, 1955a,b,c; 1979).

The necessity for therapeutic use of antimicrobial agents in the treatment of overt disease in animals has not been questioned. However, the continuous use of subtherapeutic levels of antimicrobials in animal feeds for growth promotion, improvement of feed efficiency, and disease prophylaxis has been criticized as posing dangers to human health by making an important quantitative contribution to the pool of antimicrobial-resistant bacteria that may be transferred to the human population. Possible "qualitative" effects of the selection pressure imposed by subtherapeutic usage on resistance profiles or transfer mechanisms are discussed below and by O'Brien (Appendix I).

For regulatory purposes, the Food and Drug Administration (FDA) defines subtherapeutic use as the administration of doses less than or equal to 200 g of antimicrobial per ton of feed for 2 weeks or longer. However, the FDA has approved the marketing of some antimicrobial agents for use at levels below 200 g/ton to treat certain diseases (Animal Health Institute, 1979). Therefore, the current definition of subtherapeutic use in animal feeds encompasses certain uses that are therapeutic in intent in addition to those for prophylaxis and the improvement of growth and efficiency of feed conversion.

In the hope of preserving the effectiveness of the antimicrobial agents that are important in the therapy of human diseases, some governments (e.g., the United Kingdom) have regulated the use of these agents as growth promotants in animal feeds (Swann et al., 1969). Antimicrobials used to treat humans are still approved in those countries for use in animals on veterinary prescription.

Similar actions have been under consideration in many other countries, including the United States. Since penicillin and the tetracyclines are effective and widely used in the therapy of human disease, the FDA has proposed restriction of their subtherapeutic use in animal feeds.

This committee has attempted to determine if human health is affected by the subtherapeutic use of antimicrobials in animal feeds and to ascertain what additional information is needed to make a more definitive determination.

NATURE OF THE SELECTION PRESSURE IMPOSED BY ANTIMICROBIALS

It is important to distinguish between the effects of an antimicrobial drug on a single antimicrobial-sensitive microbial strain and the effects on a heterogeneous mixture of species or strains. When antimicrobial drugs are brought into contact with multiplying susceptible microorganisms, the organisms are generally inhibited from multiplying further or are killed. When the susceptible organisms constitute a portion of the total microbial flora that is exposed to the drugs, the elimination of the susceptible organisms is generally followed by some degree of compensatory multiplication of the more resistant or nonsusceptible strains. Such a shift in the composition of the enteric flora may facilitate infection by a pathogen (Seelig, 1966).

Another important consideration in the evaluation of possible effects on human health is the possibility of "qualitative" as well as quantitative changes in resistance brought about by the continuing selection pressure exerted by subtherapeutic levels of antimicrobials in animal feeds. To date, most research has been focused on quantitative changes, i.e., changes in the prevalence of resistance, primarily because techniques to study qualitative changes have only recently been developed. Qualitative changes could include the development of new combinations of resistance genes, combination with genes for other characteristics, e.g., toxins, the spread of such plasmids to new hosts and, possibly most seriously, the evolution of more efficient, wider host-range transfer mechanisms. The evidence for such changes is discussed by Jacoby and Low (Appendix C). The implications of this "molecular epidemiology of plasmids" are considered by O'Brien (Appendix I). Another change that could be regarded as qualitative is the shift in the composition of the gastrointestinal flora under the selection pressure of subtherapeutic dosages of antimicrobials. This shift will produce changes in the interactions of the flora components with each other and with the host. Little is known about the composition of the

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gastrointestinal flora in either humans or animals, especially the anaerobic components (Savage, Appendix D). Therefore, because of the lack of data and methodology to carry out in-vivo experiments, it is not possible to assess the significance for human health of such shifts.

Investigators who have studied resistance in the gastrointestinal flora of humans or animals have generally observed changes in the prevalence of R factors in E. coli or its close relative Salmonella because these organisms are easy to culture and manipulate and because they can be pervasive pathogens in both humans and animals. The significance of resistance in these two species may not be the same since their "ecology" is different. This is discussed briefly in the next section.

A thorough assessment of the significance for human health of various uses of antimicrobials requires knowledge of the consequences of the selection pressures imposed by intermittent, therapeutic doses versus subtherapeutic continuous feeding and of different routes of administration. Unfortunately, there are insufficient data comparing these regimens. Such data would have been of invaluable assistance to the committee during its deliberations.

MECHANISMS AND TRANSFER OF RESISTANCE

In most instances bacterial resistance to antimicrobial agents is conferred by extrachromosomal genetic elements called plasmids. Those conferring resistance are called R factors or R plasmids (Jacoby and Low, Appendix C). These plasmids are widely distributed among bacterial species, including those that are pathogenic in humans and animals. Some plasmids transfer between species of different genera.

Two general types of R plasmids have been identified on the basis of their transmissibility characteristics:

Large R plasmids harbor approximately 100 genes and are transmissible to other cells by a process called conjugation or bacterial mating. Approximately 25 plasmid genes code for functions that are required for transmissibility, several other genes code for replication functions, and from four to six genes generally code for resistance to antimicrobials. The functions of other genes on the larger R plasmids have not been identified.

Small R plasmids usually harbor approximately 10 genes. These plasmids do not carry the genes for transmissibility and are not transmissible when by themselves. However, a transmissible plasmid

may sometimes mediate the transfer of an otherwise untransmissible one when present simultaneously in the same cell.

Some pairs of R plasmids cannot coexist stably in the same host cell, a phenomenon referred to as "incompatibility." This simple criterion has enabled investigators to differentiate R plasmids into more than 40 incompatibility groups. The mechanism of incompatibility is not yet understood. It may be related to the control of plasmid replication.

Different R plasmids may represent a group of genetic elements of diverse origin. However, even if located on plasmids in different incompatibility groups, the genes that confer resistance to specific antimicrobials are very similar. This may be explained by recent observations that certain antimicrobial resistance genes are located on segments of DNA (transposons) that may spontaneously translocate from one plasmid to another, thereby disseminating the resistance gene(s) among various plasmids (Kleckner, 1977). Factors that promote or inhibit the transfer of R factors *in vivo* are not completely understood (Jacoby and Low, Appendix C).

The biochemical mechanisms of resistance are known in most instances. R plasmids confer resistance to antimicrobials either by encoding for enzymes that chemically modify and thus inactivate the agent, by specifying a substitute metabolic enzyme that is insensitive to the agent, or by specifying a decrease in cell permeability to the agent. All three types of resistance mechanisms may be determined by the same plasmid, whether transmissible or nontransmissible. Thus, R plasmids are endowed with genes that increase the probability of survival of host cells in the presence of combinations of antimicrobials. The continued spread among bacteria of resistance to more than one antimicrobial and the further acquisition of additional resistance genes by individual R plasmids results from the selection pressure imposed by the use of antimicrobials.

A number of factors affect the transfer rates of plasmids between species, e.g., the frequency with which R^+ enteric bacteria come into contact with other bacteria and the environment in which they meet. The logistics of transfer of bacteria from animals to humans and of interbacterial transfer of plasmids may be different for different organisms. Salmonellae are generally infrequent, abnormal components of the flora of animals and humans, but when present they occur in enormous numbers that increase the potential for transfer at such times. *E. coli*, compared to the anaerobic flora, usually constitute a numerically small proportion of the gastrointestinal flora of animals and humans, but its continuous

presence (Savage, Appendix D) offers a different potential for transfer.

Bacterial genetic aspects of drug resistance have been reviewed by Jacoby and Low (Appendix C). The dissemination of drug-resistance genes among diverse bacterial genera is also discussed by these authors as well as by O'Brien (Appendix I). The epidemiology of plasmid transfer has not been studied in sufficient detail.

Whatever motivation lies behind the use of antimicrobial agents in any specific situation, the consequences will be the same. Administration of an antimicrobial will result in a selection pressure favoring an increase in the prevalence of resistant organisms.

THE USE OF ANTIMICROBIALS IN HUMAN MEDICINE

The use of antimicrobials in both hospitalized and ambulatory patients is extensive. Kunin (1979) reported that between 23% to 37.8% of hospitalized individuals receive them. Finkel (1978) estimated from dispensed prescription data and FDA certification records that approximately 190 million prescriptions for the major antimicrobials were filled for ambulatory patients in the United States in 1977. This is nearly one course of treatment per year for each person in the United States and includes approximately 43 million prescriptions for tetracycline.

The consequences resulting from the administration of antimicrobials to humans have been examined by Finland (1979). Hartley and Richmond (1975) reported that oral intake of tetracycline leads to the emergence of a predominantly tetracycline-resistant coliform gastrointestinal flora within 48 hours in those treated. The excretion of resistant organisms continues at least 10 days after the treatment is terminated (Richmond, 1975).

A complete evaluation of the increased prevalence of resistance to antimicrobials would require consideration of not only the contributions from subtherapeutic and therapeutic use in animals but also the extent to which these agents are administered to humans. Richmond and Linton (1980) studied the use of tetracyclines in the County of Avon in England and its possible relation to the excretion of tetracycline-resistant bacteria. They estimated that one in 130 individuals in the county carried a large proportion of tetracycline-resistant organisms in their alimentary tracts. Examination of swabs from sewers in predominantly residential areas of Bristol (in Avon) (Linton et al., 1974) indicated that approximately 3% of the isolated

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coliforms were resistant to tetracycline. Richmond and Linton (1980) concluded that, ". . . there hardly seems a need to postulate a veterinary source for the resistant coliforms encountered in the human population. This is not to say that resistant E. coli of animal and poultry origin cannot reach the human population: clearly they can and do (Linton et al., 1977). And some resistant salmonellae of animal origin certainly seem to have caused serious human epidemic disease (Anderson, 1968[b]). But whether the use of antibiotics in the animal and poultry rearing industries has a major quantitative impact must be questionable; . . ."

The approach adopted by Linton and Richmond is necessarily indirect and requires a number of approximations and assumptions. Although no similar studies have been conducted in the United States, the data reported by Kunin (1979) and Finkel (1978) on prescriptions in this country indicate that the administration of antimicrobials to humans is widespread.

THE USE OF ANTIMICROBIALS IN AGRICULTURE

The livestock and poultry industry has undergone dramatic changes since 1950. Operations that were extensive became more intensive. There were increases in the size of facilities, the number of animals reared, and a move toward centralization. Socioeconomic changes, as well as advances in biomedical sciences, nutrition, engineering, and management, have all contributed to this evolution.

Shortly after antimicrobials had been discovered and their therapeutic use in humans and animals had begun, investigators learned that the addition of antimicrobials to animal feed was effective in growth promotion, improvement of feed conversion, prophylaxis, and treatment of certain diseases. These effects of antimicrobials are especially useful when animals are stressed either by intensive husbandry practices or shipment.

The use of antibiotics (and most probably sulfonamides) in animal husbandry has steadily increased since 1950 as has animal production (Table 1). In 1978 approximately 48% of the antibiotics produced were designated for addition to animal feeds or for other (minor) uses (U.S. International Trade Commission, 1979). The motivation for such use and the economic consequences of restricting subtherapeutic concentrations of antimicrobials in feed have been dealt with in reports by the National Academy of Sciences Board on Agriculture and Renewable Resources (BARR, Appendix K) and the U.S. Department of Agriculture (1978).

TABLE 1

Antibiotic Production from 1950 to 1978 (millions of kg)[a,b]

Year	Total	Medicinal Use in Humans and Animals	Added to Animal Feed and Other Applications
1978	11.66	6.08	5.58
1977	10.48	6.35	4.58
1976	9.30	4.72	4.54
1975	8.30	4.26	4.04
1974	9.30	5.99	3.36
1973	9.43	5.72	3.72
1972	7.53	4.45	3.08
1971	8.12	4.90	3.22
1970	7.67	4.35	3.31
1969	5.99	3.36	2.63
1968	4.67	2.72	1.95
1967	4.29	2.36	1.91
1966	4.40	2.45	1.91
1965	3.40	2.13	1.27
1964	2.95	1.77	1.18
1963	3.04	1.91	1.13
1962	2.86	1.81	1.04
1961	2.31	1.50	0.82
1960	2.13	1.36	0.77
1959	1.68	1.04	0.64
1958	1.59	1.18	0.41
1957	1.47	1.08	0.39
1956	1.24	0.89	0.35
1955	0.95	0.71	0.24
1954	1.05	0.83	0.22
1953	0.94	0.74	0.20
1952	0.79	0.67	0.12
1951	0.69	0.58	0.11
1950	0.39	0.39	Mentioned, but no figure

[a]Data extracted from reports of the U.S. International Trade Commission (1951-1979).

[b]Values exclude production of sulfonamides.

The mechanisms by which antimicrobials improve growth and the efficiency of feed conversion are not fully understood. Some suggested effects include modification of host metabolism, nutrient sparing or alteration of nutrient absorption, and selective activity against microorganisms (BARR, Appendix K).

Total amounts used and patterns of usage, which vary with species and geographic location, are described in reports by BARR (Appendix K) and the Animal Health Institute (1979). Information on the total amount of specific antimicrobials administered for each application listed above does not appear to be available.

Certain antimicrobials (streptomycin, tetracyclines, penicillin) are also used to control plant pathogens. Although this application might also have consequences for human health, the amounts used are much smaller (Goodman, Appendix B).

The addition of subtherapeutic amounts of antimicrobials to animal feeds continues to be of concern because of its implications for human health and because some believe that this use is unessential. In the United States therapeutic concentrations of drugs are given to livestock extensively, with or without veterinary prescription. Treated animals are not always isolated from untreated ones, and animal-to-animal transfer of R^+ organisms is known to occur (Levy, 1978). Since most herds and flocks receive antimicrobials somewhere in the production chain either for growth promotion, prophylaxis, or therapy, it is difficult to identify slaughtered livestock that have not been given antimicrobials or have not been exposed to animals that had.

DEFINITION OF HUMAN HEALTH HAZARD

The difficulty of determining whether human health is affected by the subtherapeutic use of antimicrobials in feeds is compounded by the diversity of opinions concerning the definition of a hazard to human health. Some view an increase in the pool of antimicrobial-resistant bacteria or an increase in Salmonella shedding by food animals as a source of danger. Others maintain that a significant hazard to health exists only if antimicrobial-resistant organisms can be shown to be transmitted to animal handlers or to meat processors. Others attach importance only to the passage of resistant microorganisms to meat consumers. Still others view these circumstances as unrealized, potential hazards. These scientists insist that incremental morbidity and, perhaps, excess mortality that can reasonably be attributed to resistant

organisms resulting from subtherapeutic use of antimicrobials must be documented before risk to human health from this cause can be substantiated. This divergence of opinion was reflected in the committee itself.

There are no data from which to predict quantitatively the change in the morbidity and mortality of humans that might result from an increased prevalence of resistant bacteria in animals or from the transfer of these organisms to humans. Although a measurable risk to human health cannot be ascribed to these phenonema, they remain plausible potential hazards since some of the individual steps in the transmission chain to humans have been independently demonstrated but not quantified (see Chapter 3).

An increased prevalence of resistant bacteria may result from the administration of both therapeutic and subtherapeutic levels of antimicrobials to animals and human beings. Thus, the question is raised whether antimicrobials used subtherapeutically in the meat industry add measurably to the carriage of resistant organisms, the incidence of clinical illness, or the number of complications resulting from antimicrobial resistance in the treatment of diseases. The committee could find little relevant information on the relative selection pressures for antimicrobial resistance exerted by continuous low-dose feeding versus intermittent higher, therapeutic doses. This subject is examined later in this report.

Other possible hazards to health (also discussed below) received committee attention but were eliminated from further deliberations because they were not central to the major issue or because there was insufficient information.

Katz (Appendix E) prepared for committee consideration an assessment of the potential hazards to humans presented by residues of antimicrobials in livestock and poultry meat products. Surveys have shown that slaughtered animals may contain residues of penicillin or tetracycline that probably resulted from inadequate withdrawal times or large dosages (Huber, 1971). More recent surveys (Katz, Appendix E; Mussman, 1975; USDA, 1979) indicate that the residues resulting from penicillin or tetracycline used as feed additives were generally below the limits currently permitted by the FDA. Residues of tetracyclines were undetectable in animals slaughtered 1 to 5 days after withdrawal of antimicrobial-containing feed. The small amounts of these residues in the muscle tissues of animals do not survive normal food preparation because of heat inactivation during cooking (Katz, Appendix E).

Katz (Appendix E) reported that there has been concern that the therapeutic use of penicillin has resulted in significant residues. However, he noted that current regulations governing the subtherapeutic use of penicillin as a feed additive appear to result in very low or infrequent residues in meat.

Katz concluded, "It is doubtful that antibiotic residues or their degradation products will provide any selective pressure on enteric bacteria contaminating the carcasses of animals." The committee concurs with this assessment. It believes that further studies of the effect on human health resulting from penicillin and tetracycline residues in meat would not elucidate the hazard from subtherapeutic levels of antimicrobials in animal feeds.

The committee also viewed an assessment of the immunological consequences to humans resulting from penicillin and tetracycline residues in livestock and poultry meat products (Adkinson, Appendix J). Although pertinent information is limited, the committee concurs with Adkinson's conclusion that "there is little reason to believe that foodstuffs obtained from animals fattened with antibiotic-supplemented feed impose a significant risk to human health by contributing to antibiotic-induced allergic reactions."

Adkinson indicated that further investigations in several areas could provide information that would be useful in clinical situations. However, the committee believes that immunological problems arising from the use of antimicrobials in animal feeds are not a serious health risk for the general population.

In attempting to define the possible hazards to human health, the committee wished to know how the acquisition of antimicrobial resistance affected the virulence of pathogens infecting humans and animals. Since limited conclusive information appeared to be available (Jacoby and Low, Appendix C), the committee decided not to review this topic in depth.

CHAPTER 2

MEASURING EFFECTS ON HUMAN HEALTH FROM THE SUBTHERAPEUTIC USE OF ANTIMICROBIALS IN ANIMAL FEEDS

A complex chain of events begins with the addition of subtherapeutic levels of antimicrobials to animal feed, proceeds to the selection of bacteria bearing R plasmids in the animal gut and the transfer of these bacteria to humans (or their plasmids into the flora of humans), and ends with possibly adverse effects on human health.

The entire chain can be thought of as a stochastic process. All steps of the chain are apparently possible, but have not been quantified. For example, the extent of the normal transfer of R^+ organisms between animals or between animals and humans has not been adequately measured.

The entire length of this chain is germane to this study, i.e., how does a change in the addition of antimicrobials to animal feed at the beginning of the chain alter the probability or severity of diseases or the ability to treat them at the end of the chain?

A number of complications can arise when attempting to resolve this question. This is illustrated by the following series of questions that should be addressed when treating a hospitalized patient suffering from septicemia due to Salmonella.

1. Is the Salmonella strain resistant to antimicrobials?

2. Is the resistance plasmid mediated?

3. Was the Salmonella resistant to drugs when the infection occurred or did it acquire resistance through transfer of an R factor from the patient's resident microbial flora?

4. Did the patient acquire the Salmonella infection from contaminated meat or from another person?

5. If the infection was acquired from meat, had the animal received an antimicrobial?

6. Was the antimicrobial given to the animal for growth promotion, for prophylaxis, or for treatment of an illness?

7. If the Salmonella acquired resistance by R-factor transfer from E. coli, was the resistant E. coli selected by an antimicrobial used in previous treatment or did the patient acquire a resistant E. coli strain from another person or from ingestion of contaminated meat? If the resistant strain was acquired from another person, was that person infected via the food chain and did the food chain begin with an animal that had received subtherapeutic doses of antimicrobials?

Determination of the answers to all of these questions for an individual patient is obviously very difficult and may well be impossible.

Antimicrobial use varies greatly among producers of cattle, swine, and poultry, within each of these subsets of the industry, and among different parts of the country. Moreover, the distribution channels are long and complex. Consequently, a consumer may purchase meat that was processed in a distant plant from animals raised in a still more distant place.

The beef consumed by a household may have come from a feedlot in which antimicrobials were used only to treat sick animals, but its pork may have come from pigs fed tetracycline for growth promotion. Even if Salmonella may be judged more likely to have come from one source than from another, it may have acquired resistance by plasmid transfer from E. coli derived from either source.

EPIDEMIOLOGICAL STUDIES

The definitive epidemiological study of effects related to the subtherapeutic use of antimicrobials in animal feeds should encompass all aspects of meat production from animal breeding to consumption.

Methods

The committee examined the approaches that could be taken to relate the subtherapeutic use of antimicrobials in animal feed to the risk of increased morbidity and mortality in humans. It then grouped these approaches into six categories.

Cross-Sectional Studies (Population or Prevalence Surveys). Total communities, random samples of a total community, or selected subsets of a population may be identified and asked

to provide answers to questions, undergo laboratory or clinical procedures, or both. Such surveys are conducted once, and the findings are customarily presented as prevalence ratios of a disease or condition between populations. Differences in the prevalence of the disease or condition in demographic subgroups may be interpreted as reflecting differences in risk caused by the exposure under study. Specifically, populations that have had considerable exposure and others with minimal exposure to animals receiving antimicrobials via their feed could be surveyed to determine the prevalence of some specific conditions, e.g., urinary tract infection caused by R^+ organisms. The results of such surveys may be confounded by differences in prevalence of some associated causal or risk factors, such as socioeconomic status. For instance, elevated rates of diarrheal disease in farm workers handling feed containing antimicrobials, when compared to rates in nonfarm groups, may be due as much to differences in sanitation in the work and home environments as to the acquisition of resistant pathogens from animals.

Surveys are conducted frequently because they are relatively inexpensive and generally quite rapid. Survey data are generally unreliable in identifying the differences between statistical association and cause and effect, partly because the effects of time are difficult to incorporate into a cross-sectional survey. This weakness presents a particular disadvantage in any situation that changes over time.

Case-Comparison Studies. The characteristics of patients with a disease or other condition may be compared with those of a group of people who are free of the disease or condition, but whose other characteristics, such as age, sex, race, and history of exposure to therapeutic levels of antimicrobials, are similar. The comparison subjects are often selected from the same clinical setting in which the cases were discovered, but may be selected from groups living in the same neighborhood or perhaps by a random sampling of the general community. The characteristics of both groups and the exposure to which they have been subjected are surveyed retrospectively. For instance, patients with a disease believed to be attributable to resistant microorganisms can be matched with comparison subjects. Exposure to animals, to animal products, or to feed containing antimicrobial agents is the determined retrospectively. The efficiency of such a study may be improved by careful selection of comparison subjects to match the characteristics of the cases. The relative risk associated with certain characteristics, such as degrees of exposure to antimicrobials in animal feed, may be estimated by calculating odds ratios. Such studies may be relatively inexpensive and can be conducted

rapidly, but they require careful predetermination of the characteristics to be matched and investigated retrospectively. If important variables are missed or mismatching occurs, results may be interpreted incorrectly.

Cohort Studies. Variables related to risk of a population or a selected subset of a population may be identified and characterized, and the study group followed over time to observe new cases of illness. For instance, generally healthy individuals with high exposure to animal feeds containing antimicrobials, or to meat from animals that consumed such feed, and other members of the same community without such exposure may be followed for a year or longer. Populations with different exposures can be examined for differences in incidence or severity of diseases or for difficulties encountered during their treatment. The differences in these measurements will reflect the risk associated with the different exposures.

Cohort studies are generally considered to be the most effective approach to establishing differences in risk associated with various conditions, but they are expensive and time-consuming. Moreover, they require careful recordkeeping, meticulous followup of the cohort, and attentiveness to ensure that differences in medical care or inaccurate definitions of morbid events do not confound the study.

Experimental Studies. After a defined study population is identified and characterized, an appropriate subgroup is subjected to an experimental procedure. The test group is then followed to observe the effects of the procedure. The remainder of the initial group serves as a control. Experimental studies, particularly when the exposed group is randomly selected, are very effective in establishing the cause-and-effect relationship between the experimental variable and the observed outcome. Thus, if it were possible to select a large number of subjects and randomly assign them to groups that are or are not exposed to animals fed antimicrobials or to the products of those animals, useful information would undoubtedly accrue.

The performance of experimental studies with humans may be questioned on ethical grounds if there is any known or theorized hazard of the exposure being investigated. Moreover, such controlled trials are frequently very difficult to conduct because the subjects may not adhere to the regimen to which they have been randomly assigned. Experimental studies cannot yield definitive information on the likelihood that similar effects would result at similar frequencies under natural conditions.

Investigation of Epidemics. Outbreaks of a disease may be investigated retrospectively with epidemiological methods. Such investigations cannot quantitate the prevalence of diseases since only reported cases can be investigated. Nevertheless, they can be useful in establishing a probable chain of transmission.

Case Reports/Case Series. Although not an epidemiological method, reports of individual cases or series of cases may also establish that a particular sequence or chain of events is possible. They cannot be used to predict the prevalence of a disease or condition or the risk of any practice.

WHAT IS THE IDEAL STUDY?

The ideal study would start with the selection of animal-rearing facilities marked by sharply different practices in antimicrobial use--i.e., no use; subtherapeutic use in feeds for improving growth and feed conversion and for prophylaxis; intermittent therapeutic use; and both subtherapeutic and therapeutic use. A substantial number of facilities in each selected category should be studied to ensure that any observed differences in flora are more likely to be related to differences in antimicrobial use rather than to other confounding variables.

Fecal specimens should be examined from a large enough sample of animals in each flock or herd to monitor changes in the proportion of animals carrying Salmonella spp. and E. coli with resistance and the percentage of these resistant organisms in each animal's flora. Ideally, full microbiological characterization of the flora, both aerobic and anaerobic, should be performed for both animals and humans. Special attention should be given to changes in those indices associated with intermittent therapeutic applications of antimicrobials.

Similar studies should be conducted of employees, their families, and a comparison group of neighbors employed in other occupations to determine whether their microflora follow patterns like those observed in animals.

At the next several steps in the production chain--the slaughterhouses and wholesale and retail butcher shops--bacteriological studies of carcasses, the work environments, employees, their families, and their neighbors should be pursued over time to determine whether changes in the indices of drug resistance occurring in the production facilities can be traced through the processing plants. The processing plants should, of course, receive meats from only one of the specified categories of animal producers.

Finally, these same investigative techniques should be used in comparable communities where the meats and meat products are sold, prepared, and consumed, still separated by the type of antimicrobial use in animal feeds at their sources. Selecting comparable communities may be difficult since many factors need to be matched, e.g., the degree of hygiene in butcher shops. Samples of households should be selected, and the enteric bacteria of the family members should be characterized. The sampling would permit community-wide estimates of the prevalence of both resistant and susceptible Salmonella infection, as well as the prevalence of colonization with resistant E. coli. A system of defining, identifying, and recording illnesses should be established in order to compute the rates of disease caused by Salmonella and to determine the special problems that result from illnesses attributable to resistant strains.

Attempts should be made to trace the spread of Salmonella infection in these communities, identifying wherever possible cases attributable to contact with or consumption of contaminated meat and those due to secondary spread from person to person.

The nature of antimicrobial resistance in other pathogens causing illness in these communities should also be investigated to ascertain the extent to which R factors arising from therapeutic and subtherapeutic antimicrobial use in animals are transferred between microbial species and constitute a health problem.

A characterization of the R plasmids, which can be achieved by physical, genetic, or enzyme techniques (Jacoby and Low, Appendix C; O'Brien, Appendix I), could provide corroborative evidence of the direction of transmission and information on qualitative changes in resistance.

If the inquiries conducted on the breeding farms and the feedlots reveal no differences in the prevalence of resistant organisms in animals related to the differing use of antimicrobials, the study could be terminated. If such differences are apparent, further work would be needed. The second phase of the study would determine the occupationally associated risk of acquisition of resistant organisms and provide some information on the likelihood of spread from these foci. The carrier rates in samples from the neighborhoods of the abattoir workers should provide data on the prevalence of resistant enteric bacteria in general communities, as would the information derived from the household samples in the third phase. The community-based studies would relate the carrier rates in a community to the use of antimicrobials in animals and measure the associated burden of disease (see Table 2-1).

TABLE 2-1

The Ideal Comprehensive Study Design

PHASE 1: BREEDING FARMS AND FEEDLOTS

Study Groups	Study Procedures
Herds and flocks in four categories of antimicrobial use: no antimicrobial use subtherapeutic use only therapeutic use only both subtherapeutic and therapeutic use	Bacteriological indices: Prevalence of carriage of antimicrobial-resistant *Salmonella* spp./relative frequency of resistant organisms in each specimen
Animal handlers Family members	Prevalence of carriage of antimicrobial-resistant *E. coli*/relative frequency of resistant organisms in each specimen
Neighbors	Characterization of fecal flora and plasmids

PHASE 2: SLAUGHTERHOUSES, PROCESSING PLANTS, AND RETAIL BUTCHER SHOPS

Study Groups	Study Procedures
Carcasses	Bacteriological indices (as above)
Meat handlers Family members Neighbors	Human illness caused by bacteria

PHASE 3: COMMUNITIES

Study Groups	Study Procedures
Households selected on the basis of probability sample	Bacteriological indices (as above)
Cases of salmonellosis	Tracing of source of infection
Other cases of infectious diseases caused by bacteria	

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FEASIBILITY OF A COMPREHENSIVE STUDY

The committee reviewed methods for gathering information on the effects of various exposures on subsequent morbidity and mortality and for determining the specific needs for information on the subtherapeutic use of antimicrobials in animal feeds. It concluded that the comprehensive study described above could not be realized or even approximated. This decision reflects a number of facts, which are discussed below:

The use of antimicrobials differs markedly for the three major meat animals--cattle, swine, and poultry. Moreover, for each type of animal the use of antimicrobials varies in various parts of the country and at different times, e.g., with seasons or weather conditions. Moreover, it is often not possible to differentiate whether the antimicrobials had been given for growth promotion, prophylaxis, or treatment of manifest illness. In much of the industry, only insignificant numbers of animals have never received any antimicrobials, and in normal rearing and processing operations, it is not practical to identify these animals. During shipment from breeding farms to feedlots, groups of animals with different exposures to antimicrobials are often combined. The likely exchange of bacteria between animals under these circumstances further hinders the identification of the origin of any observed R^+ organisms.

The processing of meats and meat products also contributes to the mixing of meats from animals with different antimicrobial histories. Cross-contamination can also occur in these processes, e.g., via cutting boards and instruments. Currently, there is no hope of identifying communities in which residents can purchase only the meat of animals that had been exposed to only one regimen of antimicrobial usage.

The diseases and conditions likely to result from resistant microorganisms in the general population exposed to meat are relatively rare. Overt diarrhea caused by Salmonella, acute urinary tract infections in young women, and other illnesses related to infections with enteric pathogens all have extremely low incidence rates. Thus, any study attempting to relate an increase (possibly a small increase) in morbidity or mortality to exposure to R^+ organisms on meat (whether selected by subtherapeutic or therapeutic antimicrobial use) would be so massive that it would probably be unmanageable. Additionally, the chain of events linking antimicrobial-resistant bacteria in the animal gut to overt human infections is extraordinarily difficult to trace in any given case. This difficulty would increase the uncertainty in the study and jeopardize the validity of any risk estimates that might be developed.

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ALTERNATIVES TO A COMPREHENSIVE STUDY AND THEIR DEFICIENCIES

Less comprehensive approaches, although more realistic, cannot provide direct evidence of a consistent chain of causation from subtherapeutic antimicrobial use in animal feeds to human illness. Moreover, the fragmentary data that are available (discussed below) not only suffer from deficiencies of method and design but also leave gaps that can be bridged only by conjecture or speculation. Better research may repair the former, but little can be done about the latter. Some deficiencies of the narrower studies are listed below:

- Studies of the prevalence of antimicrobial-resistant bacteria in animals cannot be used to determine the extent of the transmission of R^+ enteric organisms from animals to human populations.

- In studies of the prevalence of R^+ organisms in farm workers, the R^+ bacteria acquired directly from animals cannot easily be distinguished from those R^+ organisms resulting from the selection pressure exerted by ingested or inhaled antimicrobials from the feeds.

- Studies of the prevalence of resistant organisms on carcasses or in abattoir workers do not provide direct measurements of the extent to which these organisms are transmitted to the general population, nor can they distinguish the effects of subtherapeutic use from the therapeutic use of antimicrobials.

- Studies comparing the prevalence of R^+ organisms in meat-eaters and vegetarians cannot differentiate whether observed differences in the meat-eaters can be attributed to the selective pressure for resistance exerted by the subtherapeutic use or by the therapeutic use of antimicrobials in the animals consumed. Such studies would also need to take into account the use of antimicrobials in the subjects themselves or in persons in their immediate environment.

- Studies on the prevalence rates of R^+ organisms in different populations can only imply the causes for differences in rates. They do not relate R^+ prevalence rates to increases in morbidity, mortality, or complications in therapy caused by resistance in the pathogen.

The foregoing discussion makes it clear that isolated studies on parts of the transmission chain cannot be used to quantitate the overall effects on human health resulting from the subtherapeutic use of antimicrobials in animal feeds.

This committee therefore concludes that it is not possible to conduct a feasible, comprehensive direct study of the effects on human health arising from the subtherapeutic use of antimicrobials in animal feeds. However, after examining some of the issues and the research conducted to date, the committee outlined several studies that might quantitate some of the stages in the chain of causation on which speculation of hazard is based.

The studies are presented as an indication of what the committee believes to be the most fruitful approaches. They will not provide a direct assessment of the effects on human health resulting from subtherapeutic levels of antimicrobials. Chapter 4 contains descriptions of these studies and some caveats on the interpretation of their results.

CHAPTER 3

CRITICAL REVIEW OF THE EPIDEMIOLOGICAL LITERATURE

Effects on human health resulting from the use of antimicrobials in animal feeds have been reviewed by distinguished scientists in the United States and Europe. Reports prepared by the Swann Committee (Swann et al., 1969), the Food and Drug Administration (FDA) Task Force (FDA, 1972), the FDA Environmental Impact Statement staff (FDA, 1978), the Office of Technology Assessment (1979), and the World Health Organization (1974, 1976, 1978) provide extensive coverage of the issues and background information. The majority opinion expressed in each report was that an increased prevalence of antimicrobial-resistant organisms presents a threat to human health and that the subtherapeutic use of antimicrobials increases the prevalence of R^+ organisms in animals. In all of the reports authors were not able or did not attempt to quantitate or estimate the relative contribution of the subtherapeutic use of antimicrobials in animals to health problems in humans caused by R^+ organisms--one of the objectives of this study. Majorities of the individuals involved in these various studies recommended policies aimed at restricting the subtherapeutic use of antimicrobials in animal feeds, particularly for those agents used in the therapy of diseases in humans.

The therapeutic use of antimicrobials in both animals and humans has been shown to result in an increased prevalence of resistant bacteria (Anderson, 1968a; Finland, 1979; Mercer et al., 1971). Similar results have been demonstrated for subtherapeutic use in both animals and humans (Goldberg et al., 1961; Savage, Appendix D; Siegel, 1976; Sprunt, 1977).

There is little evidence to indicate the quantitative contributions of these usages to effects on human health attributable to antimicrobial-resistant bacteria. The epidemiological literature on this topic is reviewed below.

INVESTIGATIONS OF EPIDEMICS

A number of investigations of epidemics have indicated that antimicrobial-resistant bacteria from animals can cause infections in humans (Anderson, 1968a; Anderson and Datta, 1965; Center for Disease Control, 1977; Fish et al., 1967; Lyons et al., 1980; Rowe et al., 1979; Threlfall et al., 1978a, b). Some of these epidemics, involving Salmonella typhimurium phage type 1 (Anderson and Datta, 1965), phage type 29 (Anderson, 1968a), and phage types 204 and 193

(Rowe et al., 1979; Threlfall, 1978a,b), took place in the United Kingdom. One case in Canada was also caused by S. typhimurium (Fish et al., 1967). Another outbreak, which took place in Connecticut, involved S. heidelberg (Center for Disease Control, 1977; Lyons et al., 1980).

In the earlier outbreaks in the United Kingdom, there was substantial evidence indicating that the resistant bacteria were selected by indiscriminate therapeutic use of antimicrobials in animals (Anderson, 1968a; Anderson and Datta, 1965). The value of phage typing, as it is used in the United Kingdom, is its clear demonstration that the entire sequence of events involved in transmission of R^+ organisms to humans via food is possible. In the Connecticut outbreak, the investigators demonstrated that the reservoir of the multiply resistant Salmonella infection was likely to have been harbored by a group of 1-week-old calves owned by one of the ill persons. Lyons et al. (1980) indicate that these calves had been treated by their owner for the diarrhea that they had when brought to the owner's farm. In the Canadian case, the patient became ill approximately 1 week after illness was observed in a cow with which he had had direct physical contact. The cow had been treated therapeutically with antimicrobials by the owner at the onset of the illness. Organisms identified as S. typhimurium phage type 10 with identical antimicrobial-resistance profiles were isolated initially from the cow and later from the patient. Although reports of these epidemics document the transfer of resistant bacteria from animals to humans, there are no studies to quantitate the frequency of that occurrence.

Furthermore, not all observations have reinforced the suggestion that a reservoir of resistant bacteria in animals provides the major source of resistant bacteria in humans. In a study conducted in Omaha, Nebraska between 1968 and 1978, Meyer and Lerman (1980) documented the rise and fall in prevalence of resistant strains of Shigella sonnei, which was a predominant pathogen in humans during that period. In 1973, they observed a peak in the prevalence of resistance to ampicillin and a similar pattern of resistance to five other antimicrobials. There was no evidence that the use of ampicillin in humans had changed during the course of the study.

Cherubin et al. (1980) reported a very similar pattern of rise and fall of resistance to ampicillin for Salmonella typhimurium isolated in New York City from 1965 to 1968. He attributed this pattern in humans to an epidemic of a resistant strain but pointed out that isolates from calves and other animals in New York State did not exhibit a similar change. Calves continued to harbor ampicillin-resistant strains at a prevalence that increased annually from 1972

to 1978. Thus, there is evidence for waves of resistant enteric flora in humans and in animals that do not correlate with each other or with patterns of antimicrobial usage in humans. This suggests a substantial degree of separation of reservoirs in humans and animals.

POPULATION SURVEYS

By conducting retrospective health surveys of farm families, rural families with no animals, and urban families, all having had a recent hospital admission, Smith et al. (1974) attempted to determine whether association with farm animals was connected with greater risk of disease. Comparing admission diagnoses, they found no significant differences and no preponderance of antimicrobial-resistant bacterial disease. However, since there were insufficient numbers of subjects in the survey and the study design was not satisfactory, no general conclusion can be drawn.

In other attempts to determine the possible consequences to human health from the subtherapeutic use of antimicrobials in animal feeds, investigators have studied the relative prevalence of antimicrobial-resistant bacteria among various groups or have attempted to demonstrate the possibility of particular steps in the chain connecting the antimicrobials in animal feeds to increased carriage of R^+ bacteria in humans. Discussions of many of these studies are contained in the consultant reports to the committee (Appendixes A-J). Those that deal with the epidemiological aspects of the question are reviewed briefly below. Limitations on the inferences that can be drawn from these narrow studies are detailed in Chapter 2.

As far as the committee could determine, there have been no adequate attempts to relate an increased carriage of R^+ organisms to putative increased morbidity and mortality or dilemmas caused by resistance in the treatment of infection. Thus, predictions of the magnitude of any possible risk cannot be made from results of studies on particular stages or steps in the transmission chain.

The prevalence of resistant enterobacteria in groups with varying exposure to domestic livestock has been investigated by Betinová (1972), Dorn et al. (1975), Fein et al. (1974), Linton et al. (1972), Siegel (1976), Siegel et al. (1975), Smith and Crabb (1961), Smith et al. (1974), Wells and James (1973), Wiedemann and Knothe (1971), and Woods et al. (1972).

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Smith et al. (1974) compared the proportion of E. coli with resistance to antimicrobials among several groups of Iowa families and their livestock. They compared rural families with and without livestock, urban families, and both urban and rural families in which one member had recently been discharged from hospital inpatient status. Results generally indicated that the proportion of E. coli with resistance to four or more antimicrobials was greatest among families with livestock, regardless of their recent association with hospitals, and lowest among both urban and rural groups without a recent hospital association or proximity to livestock. These results include neither quantification of the transfer of resistance between animals and humans nor specification of resistance patterns. Since all livestock raised by these families had received some antimicrobials, some members of these families had been exposed to both the livestock fed antimicrobials and the feed containing the antimicrobials. However, the study does suggest that persons working with livestock that are receiving antimicrobials do harbor more resistant E. coli than do families not exposed to livestock, independent of recent hospital exposures. Unfortunately, the history of antimicrobial use by these humans was not documented.

Comparing rural and urban dwellers in England, Linton et al. (1972) found a much higher proportion of drug-resistant coliform bacilli in the feces of rural adults working with livestock than in rural adults not so employed. Urban adults harbored an intermediate proportion of resistant organisms. Both urban and rural children less than 5 years old harbored the highest proportion of all groups tested. Although this finding suggests an association between working with livestock and increased levels of resistant organisms, the authors supplied no information on antimicrobial usage in either animals or humans nor was there a comparison of specific resistance patterns between the flora of the animals and that of the humans exposed to them.

Dorn et al. (1975) conducted a small study comparing the specific resistance patterns between Missouri farm families raising beef and hogs and their animals and between families both raising and consuming home-raised meat and their animals. They found a significant association between resistance patterns in animals and families who consumed home-raised meat but not between patterns in animals and families who did not consume meat they raised. They concluded that the exposure of humans to E. coli from animals through consumption of meat was more plausible than mere contact with animals as an explanation for interspecific crossover of transferable drug resistance.

Siegel et al. (1975) compared the proportion of antimicrobial-resistant coliforms in five groups of Illinois persons with varying exposure to antimicrobials and animals fed antimicrobials. They found that the proportion of the enteric flora with resistance to antimicrobials in these groups ranked in the following decreasing order: (1) people working on farms in contact with farm animals receiving antimicrobials in feed, (2) people residing on the same farms but not in contact with the animals, (3) people treated with antimicrobial drugs, (4) untreated people residing with treated individuals, and (5) untreated people not residing on livestock farms. In general, groups 1, 2, and 3 had similarly high proportions of coliforms with resistance to oxytetracyclines, dihydrostreptomycin, and ampicillin, group 5 had the lowest proportion, and group 4 was usually intermediate. These results indicate that living on farms, raising livestock fed antimicrobials, or being treated with antimicrobials resulted in approximately equivalent proportions of organisms with resistance to these three antimicrobials and that persons without direct exposure to antimicrobials or animals fed antimicrobials have fewer resistant organisms. Since Siegel and his colleagues presented few data concerning specific antimicrobials used in humans or animals or specific matching resistance patterns, it is not possible to evaluate the role of feed containing subtherapeutic levels of antimicrobials in increasing the prevalence of resistant bacteria in either animals or people.

From studies conducted in the Federal Republic of Germany, Wiedemann and Knothe (1971) indicated that both farm workers and city dwellers carried enterobacteria with resistance to antimicrobials (in some cases plasmid-mediated), but that the proportion of the total enterobacterial flora with resistance was higher in farm workers. The significance of the results they obtained by comparing the percentages of farm workers, their relatives, feed handlers, city dwellers, slaughterhouse workers, chickens, calves, and pigs carrying resistant Enterobacteriaceae cannot be evaluated since the number of subjects in each category was not reported.

Each of the studies noted above suffers from various methodologic deficiencies that should be avoided in future efforts. None adequately quantitates the nature and extent of exposure to antimicrobials. Particularly lacking are data pertaining to those drugs given therapeutically to the study subjects. Moreover, the studies do not specify the type of antimicrobial given to the animals. Most of the investigators do not adequately describe the contact between the animals and the subjects. Since these studies are cross-sectional, they can indicate whether an association exists between an increased prevalence of resistant bacteria in humans and contact with animals, or direct contact with feeds containing antimicrobials, but cannot establish that such contacts cause the increase. In only one study

was there an attempt to match the specific resistance patterns of E. coli from animals to those from exposed humans. None of the studies adequately documents the health status of the study subjects.

Despite their limitations, these studies lead to certain conclusions. Animals fed antimicrobial agents for extended periods or treated with antimicrobials develop resistant enterobacteria. People in close contact with those animals and their antimicrobial-containing feed are more likely to harbor resistant organisms in their gut flora than those without contact with animals. Patients who have received therapeutic drugs have a similar or even greater likelihood of carrying resistant organisms. Children who have not personally received antimicrobials also seem to have a high prevalence of R^+ organisms in their flora for reasons that are currently not clear. Future studies can be based on the assumption that these conclusions have been reasonably well established.

Two more surveys that deserve attention compared the prevalence of resistant E. coli in meat-eaters and in those who did not eat meat. Such a comparison would indicate if eating meat from animals that were presumed to have received antimicrobials was associated with an increased carriage of R^+ organisms. In a study by Guinée et al. (1970) the groups selected as meat-eaters were military kitchen personnel and office workers; those who did not eat meat were vegetarians and infants less than 6 months of age. Because the groups were relatively small and not strictly comparable, the conclusions drawn from these studies are questionable. But the percentages of the 77 vegetarians and 87 infants yielding resistant E. coli were not significantly lower than those in the meat-eating groups. This study does not provide evidence that eating meat is associated with an increased intestinal carriage of resistant coliforms.

Lebek (1972) reported the distribution of R factors among E. coli isolated from feces of healthy and ill human subjects. The percentages of various groups studied having R^+ organisms in their fecal flora were: hospital patients, 84% (74 of 88); healthy nurses, 82% (82 of 100); healthy soldiers, 51% (26 of 51); healthy 6-9 year olds, 65% (66 of 101); and healthy vegetarians, 57% (16 of 28). The vegetarians were described as "belonging to a religious community" that "takes no drugs and lives on a vegetarian diet," and the children, according to their parents, had not received antimicrobial drugs during the 2 years preceding the investigation. Although it is difficult to determine if the groups were strictly comparable, the results again do not support the concept that eating meat is associated with an increased intestinal carriage of resistant coliforms.

EXPERIMENTAL STUDIES

In the experimental studies described below, investigators have examined aspects of the transfer of resistant bacterial strains from animals to humans. By themselves these studies do not allow quantitative prediction of risk for the general population.

Hirsh and coworkers (Burton et al., 1974; Hirsh et al., 1974) examined the effects of varying doses of oxytetracycline (OTC) on colonization of the gut in humans following ingestion of resistant E. coli of bovine origin. No differences were found in length of time the organisms were excreted by those fed either 0 or 50 mg OTC/day, but 1,000 mg/day did potentiate the establishment of tetracycline-resistant E. coli.

In more direct experiments, Smith (1969) fed to a human various doses of resistant E. coli of animal origin containing unique resistance markers. The resident strains occasionally acquired resistance from the animal donor strains but the resistance was not maintained.

In an experiment that simulated the most likely exposure to be encountered by the general public, Linton (1977) studied five humans who, over a period of 3 months, handled, cooked, and ingested 15 chickens that had been sampled for E. coli. One of the five subjects was clearly colonized after handling but before eating. The colonization was transient. As in the experiment by Smith, plasmid-mediated resistance from E. coli in chickens was transferred to a strain of E. coli in the original resident flora of the human host.

In "feeding" studies, highly artificial conditions are sometimes created, e.g., huge doses are sometimes fed to a subject in a medium such as bicarbonate. Hence, results must be extrapolated with caution.

In two more experiments, investigators attempted to determine whether the administration of low doses of tetracycline to animals resulted in the transfer of resistant bacteria to humans. Hirsh and Wiger (1977) studied the fecal flora from 30 calves, 16 of which were fed 350 mg/day of tetracycline, and from their 20 handlers. A low level of transfer was found irrespective of whether the calves were receiving tetracycline.

Levy and coworkers (Levy, 1978; Levy et al., 1976a,b) observed an increased prevalence of antimicrobial-resistant intestinal bacteria in a farm family in contact with chickens fed tetracycline and the tetracycline-containing feed. Later, they detected resistance in organisms isolated from chickens not fed antimicrobials and from farm personnel other than the family. Of particular interest was the reversibility of the apparent selection for resistant organisms

after 9 months of usage. This was indicated by the finding that resistant organisms were not observed 6 months after discontinuance of the supplement. Furthermore, the use of only one antimicrobial (tetracycline) did lead to selection of strains with multiple resistance. There were some problems with the design of this study. More appropriate controls could have been used, and the sampling frequencies could have been more nearly equal. Resistance patterns reported in the earlier paper by Levy et al. (1976a) did not always include resistance to tetracycline, the agent in the chicken feed exerting the selection pressure.

When the results of these studies are examined together, they suggest that supplementation of animal feeds with antimicrobials can select for resistance in the enteric organisms of animals. This resistance can be transferred to humans who are in contact with these animals and their feeds or to those who handle the carcasses, e.g., those who prepare food for cooking. The resistance profiles selected may include resistance to agents in addition to those added to the feed since resistance genes are often linked. In some studies the prevalence of resistance has been shown to decline when supplementation is discontinued. In other instances herds receiving feed believed to be free of antimicrobials and with no recent history of antimicrobial therapy have a high prevalence of R^+ organisms (Smith et al., 1974). This may be attributable to contamination of the feed (Siegel, 1976). In January 1980, this topic was discussed at a conference sponsored by the U.S. Department of Agriculture on the contamination of feed by the sulfonamides. However, it cannot be predicted with certainty that the overall prevalence of antimicrobial-resistant organisms in animals will decrease to low levels if the subtherapeutic use of antimicrobials in feed is ended.

A large and complicated study was designed and conducted by Siegel (1976). He attempted to use bacteriophage typing to identify E. coli strains so that he could follow their transfer from various animal sources to humans--on the farm, in the slaughterhouse, or in the surrounding community. Despite some methodological problems, the study indicates that swine were the probable source of bacterial phage types that were found in poultry and beef cattle. These phage types were the same as those found most often in humans on farms. Presumably, they also originated from the pigs. Some of the farm strains that were prevalent on the carcasses were also found in slaughterhouse workers and in the slaughterhouse. There was evidence that other strains--some of which were carried by the workers or were present in the slaughterhouse--were transferred onto the carcasses at this point. Analysis of the flora of the consumers of the pork products was more difficult. Although pork consumers shared some common phage types with the workers in the slaughterhouse and

on the farm, the route and extent of transfer was impossible to measure. This type of observational study is limited in what it can demonstrate, since it cannot indicate the direction in which organisms transfer. Genetically marked organisms, such as those used by Smith (1969) and Hirsh et al. (1974), might provide a better technique for following transmission.

CASE REPORTS

Some case reports provide evidence for the transfer of plasmid-mediated resistance under normal conditions. Petrocheilou and coworkers (1977, 1979) described tetracycline-resistant plasmids found in a number of E. coli strains from a woman who had received prolonged tetracycline treatment for acne vulgaris. Her husband, who had received no antimicrobial therapy, also harbored such strains. The two E. coli strains were indistinguishable as were the plasmids they carried. This observation suggests that E. coli carrying R plasmids may spread from individuals under treatment to untreated close contacts.

The study by Neu et al. (1973) suggested that specific resistance patterns could be transferred (in the intestines of one patient and in the urinary tract of another) from one organism to another. The transfer was suggested by the similar spectra of antimicrobial resistance. Corroboration of the transfer by plasmid DNA homology studies, such as those reported by Petrocheilou and coworkers (1977, 1979), was not attempted in the study of Neu et al. (1973).

Brumfitt and coworkers (1971) studied urinary tract infections caused by E. coli in patients living at home. Seven (19%) of 37 female patients had infections characterized by resistant E. coli and 23 (62%) carried some resistant E. coli in their fecal flora. In eight patients who had predominantly resistant (> 60%) E. coli fecal flora, five had urinary tract infections characterized by resistant E. coli. In 15 patients whose fecal flora contained a lower proportion of resistant E. coli (0-50%), only one patient had a urinary tract infection characterized by a resistant strain. From these findings, the investigators concluded that resistant strains of E. coli, when carried in the intestine, were neither more nor less likely to infect the urinary tract than were nonresistant strains.

SUBTHERAPEUTIC USE IN HUMANS

The committee also examined reports pertaining to the consequences of long-term administration of subtherapeutic levels of antimicrobials to humans to learn if there were adequate data which,

upon extrapolation, might allow conclusions to be drawn about the effects on human health that could result from subtherapeutic levels of antimicrobials in animal feeds.

Goldberg et al. (1961) demonstrated that low dosages (10 mg per day) of OTC increased the prevalence of resistant bacteria in prison volunteers. Some subjects who did not receive OTC shed resistant coliforms. In those individuals whose resistant bacterial flora was increased by OTC, the prevalence of resistant coliforms returned to normal within 2 months after the OTC treatment was stopped.

Haight and Pierce (1954) reported a study in which naval recruits were given 250 mg of chlortetracycline or 100,000 units of procaine penicillin per day orally for 7 weeks. Those receiving antimicrobials gained more weight than did the controls.

There are a number of categories of infections for which antimicrobials have been used prophylactically in humans (Utz, Appendix A). A major use is the administration of tetracyclines to control acne in adolescents (Schmidt et al., 1973). Antimicrobials are also used in the prevention of endocarditis subsequent to rheumatic fever (McVay and Sprunt, 1953). Valtonen et al. (1977) have described the effects on enteric bacterial flora that result from neomycin prophylaxis in patients with hypercholesterolemia.

The committee examined many original research reports and a number of review articles on the subtherapeutic use of antimicrobials in humans including the following. Many papers on the use of tetracyclines to control acne have been reviewed by the American Academy of Dermatology (1975). Jukes (1973) described a variety of reports on the long-term administration of antimicrobials to infant. and children or to patients with tropical sprue. Reports on the subtherapeutic and prophylactic use of antimicrobials in humans have been reviewed extensively by the Council for Agricultural Science and Technology (in press) and Pfizer, Inc. (1978).

The subtherapeutic or prophylactic administration of antimicrobials to humans generally results in an increased prevalence of resistant organisms in the recipient. Sprunt (1977) demonstrated that this increase is lower when intermittent doses are injected intramuscularly than when prophylaxis is administered orally. Moreover, she reported that lower doses eliminate only a small portion of the resident flora, thereby permitting fewer resistant organisms to survive and multiply.

In most of these studies no added health risk attributable to resistant organisms was recorded. However, the number of individuals involved in any one study of prophylaxis was small, and the study designs did not have as their primary objective the detection of adverse health effects of antimicrobial use. Thus, it is not possible to quantify the risk of infection from resistant bacteria resulting from such antimicrobial usage. Risks from prophylactic use are indicated in a recent report of two patients who developed endocarditis due to resistant viridans streptococci after undergoing oral penicillin prophylaxis subsequent to rheumatic fever (Parrillo et al., 1979). How commonly this happens is not known. Since reports of such occurrences are rare, the committee believes that they probably occur infrequently.

EXPERIENCE WITH REGULATIONS IN OTHER COUNTRIES

Surveys of resistance in isolates from animals and humans have been conducted in several countries following the institution of various regulations governing the addition of antimicrobials to animal feeds. The reports of these surveys should be reviewed although they contain information that is far from conclusive. Moreover, surveys of the prevalence of resistance do not necessarily indicate "qualitative" changes in resistance, e.g., new combinations of resistance, more efficient transfer mechanisms, or a wider potential host range for a new plasmid.

In the United Kingdom the regulations recommended by the Swann committee (Swann et al., 1969) were implemented in 1971 although no baseline data on antimicrobial use or resistance patterns had been collected. The development of the regulations and their effects have been reviewed by Braude (1978), Linton et al. (1977), and Smith (1977). The "Swann regulations" restricted primarily feed supplementation with antimicrobials that have value in the therapy of infections in humans. However, the restricted antimicrobials have remained available through veterinary prescription for use in animals. Smith (1977) reported that "in the four years since [the 'Swann'] prohibition, the amount of tetracycline-resistant E. coli in the pig population might have decreased slightly but the incidence of pigs excreting these organisms (100% in 1975) had not." In the United Kingdom, Sojka et al. (1977) reported that from 1971 to 1974 there was no evidence of a consistent decline in total resistance; however, they reported a small increase in resistance to tetracycline in salmonellae isolated from animals.

Since 1974, 30%-35% of the Salmonella isolates from humans in the United Kingdom have been resistant to antimicrobials. Recently, there has been a rise in multiple resistance due to a particular prevalent phage type. In 1965, also in the United Kingdom, approximately 40% of the Salmonella isolates from bovines were resistant. By 1978 this had risen considerably: approximately 70% of the Salmonella isolates from bovines were resistant (L. Ward, Central Public Health Laboratory, United Kingdom, personal communication). Linton et al. (1977) and Braude (1978) point out that there is little indication that the overall sales of antimicrobials for veterinary use have declined as a consequence of the Swann regulations. Farm animals may well be receiving the same amounts of antimicrobials as in the past, ostensibly for different purposes, i.e., prophylaxis or treatment of disease rather than for growth promotion, and possibly by alternative routes of administration (Braude, 1978).

Regulations developed by the European Economic Community to control the use of antimicrobials in animal feeds came into force in 1974. They proscribed the addition of tetracyclines to feed, a practice that had been increasing in the Netherlands since the 1960's. Subsequent to this prohibition, a decrease in the prevalence of tetracycline-resistant strains of Salmonella in pigs and humans was reported by van Leeuwen et al. (1979). It is difficult to attribute this decrease unequivocally to the ban on tetracyclines as feed additives since an epidemic of one antimicrobial-resistant phage type (505) of Salmonella typhimurium contributed greatly to the prevalence of resistant strains in the early 1970's. It is questionable whether the decrease in resistance to tetracycline after 1974 was due to the change in regulations or to the cessation of the epidemic of this particular resistant strain in both humans and pigs. The prevalence of resistant strains before that epidemic was similar to the levels after regulations came into force. A similar decrease in resistance was not observed in isolates from calves for which tetracycline is still used therapeutically. Phage type 505 of S. typhimurium was not prevalent in this species. There are no data to show if the use of tetracycline has in fact decreased during the period studied by van Leeuwen and colleagues (1974-1978).

In the Federal Republic of Germany, Bulling and coworkers (1973) and Stephan et al. (1976a,b, 1977a,b) reported a decline in tetracycline-resistant S. typhimurium and S. panama in calves and pigs since the 1974 ban. There are no comparable data on isolates from humans in Germany nor on antimicrobial use in that country.

A specific strain of S. typhimurium (phage type 505) was a major contributor to the resistance pattern in the Netherlands and the Federal Republic of Germany. Since the epidemic caused by that

strain has now dissipated, it remains to be seen whether the trend of reduced prevalence of resistance will continue.

Data from Europe do not indicate whether restrictive regulations have actually reduced or averted hazards to human health.

CONCLUSIONS DRAWN FROM THE LITERATURE

Relatively little research on the subtherapeutic use of antimicrobials in animal feeds and the use of antimicrobials in animals generally is truly epidemiological. Reports are often based on regrettably few subjects observed for brief periods. The findings of such research are fragmented bits of information concerning isolated sections of the meat production system. Therefore, they do little to resolve the question: does the subtherapeutic use of antimicrobials in animal feeds relate to excessive morbidity and mortality of humans? However, the data indicate that antimicrobial-resistant organisms transfer from animals to humans who have been in contact with them on farms. Moreover, abattoir workers have been shown to harbor the same phage types as found in farm animals. The extent of subsequent person-to-person exchange has not been adequately determined. Furthermore, there is no evidence to prove that resistant bacteria are more prevalent among people consuming meat and meat products than among other groups.

There are no data linking human illness with the subtherapeutic use of antimicrobials in any aspect of animal husbandry, but the absence of information is certainly not to be equated with proof that the proposed hazards do not exist. For many questions pertaining to the subtherapeutic use of antimicrobials, the research is inadequate or nonexistent. The committee discussed in detail how this situation might be remedied. Its suggestions are contained in Chapter 4.

CHAPTER 4

STUDY POSSIBILITIES

The committee considered ways to remedy deficiencies in the information that has been used to support claims that the subtherapeutic use of antimicrobials in animal feeds creates a hazard to the health of humans. The studies suggested in this chapter are not to be interpreted as proposals of work that would provide a sufficient basis for an acceptable quantitative assessment of any risk to human health since the remaining gaps in knowledge would still have to be bridged by conjecture or speculation. Rather, the committee believes these studies to be the most fruitful approaches to quantifying some of the stages in the chain of events from which health hazards might result (see Chapter 2) so that speculations concerning such hazards may be more firmly based.

Study 1 should identify relative contributions of subtherapeutic and therapeutic antimicrobial regimens to the emergence of resistant enteric flora in animals. Studies 2 and 3 are designed to assess the extent to which carriage by humans of bacteria having R factors is associated with meat consumption or occupational exposure to bacteria from animals in abattoirs. Study 4 addresses the relationships between carriage of or occupational exposure to R^+ organisms and increased morbidity from urinary tract infections. Each proposal points out the limitations of the study and indicates what conclusions can be drawn from the data to be collected. Before these studies commence more detailed protocols should be evaluated by groups of individuals with expertise in the disciplines that are relevant to each study.

STUDY 1--THE EFFECTS OF SUBTHERAPEUTIC AND THERAPEUTIC DOSES OF ANTIMICROBIALS ON THE PREVALENCE OF R^+ ENTERIC ORGANISMS IN ANIMALS

In this study, the relationship between the appearance of resistant Enterobacteriaceae and the pattern and dose of antimicrobials used in the feed of food animals or otherwise administered to them could be examined. The proportion of *E. coli*, salmonellae, and other Enterobacteriaceae carrying R plasmids should be measured in beef cattle, hogs, and chickens before, during, and after they are fed and/or treated with various doses of tetracycline at growth promotional, prophylactic, and/or therapeutic levels. This study will clarify the contribution of subtherapeutic and therapeutic dosing regimens to the emergence of resistant enteric flora in animals.

Experimental Study Design

The committee regards the ability to obtain animals with a very low, preferably zero, incidence and proportion of R^+ enteric bacteria in their gut flora as central to the usefulness of this study. Alternative strategies, which could be adopted if it proves impossible to meet this criterion, are discussed under "Interpretation of Results."

Within each animal species, individual animals not previously exposed to antimicrobials should be allocated randomly to pens, and the pens should be assigned randomly to treatment groups, each of which contains more than one pen of animals. The number of animals in each pen and the number of pens may differ among the three animal species and should be specified so that the samples are of sufficient size for investigators to detect meaningful differences in the proportions of animals carrying tetracycline-resistant organisms among the various treatment groups.

Prior to random allocation, several fecal samples should be collected from each animal to establish the baseline for total flora, for the prevalence of R^+ organisms, and for the rate at which such pathogens as Salmonella are shed. Fecal specimens should be examined for a short period after penning in order to monitor exchange of bacteria among animals in each pen. Specimens should be examined at regular intervals thereafter to monitor the changes occurring during the feeding period. Bacteriological procedures should be determined on the advice of persons with expertise in the field of veterinary microbiology.

Animals should be divided into treatment groups as follows:

1. No antimicrobials. These animals should be given no antimicrobials of any sort during the feeding period.

2. Subtherapeutic levels of tetracyclines. These animals should receive tetracyclines for a period similar to that during which the antimicrobials are administered during typical rearing conditions for that species. Three dose levels should be given to different groups:

    a. A level no greater than the minimum regarded as necessary to elicit growth promotion or more efficient feed conversion.

    b. The usual level of antimicrobials fed to the particular animal species for growth promotion, improvement of feed conversion, and disease prophylaxis.

c. A level substantially above the usual feeding level but not above regulatory definition of a maximum subtherapeutic dose.

3. Therapeutic doses of tetracycline. These animals should not receive subtherapeutic antimicrobials. A simulated typical course of tetracycline therapy should be administered to the group at a specified time during the feeding period in accordance with accepted veterinary practices for the test species. If it is decided that tetracycline should be given to only some animals in the group, this must be recorded.

4. Subtherapeutic plus therapeutic doses of tetracyclines. These animals should be fed the usual subtherapeutic levels (2b) of tetracycline during the entire feeding period and should be treated with a simulated therapeutic course of tetracycline, thus combining 2b and 3 above.

In the event of sickness requiring the use of therapeutic doses of antimicrobials in any treatment group, all animals judged (on predetermined criteria) to be sick should be permanently removed from the experiment and from contact with the remaining test animals and should be treated as indicated by prudent veterinary practice.

Antimicrobials should be discontinued in all animals prior to slaughter at the time designated by current regulations to ensure that residual levels do not exceed those permitted in carcasses. Monitoring of R^+ bacteria should be continued in some animals for a period after the animals normally would have been slaughtered to observe all changes in R^+ prevalence after antimicrobials are withdrawn.

Design of data acquisition. The design of the quantitative culture techniques should enable investigators to detect a biologically important difference in the number of tetracycline-resistant organisms in fecal specimens from the various treatment groups. These techniques should be designed by persons with expertise in microbiology and in biostatistics as it relates to the analysis of microbiological data. The protocols and analytical methods should be specified in advance.

Data to be collected:

1. The number of animals in each group carrying Enterobacteriaceae with R factors mediating tetracycline resistance should

be recorded. For each stool specimen, the Enterobacteriaceae should be enumerated and serotyped, and the percentage carrying R factors mediating resistance to tetracycline should be ascertained by quantitative culture techniques.

2. Salmonellae shed by each animal should be enumerated and serotyped.

3. The weight of each animal should be recorded at the beginning, at the end, and at regular intervals during the experiment.

4. Feed should be analyzed chemically for a range of antimicrobials prior to use to avoid contamination and to ensure that the desired levels of tetracycline are achieved when supplementation is intended. Contaminated feed should be discarded to avoid the selection (by antimicrobial agents other than tetracycline) of organisms carrying plasmids on which resistance to tetracycline is linked to other resistances. The feed consumed in each pen should be recorded, and the consumption of antimicrobials by individual animals should be calculated.

Site conditions: One or more sites, such as an agriculture experiment station or veterinary college, should be selected. They should enable investigators to meet the following criteria:

1. Test animals should be maintained in a controlled experimental environment, but they should be handled in a manner that simulates current growing and finishing practices before marketing.

2. The sites should contain isolation facilities to prevent transfer of bacteria among pens. Different personnel will be needed for each pen to prevent cross-infection between pens.

3. Direct veterinary supervision during the course of the experiment should be available.

4. There should be local slaughtering facilities in which the bacteriological characteristics of carcasses produced during this experiment can be monitored.

Observational Studies

These studies require the cooperation of commercial operations that use antimicrobials in a pattern similar to some or all of those used in the experimental study design described above. Fecal samples and feed samples should be collected, and the feeding history and weight gains of the animals throughout the feeding period should be recorded. Fecal samples should be analyzed microbiologically with the same methods that were proposed in the experimental study design. The data obtained from these groups should be analyzed in a manner that will enable investigators to compare the appearance of R^+ organisms in these groups to that of the groups in the experimental study design.

Costs

The numbers of animals used in the following calculations are for illustrative purposes only. They should not be interpreted as the committee's recommendation for quantities needed to obtain statistically meaningful results. Investigators should determine the number to be used in accordance with the principles indicated in the study design outlined above. Animals should be observed past the usual marketing time in order to assess the full consequences of antimicrobial withdrawal on the microbial flora. The costs of acquiring specimens are included under Veterinary Supervision estimates. Partial costs may be recovered by sale of the animals.

EXPERIMENTAL STUDY: BOVINES

Costs are based on six groups of 20 animals.

Purchase of Animals:

In order to acquire animals with known antimicrobial exposure histories, it may be necessary to pay more than the going market rate, which varies with time.

Acquisition of 120 400-lb calves at approximately $1.25/lb will cost:

120 x 400 lb x $1.25/lb	= $ 60,000	

Feed and Care Costs:

120 x 240 days x $5.00/day	= 144,000	

Veterinary Supervision (2 h/day):

2 h x 240 days x $30/h	= 14,400	
Animal Husbandry: Total Direct Cost	=	$218,400

Laboratory Services (based on one specimen/animal every 3 days):

80 specimens/animal x 120 animals x $25/specimen	=	240,000
Total Direct Costs	=	458,400
Overhead (estimated 50%)	=	229,200
BOVINES, TOTAL COST		$687,600

EXPERIMENTAL STUDY: SWINE

Costs are based on six groups of 20 animals.

Purchase of Animals:

Acquisition of 120 specific pathogen-free animals, approximately 4 weeks old, will cost:

120 x $180/animal	= $ 21,600	
Feed and Care Costs:		
120 x 180 days x $2.00/day	= 43,200	
Veterinary Supervision (2 h/day):		
180 days x 2 h x $30/h	= 10,800	
Animal Husbandry: Total Direct Cost	=	$75,600
Laboratory Services (based on one specimen/animal every 3 days):		
60 specimens/animal x 120 animals x $25/specimen	=	180,000
Total Direct Costs	=	$255,600
Overhead (estimated 50%)	=	127,800
SWINE, TOTAL COST		$383,400

EXPERIMENTAL STUDY: CHICKENS

Costs are based on six groups of 50 chickens.

Purchase of Animals:

Acquisition of 300 specific pathogen-free chicks will cost:

300 x $15	= $ 4,500	

Purchase cost may be lower if chicks are reared from eggs rather than obtained already hatched.

Feed and Care Costs:

300 chickens x $0.50/day x 100 days	= 15,000	

Veterinary Supervision (2 h/day):

100 days x 2 h/day x $30/h	= 6,000	
Animal Husbandry: Total Direct Cost	=	$ 25,500

Laboratory Services (based on one specimen/animal every 3 days):

34 specimens/animal x 300 animals		255,000
x $25/specimen	=	
Total Direct Costs	=	280,500
Overhead (estimated 50%)	=	140,250
CHICKENS, TOTAL COST		$420,750

TOTAL COSTS - STUDY PROPOSAL 1: EXPERIMENTAL STUDY

Bovines	=	$687,600
Swine	=	383,400
Chickens	=	420,750
		$1,491,750

Any costs recovered from the sale of the animals could be returned to the contractor.

OBSERVATIONAL STUDY

Cost for the observational study will be dependent upon the arrangements that can be made with those commercial concerns willing to cooperate in the study and the sizes of the groups to be observed.

Interpretation of Results

There are a number of possible results from this study:

Animals receiving antimicrobials may have no greater prevalence of R^+ organisms and no more Salmonella with pathogenicity for humans than animals not receiving antimicrobials. This is an unlikely result, given data to the contrary. However, if such a result did occur, there would be no evidence to indicate that the subtherapeutic use of antimicrobials might affect human health.

The prevalence of R^+ organisms or Salmonella with pathogenicity for humans may be similar in animals on subtherapeutic regimens of antimicrobials and those on therapeutic regimens, and these prevalences might be greater than that in the untreated animals. In this instance there would be no evidence to indicate that subtherapeutic use of antimicrobials increases the possible hazard to humans over that from the therapeutic use of antimicrobials.

If the prevalence of either R^+ organisms or of pathogenic Salmonella resulting from the subtherapeutic use of antimicrobials significantly exceeds that from therapeutic use, then one still cannot infer that the subtherapeutic use of antimicrobials represents a definite risk of human disease. Further studies would be required to measure the effects on human health resulting from increased prevalence in animals of R^+ organisms or of Salmonella with pathogenicity for humans.

If animals with a low initial prevalence of R^+ enteric organisms cannot be obtained, the study should be modified to determine if the feeding of strictly monitored antimicrobial-free feed results in a decline in R^+ prevalence.

The validity of such conclusions depends upon the degree to which the experimental conditions, antimicrobial regimens, etc., actually parallel production practices. This can be ascertained by including the observational study in the overall design.

STUDY 2--STUDIES OF VEGETARIANS AND NONVEGETARIANS

This study would measure the extent to which carriage of bacteria with R factors is associated with meat consumption. It begins with the hypothesis that consumption of meat contaminated with antimicrobial-resistant bacteria from animals would result in meat-eaters having a higher prevalence of antimicrobial-resistant enteric bacteria than do vegetarians. Two related studies are described in Chapter 3.

Study Design

Groups of vegetarians and nonvegetarians should be compared for prevalence of resistant Enterobacteriaceae in their fecal flora. The groups must be carefully controlled for factors such as antimicrobial usage, age, socioeconomic status, family size, age of children, and pets. Seventh Day Adventists and Mormons would provide two convenient study groups. In this study the prevalence of R^+ factors is monitored by the use of resistance to tetracycline because of its likely frequency in the population and its therapeutic significance. Moreover, it is the subject of the proposed restrictions by the Food and Drug Administration (FDA).

Data to be Collected

The number of persons in each group carrying Enterobacteriaceae with R factors mediating resistance to tetracycline should be determined. The sample size should be sufficiently large for investigators to identify a meaningful difference, if it exists, in the rate of colonization by organisms with resistance to tetracycline.

The percentage of Enterobacteriaceae that carry R factors mediating resistance to tetracycline in each fecal specimen should be ascertained by quantitative culture techniques. These techniques should be designed to detect a biologically important difference in the prevalence of tetracycline resistance factors in stool specimens obtained from the groups being compared.

Costs

A preliminary survey should be conducted to ascertain approximate prevalence rates of R^+ carriage. A pilot study of 100 vegetarians and 100 nonvegetarians would cost approximately $100,000 for epidemiological and laboratory services. The size and desirability of the full survey should be decided on the basis of the results of the preliminary survey.

Interpretation of Results

If the prevalence of organisms with R factors is the same in vegetarians as in meat-eaters, there would be no support from this study for the belief that either the therapeutic or subtherapeutic use of antimicrobials in animals affects human health via R^+ organisms on or in meat.

If meat-eaters have a greater prevalence of R^+ bacteria than do vegetarians, it is possible to infer that the excess of antimicrobial-resistant bacteria is associated with the ingestion of meat, but it is not possible to differentiate between the effects of subtherapeutic and therapeutic uses of antimicrobials in meat sources. Other confounding variables would include the handling of meat products and contamination of cooking utensils or work surfaces. If meat-eating is associated with a greater R^+ prevalence, then further studies would be needed to determine if the excess of R^+ organisms results in excess morbidity or mortality or complicates the treatment of diseases and to determine the influence on R^+ prevalence of other aspects of diet.

STUDY 3--STUDIES OF ABATTOIR WORKERS, THEIR FAMILIES, AND NEIGHBORHOOD CONTROLS

This study would measure the extent to which occupational exposure of humans to bacteria from animals is associated with carriage by humans of bacteria with R factors. Secondarily, it would gauge the spread of these bacteria or R factors among humans who are in close contact with one another.

Abattoir workers are exposed to large numbers of bacteria from animals but are not exposed to antimicrobial-containing feeds. Consequently, they are exposed to the organisms (which are likely to be resistant in animals fed antimicrobials), but unlike farm workers they are not exposed to dusts containing antimicrobials that might be ingested or inhaled, thereby exerting a selective pressure favoring resistant enteric or respiratory tract bacteria. Thus, abattoir workers can be used productively to evaluate the propensity of bacteria from animals to colonize humans and to study the secondary spread of such bacteria and/or R factors via contact spread to family members. If secondary spread occurs, family members would be expected to have an intermediate but increased R factor carriage rate compared to controls.

Study Design

Groups of abattoir workers involved in the processing of poultry, pork, and cattle, their household contacts, and neighborhood controls should be compared for the prevalence of R^+ Enterobacteriaceae in their stool flora. The groups must be carefully controlled for factors such as antimicrobial usage, age, socioeconomic status, family size, age of children, and household pets. In this study the prevalence of R factors is monitored by the use of resistance to tetracycline as a marker for the same reasons given under Study 2.

Data to be Collected

The number of persons in each group carrying Enterobacteriaceae with R factors mediating resistance to tetracycline should be determined. The population groups studied should be large enough to provide a high probability for identifying a meaningful difference, if one exists, in the carriage rate for the tetracycline resistance factor. Information on the task performed by the abattoir workers should be collected in order to determine their potential for exposure to enteric organisms.

For each stool specimen, quantitative culture techniques should be used to determine the percentage of Enterobacteriaceae that carry R factors mediating resistance to tetracycline. These techniques should be designed to detect a biologically important difference in prevalence.

Costs

A preliminary survey should be conducted to ascertain approximate prevalence rates of R^+ carriage. The rates found in such a survey would determine the size and desirability of a full survey. A pilot study of 100 abattoir workers and 100 controls would cost approximately $100,000 for epidemiological and laboratory services. The size and desirability of the full survey should be decided on the basis of the results of the preliminary survey.

Interpretation of Results

By comparing rates at which R^+ bacteria are carried by abattoir workers with those of their families and neighborhood controls, one can determine if any association exists between occupational exposure to bacteria from animals and an increased prevalence of R factors in the human enteric flora. Such comparisons would also allow one to evaluate spread of R factors among humans in close contact with one another.

If the prevalence of organisms with R factors is the same in abattoir workers and controls, this study would provide no support for the belief that resistant bacteria, resulting from either the therapeutic or subtherapeutic use of antimicrobials in animals, significantly affects the flora or health of humans.

It would not be possible to attribute a greater prevalence in the abattoir workers solely or in part to the subtherapeutic use of antimicrobials in feeds. Further studies would be needed to determine

if any observed excess of R^+ organisms in abattoir workers resulted in excess morbidity or mortality or in complications in the treatment of diseases.

RATIONALE FOR CONDUCTING STUDIES OF MORBIDITY AND MORTALITY IN HUMANS

The three studies described above are intended to serve as indicators of the development and transfer of resistant enteric organisms. If there is no indication of an important association between the exposure of animals to either subtherapeutic or therapeutic levels of antimicrobials and the development of resistant enteric organisms, or if exposure to bacteria from animals fails to influence the flora of humans, the possibility of detrimental effects on human health would not be sufficiently well established to justify widespread changes in the current use of antimicrobials throughout the meat industry. If either the vegetarian study or the abattoir study indicates that the carriage of resistant organisms by humans is associated with meat consumption or occupational exposure to bacteria from animals, more extensive evaluations of morbidity and mortality would be justified. One possible study is detailed below.

STUDY 4--COMPARISON OF CONTROLS WITH SUBJECTS WITH URINARY TRACT INFECTION

Laboratory screening services frequently discover urinary tract infections (UTI) by culturing urine samples obtained from women during routine medical examinations. These screenings can be linked with other microbiological tests to determine the existence of an association between R^+ Enterobacteriaceae in stool flora and the occurrence of primary UTI and to ascertain the proportion of primary UTI infection that is caused by antimicrobial-resistant Enterobacteriaceae. Brumfitt et al. (1971) have conducted a small study of these topics.

Study Design

If the results of Studies 1, 2, and 3 indicate that more extensive evaluations of morbidity and mortality are necessary, the committee recommends that a screening for primary UTI be conducted on 5,000 females who work in the meat processing industry (e.g., in poultry dressing plants) and who thus are exposed to high levels of antimicrobial-resistant bacteria. If the screening

can be conducted in a processing plant that can provide reliable data concerning the antimicrobials received by the birds that are processed, the antimicrobial-resistant profiles of the flora of the poultry carcasses should be compared with those of the isolates from humans. Such additional sampling would add to the costs of the study but would provide exceedingly useful corroborative information. In conjunction with the screening of meat processors, an equally large group of controls should be studied, e.g., the 9,000 women in East Boston, Massachusetts first studied by Kass (1978) could be resurveyed. These surveys would enable investigators to compare female meat processors who have bacteriuria with similarly infected women in an urban setting far removed from contact with livestock and to match cases and controls for age, race, parity, and antimicrobial history.

Participating screening services should use uniform procedures and criteria to detect UTI's. New cases should be asked to return for a confirmatory urinary culture, at which time a rectal swab should be obtained to determine the carriage of R^+ Enterobacteriaceae.

Women who have not received antimicrobials during the preceding calendar year should then be matched with control (non-UTI) women of comparable age, race, parity, and negative antimicrobial history as determined by the screening service. For each stool specimen from a case or control, all Enterobacteriaceae should be serotyped and the percentage with R factors should be ascertained by quantitative culture techniques. The number of cases and controls required to discriminate between no association and a meaningful difference at acceptably low probabilities for error must be specified in a detailed protocol. The control population of 9,000 should yield approximately 150 cases of bacteriuria, approximately 100-120 of which will be infected with *E. coli* (Kass, 1978).

The role of resistance selected by the therapeutic use of antimicrobials in humans should be rigorously investigated and controlled in this study. Even a negative result with a large group would enable investigators to estimate the upper bound of the risk to human health.

All confirmed cases of UTI should be studied in the following manner.

1. Each isolated infecting strain should be tested for antimicrobial susceptibility patterns, type distribution, and plasmid DNA sequence homology.

2. Each patient should be sent a questionnaire to determine:

Basic demographic data

Basic household composition

Identification of infection as a sporadic case or part of an outbreak

Occupation of patient, spouse, and household contacts

Outcome of illness--expense in terms of workdays lost, medical expenses, etc.

Present health status of subject

Antimicrobial history of subject and household members and pets during past 2 years

Hospitalization history of subject during past 2 years

Major illness of subject and household members during past 2 years

Patients with UTI characterized by bacteria with resistance to antimicrobials should be compared with patients with UTI characterized only by antimicrobial-sensitive bacteria to determine whether there are differences in antimicrobial history, in exposure to animals or carcasses that had been in contact with antimicrobials, or in exposure to feeds containing antimicrobials.

Interpretation of Results

If women with resistant enteric flora have a relatively high risk of a UTI compared to those without resistant fecal flora, the prevalence of R^+ fecal flora among new cases should be greater than that among comparable controls. The observation of such an association would favor the interpretation that the carriage of R^+ organisms, from whatever source, is indicative of an elevated risk of UTI. An insignificant association would indicate that the presence of R^+ enteric flora does not make an important contribution to this form of morbidity. This study would be sensitive to a differential virulence between resistant and susceptible enteric flora causing UTI's, but it would not elucidate the contribution of

the subtherapeutic use of antimicrobials in animal feeds to this cause of illness. Whatever the results of this study, they would not provide justification for drawing general conclusions about the likely changes in the virulence of other pathogens gaining resistance to antimicrobials.

The comparison of UTI prevalence between groups with high and low exposure to R^+ enteric organisms would provide some indication of the amount of primary UTI that is attributable to an occupational exposure. With a sufficiently large control group and high risk groups in proximity to animals or carcasses with high levels of R^+ organisms, a positive result would enable some conclusions to be drawn about total antimicrobial use in animals and UTI in humans caused by antimicrobial-resistant bacteria. No conclusion could be drawn about the relative contribution of subtherapeutic use to the increased morbidity.

Costs

If such a study were conducted over 2 years, which would allow time for training of personnel and analyzing results, an estimated $150,000 per year would be required. If the study were spread over 3 years, the total annual cost would be reduced by approximately 15%. If the information concerning the antimicrobials received by poultry passing through the processing plant can be obtained and the flora of the carcass is subsequently sampled, then additional costs will be incurred.

CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

A relatively small proportion of the research that has been conducted on the subtherapeutic or therapeutic use of antimicrobials in animal feeds is truly epidemiological. Much of the information on this subject has been generated by poorly controlled studies of small numbers of subjects observed for brief periods.

An ideal study of the effects on human health resulting from the subtherapeutic use of antimicrobials in animal feeds would be able to relate, without conjecture or speculation, antimicrobials in feed to changes in morbidity or mortality or to treatment complications caused by resistance to antimicrobials in humans who had been exposed to animals or meat products during processing, handling, or, especially, consumption. Changes in morbidity and mortality could be used to quantitate the risk of the potential hazards posed by increased prevalence of resistant bacteria, by the development of plasmids conferring multiple resistance, or by the evolution of especially efficient transfer mechanisms within the reservoir of bacteria in animals.

Because the literature provides only isolated fragments of information relating to various components of the [illegible] system, it is insufficient for assessing the difference between the use of [illegible] levels of [illegible] and the non-use of [illegible] tween the consequences of subtherapeutic and therapeutic [illegible] antimicrobials in [illegible]. Reports in the United Kingdom, the Federal Republic of Germany, and the Netherlands do not indicate clearly whether restrictive regulations have actually reduced or averted the postulated hazards to human health. Restrictions on the use of antimicrobials in the United Kingdom may well have altered the patterns of their use without significant alteration in the total amounts used or their consequences. Therefore, it is not possible to conclude from the literature that restricting only the subtherapeutic use of antimicrobials will cause a decrease in the overall prevalence of R^+ organisms in humans or animals. Furthermore, there is little information on qualitative changes in resistance in the enteric bacteria of animals or humans. For example, no data exist to indicate the extent to which new resistance combinations or more efficient transfer mechanisms have

been brought about by the subtherapeutic use of antimicrobials in feeds.

After reviewing the evidence, the committee concluded that the postulations concerning the hazards to human health that might result from the addition of subtherapeutic antimicrobials to feeds have been neither proven nor disproven. The lack of data linking human illness with subtherapeutic levels of antimicrobials must not be equated with proof that the proposed hazards do not exist. The research necessary to establish and measure a definite risk has not been conducted and, indeed, may not be possible.

The committee gave considerable thought to the necessary elements of the ideal study to measure accurately the effects on human health resulting from the subtherapeutic use of antimicrobials in animal feeds and how such a study should be designed.

It concluded that a comprehensive, all-encompassing study could not be realized or even approximated because of insurmountable technical difficulties. This decision reflected a number of facts:

- There are marked differences in both the therapeutic and subtherapeutic use of antimicrobials in the various major species of animals raised for their meat and in the different regions of the country.

- It is not clear how much the subtherapeutic use of antimicrobials, as compared to the therapeutic use, contributes to the prevalence of resistant bacteria in animals.

- Household members consume meats from animals that have been exposed to different antimicrobial agents during the course of different regimens, both subtherapeutic and therapeutic. Thus, it is not practical to determine the original selective pressure for resistant bacteria that may occur on a particular piece of meat.

- It is difficult to determine the relative contributions made by subtherapeutic and therapeutic levels of antimicrobials in animals or in humans to the pool of resistant bacteria that may affect human health.

The committee concluded that less comprehensive approaches, although more feasible, could not provide direct evidence of a consistent chain of events from animal production to meat consumption. However, it did outline a sequence of four possible studies on individual aspects of the transmission chain. The results of these studies, if interpreted with the recommended precautions, would provide a useful scientific background for policymakers. At best, however, the remaining gaps in our knowledge will still have to be bridged by conjecture or speculation.

The committee also discussed some nonepidemiological aspects of the subtherapeutic use of antimicrobials. A better understanding of the mechanisms through which subtherapeutic levels of antimicrobials produce beneficial effects may lead to development of other substances or other treatments of equal or greater effectiveness, thereby rendering this entire issue moot. For example, if the beneficial effect is caused primarily by controlling infections, then other preventive techniques such as new vaccines may yield equal benefit. If the mechanism is nutritional, i.e., nutrient sparing or an alteration of nutrient absorption, then new nutritional supplements may yield the desired result.

Plasmids in isolates from animals and humans must be characterized to assess the possibility that subtherapeutic levels of antimicrobials in animals produce qualitative changes in resistance to antimicrobials in the enteric flora of animals, changes that might subsequently affect human health.

RECOMMENDATIONS

present ... study ... errors of ambiguous design and small sample size that have caused such difficulties in interpretating the data. The proportionate contributions to resistance made by subtherapeutic and therapeutic uses of antimicrobials in animals and in humans urgently need resolution.

The committee RECOMMENDS increased and continued monitoring and surveillance of the occurrence of antimicrobial resistance in bacteria in animals, in meat and meat products, and in humans, especially in cases of human illness due to Salmonella and pathogenic E. coli. If restrictions on antimicrobial use are adopted, the committee RECOMMENDS that monitoring be continued in order to determine the effect of such restrictions.

The committee RECOMMENDS further research on:

- the mechanism of action of subtherapeutic levels of antimicrobials in feed (BARR, Appendix K) including characterization of the composition and interactions of the gastrointestinal flora (Savage, Appendix D),

- factors that inhibit the development and transfer of resistance in vivo (Jacoby and Low, Appendix C), and

- studies on the epidemiology of plasmid-mediated resistance to antimicrobials in both animals and humans (O'Brien, Appendix I).

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THE APPENDIXES

The following papers, Appendixes A through K, were commissioned by the Committee to Study the Human Health Effects of Subtherapeutic Antibiotic Use in Animal Feeds. They were used by the committee as working papers and are attached to the committee's report for information only. They do not constitute part of the foregoing report prepared by the committee. All references to these papers should be attributed to the authors, not to the committee.

A transcription of the public meeting held August 23, 1979 also formed part of the working papers used by the committee. These records may be obtained on loan from Dr. Enriqueta C. Bond, National Academy of Sciences, 2101 Constitution Ave., N.W., Room 347, Washington, D.C. 20418.