Papers and Originals

Some Principles in the Chemotherapy of Bacterial Infections*

JOHN CROFTON,† M.D., F.R.C.P., F.R.C.P.ED.

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Summary: The classification of antibacterial drugs into bactericidal and bacteriostatic groups is of clinical value. Bactericidal drugs are to be preferred when possible as they are more rapidly effective, may be synergistic when used in combination against bacteria difficult to eliminate, and are less likely to leave residual "persistent" organisms. Antagonism between chemotherapeutic agents is of little clinical importance.

The modes of action of antibacterial drugs are reviewed. The penicillins, cephalosporins, and cycloserine interfere with bacterial cell wall synthesis. Polymyxin, colistin, and nystatin affect the bacterial cell membrane. Protein manufacture by the bacterial ribosome is interfered with by the tetracyclines, chloramphenicol, erythromycin, and lincomycin, and the initiation of protein molecules by streptomycin. Streptomycin, kanamycin, and neomycin also cause the ribosome to manufacture warped proteins. Nalidixic acid, griseofulvin, and novobiocin upset the D.N.A. replication of the bacterial chromosome.

Many of the factors adverse to the success of chemotherapy are unimportant when powerful drugs are given in large doses to relatively fit patients infected with highly sensitive bacteria. But these factors become important when patients, particularly debilitated patients, are infected acutely or chronically with some of the more obstinate bacteria. Some grasp of these principles is therefore both intellectually satisfying and clinically useful.

Introduction

The greatest triumph of medicine is to prevent or abolish disease, but it may be the tragedy of a great originator that this very triumph tends to make him forgotten. Few people are interested in dead dragons, however grateful they may be to the rescuing St. George while their hair is actually being singed by smoke and fire. It is true enough of tuberculosis that we have scotched the snake, not killed it, and that the disease is still an enormous problem in many countries, but its present relative control in Britain might lead us to forget some of the pioneers who fought the dragon while he was in his prime.

This lecture is founded in honour of a great statesman of medicine, a man with a capacity to inspire and organize both doctors and laymen in the service of sick people. The system of co-ordinated prevention and treatment of tuberculosis which Philip initiated in Edinburgh only five years after Koch discovered the tubercle bacillus—and only five years after he himself had qualified in medicine—was copied throughout Britain

and throughout the world (Clayson, 1957; Williams, 1957). It provided the organization through which the great advances in treatment and prevention made in recent years could attain their optimal effect in minimal time. It is a measure of Philip's achievement that this lecture will mention tuberculosis only incidentally.

It is useful for the clinician, busy with his everyday practice, occasionally to step back and take a look at principles. Chemotherapy was initially a large empirical branch of treatment, but advances in molecular biology and in what one might call structural biochemistry are gradually probing the mechanisms behind empirical facts and evolving coherent and intellectually satisfying patterns. In this lecture I intend to glance at some of the principles which are emerging in chemotherapy, for though it may be that the clinician can deal with many infections with no more than a casual glance at the decorative propaganda that floods across his breakfast table, the minority, those clinical problems which are really difficult, require some knowledge of principles and some skill in applying them. I myself am merely a clinician, condemned to superficiality by the intrinsic and fascinating diversity of my trade, so all I can try to do is to attempt to interpret for other clinicians my gleanings from the much more profound studies of those undeviated by bedside responsibilities.

Bactericidal and Bacteriostatic Drugs

For a number of purposes it is convenient to differentiate between bactericidal and bacteriostatic drugs. Bactericidal drugs actively kill bacteria, which are eliminated with the aid of the body's defences (Fig. 1). Bacteriostatic drugs merely prevent the growth of the organisms, the final elimination depending largely on the body's defences. If the body's defences are insufficient or if the drug is stopped prematurely the patient's bacterial population may rise again and a relapse occur (Fig. 2).

Bacteriostatic Drugs.—These include a number of frequently used agents, such as chloramphenicol, erythromycin, novobiocin, sulphonamides, and tetracyclines.

Bactericidal Drugs.—These include most of the others, in particular the following: ampicillin, carbenicillin, cephaloridine, cloxacillin, colistin, gentamicin, kanamycin, lincomycin, methicillin, penicillin, polymyxin, and streptomycin.

The differentiation between bactericidal and bacteriostatic drugs is to some extent relative. If a bacteriostatic drug is administered on a long-term basis and drug resistance does not arise, the organisms will eventually be dealt with by the host's defences if they are prevented from growing. Their elimination may be assisted by the development of active immunity. For instance, in tularaemia contracted in the laboratory, a well-known risk, the date of onset could be rapidly identified and the optimum time to start treatment with tetracycline, a

* Based on the first Sir Robert Philip memorial lecture given at the Royal College of Physicians of Edinburgh, November 1968.

† Professor of Respiratory Diseases and Tuberculosis, University of Edinburgh, Edinburgh 10.

bacteriostatic drug, determined. It was found that the results were better and there were fewer relapses if treatment was begun not immediately after the onset but a week later (Overholt *et al.*, 1961), indicating a synergistic effect of tetracycline with the host's defences. Some bacteriostatic drugs, such as erythromycin, are bacteriostatic at low concentrations but bactericidal at high (Garrod and O'Grady, 1968). With most bactericidal drugs the rate of killing increases with concentration.

FIG. 1.

FIG. 2

Modes of Action of Chemotherapeutic Agents

In general, bactericidal drugs act in one of three ways. They either affect the bacterial cell wall, so interfering with the cell's osmotic defences and causing it to absorb water and burst; or they affect the cell membrane and so probably cause loss of vital metabolites; or they have a lethal effect on protein synthesis. Bacteriostatic drugs interfere with the manufacture of protein at an earlier phase and result in insufficient rather than warped proteins, and so prevent the growth and proliferation of the bacteria without actually destroying them. These mechanisms of action will now be discussed.

The sites of action of the principal antibiotics are indicated diagrammatically in Fig. 3, in which bactericidal drugs are shown in capital letters. The penicillins, cephalosporins, and cycloserine affect bacterial cell wall synthesis. Polymyxin B, colistin, amphotericin B, and nystatin all affect the barrier function of the cell membrane. The bacteriostatic drugs, tetracyclines, chloramphenicol, erythromycin, and lincomycin, affect the earlier stages of manufacture of proteins by the ribosome; streptomycin, kanamycin (and neomycin) cause the ribosome to miscode and manufacture wrong proteins or enzymes which may poison the cell. Nalidixic acid, griseofulvin, and novobiocin affect the deoxyribonucleic acid (D.N.A.) replication of the chromosome.

Action of Antibiotics on Protein Synthesis

General data are taken from Goldberg (1965), Carter and McCarty (1966), and Sanders and Cluff (1968) (Fig. 4).

FIG. 3.—Sites of action of antibiotics.

Messenger-ribonucleic acid (mR.N.A.) conveys the coding for the manufacture of proteins from the D.N.A. of the nucleus to the ribosomes situated in the cytoplasm, where the proteins are manufactured. Chloramphenicol interferes with the attachment of mR.N.A. to the ribosome so that the "instructions" fail to reach the ribosome and the relevant protein is not manufactured (Jardetzky and Julian, 1964). Transfer-ribonucleic acids (tR.N.A.) of different types convey amino-acids, link them to the messenger-ribosome complex, and place them in proper sequence for the manufacture of proteins. The tetracyclines (Suarez and Nathans, 1965), erythromycin, and lincomycin (Chang *et al.*, 1966) interfere, probably in different ways, with the attachment of the amino-acid-tR.N.A. complex to the ribosome.

In in-vitro studies streptomycin, kanamycin, and also neomycin cause a distortion of the 30S portion of the ribosome (Cox *et al.*, 1964; Davies, 1964; Davies *et al.*, 1964), which results in a misreading of the instructions from mR.N.A. and in consequence the manufacture of false proteins (Davies, 1965). This in itself seems unlikely to be lethal to the bacterial cell, as cells of certain strains can survive and grow, albeit sluggishly, in the presence of streptomycin in spite of making as much as 80% of faulty proteins (Gorini and Kataja, 1964). Recent work (Luzzatto *et al.*, 1968) suggests that the most critical action of streptomycin is to form aberrant complexes, involving two portions of the ribosome (30S and 50S particles), and also mR.N.A. and tR.N.A., at a stage at which all these molecules are normally associating to initiate a new protein molecule. In this way protein synthesis is completely blocked.

FIG. 4.—Antibiotics and protein synthesis.

Action of Antibiotics on Cell Wall and Membrane

Fig. 5 symbolizes a portion of bacterial cell wall and membrane. Penicillin and cephaloridine prevent the cross-linking by transpeptidization of the glycopeptide backbone of the cell wall (Strominger and Tipper, 1965). The cell wall of bacteria contains three kinds of structural units peculiar to the wall and not found in host cells: muramic acid, D-amino-acids, and sometimes diaminopimelic acid (Perkins, 1963). This accounts for the lack of toxicity of penicillin and cephaloridine to host cells. The actual destruction of the cell wall is probably effected by bacterial mucopolysaccharidases, the normal function of which is to lyse the inner part of the wall in the growing cell so as to allow the increasing cytoplasm to expand, the outer part of the wall being enlarged by a synthesis as the cell grows.

In relatively low concentrations penicillin has no inhibitory effect on protein production, so that the cell continues to

produce mucopeptidases (Rogers, 1967). This accounts for the fact that, under experimental conditions, it may be an hour or more before penicillin begins killing the bacteria; during this period the effect of penicillin is reversible if the antibiotic is removed (Pennington and O'Grady, 1967). The process also probably accounts for the antagonistic effect of chloramphenicol on the bactericidal action of penicillin under experimental conditions. Chloramphenicol interferes with the manufacture of the mucopeptidases necessary to complete the destruction of the cell wall (Rogers, 1967). In higher concentrations, however, penicillin does directly interfere with protein synthesis as well as affecting the cell wall, so that chloramphenicol is no longer antagonistic.

FIG. 5.—Antibiotics and the cell wall and membrane.

Cycloserine is an analogue of D-alanine, one of the amino-acids which forms a substrate for the formation of the pentapeptide side-chain of the cell wall backbone. Strominger and Tipper (1965) beautifully illustrate the structural resemblance between cycloserine and the amino-acid which allows the antibiotic to confuse and disrupt the building process by competitively inhibiting the relevant enzymes.

The cell membrane of bacteria probably has a very important function in controlling relationships between the interior of the cell and the environment and in containing metabolites and enzymes within the cell (Salton, 1967). Amphotericin B and nystatin bind to a sterol in the cell membrane and so interfere with its osmotic barrier activity (Sanders and Cluff, 1968). Polymyxin B and colistin have the same effect by acting as cationic detergents with an affinity for phosphate radicals (Newton, 1954; Sanders and Cluff, 1968).

Synergism and Antagonism Between Drugs

In in-vitro studies Jawetz and Gunnison (1953) showed that if two bactericidal drugs were used together in concentrations which, with each drug alone, were only marginally effective, the bactericidal effect could be dramatically increased. The same thing could be shown with experimental infections, again provided marginal doses were used. The effect was demonstrable only with certain organisms. It may be due to a double effect on two metabolic processes, but the elimination of L-form persisters (see below) might play a part (Hewitt and Deigh, 1965).

In most infections it is unnecessary to use two drugs in combination for their synergistic effect, because a single drug is highly successful provided the organism is sensitive to it. But in certain infections which are difficult to eliminate the combination of two bactericidal drugs may be particularly useful. A well-known example is the superiority of penicillin combined with streptomycin in the treatment of enterococcal bacterial endocarditis, though experimental work suggests that penicillin with kanamycin might be even more effective (Hewitt and Deigh, 1965). The same combination is often used to treat bronchial infection with *Haemophilus influenzae* in chronic

bronchitis and bronchiectasis, this organism being notoriously difficult to eliminate. In bacterial endocarditis or septicaemia due to an unusual organism which proves difficult to eliminate careful laboratory studies, utilizing different combinations of bactericidal drugs, may give the clinician very useful guidance in therapy (Garrod and Waterworth, 1962).

In general there is virtually never a synergistic effect between bacteriostatic drugs. With marginal doses the effect is purely additive. Clinically a more than adequate dose of both drugs is usually given, so that the effect is equivalent only to the more powerful component and the second drug is redundant.

Antagonism between antibiotics is of considerable theoretical interest but of no practical clinical importance. In studies in vitro and in experimental animals Jawetz and his colleagues were able to show that when a bactericidal drug was used in a marginal concentration or in a marginal dose its effect might be diminished by previous or simultaneous exposure of the organism to a marginal dose of a bacteriostatic drug (Jawetz *et al.*, 1951; Jawetz and Gunnison, 1953). In subsequent in-vitro studies this phenomenon has been confirmed for many drugs (Manten and Wisse, 1961). In most cases the explanation of the phenomenon is probably simple. Bactericidal drugs are most effective on growing cells, as the increased metabolic activity during cell growth provides maximum susceptibility to the interfering action of the drug. The previous use of a bacteriostatic drug reduces metabolic activity.

It is of some interest that chloramphenicol in vitro has been shown to diminish the bactericidal effect not only of penicillin, as already mentioned, but also of human serum when used against *Escherichia coli*, though there is no effect if chloramphenicol-resistant strains are used. The same phenomenon has been shown if chloramphenicol is given orally to patients; their sera become less bactericidal (Waisbren and Brown, 1964). Presumably the slowing of the bacterial metabolism by the effect of the chloramphenicol renders the organisms less susceptible to the antibodies in the serum.

Antagonism between drugs is almost certainly of no clinical importance, because, under clinical conditions, drugs are not given in marginal doses. A high dose of a bactericidal drug is unaffected by the simultaneous administration of a bacteriostatic one (Jawetz, 1964). There is one often-quoted report in which it is suggested that the effect of penicillin in pneumococcal meningitis was diminished by the simultaneous administration of a tetracycline drug (Lepper and Dowling, 1951). This was not a controlled trial, and the conclusions have been criticized. Other clinical studies (Ahern and Kirby, 1952; Davis, 1954) have failed to show the phenomenon.

Causes of Failure of Chemotherapy

Among the causes of failure of chemotherapy, or of relapse of an infection after an initial improvement, the following have to be considered: (1) bacterial drug resistance, (2) bacterial "persistence," (3) poor host defences, (4) poor drug absorption, (5) drug inactivation by the host's protein or flora, and (6) poor penetration of the drug into tissues or cells.

Drug Resistance

Drug resistance may be conveniently classified into three major groups: (1) *natural drug resistance* occurring in organisms which have not been exposed to the drug, at least in that particular patient; (2) *acquired drug resistance*, which results from exposure of the organisms to the drug and which may occur during the treatment of an individual patient; and (3) *transferred* (or "*infectious*") drug resistance by which genetic material conferring resistance may be transferred from a resistant to a sensitive species or strain.

Natural Drug Resistance

Natural drug resistance may be characteristic of an entire species, but sometimes, as in the case of penicillin resistance in staphylococci, is confined to particular strains of that species. It may be due to (1) the absence of the metabolic process which is affected by the drug in question, (2) a structural peculiarity, such as the absence of cell wall in mycoplasmata which renders them resistant to penicillin, or (3) the production of enzymes which destroy the drug, as in the production of penicillinase by staphylococci. When one considers that a number of antibiotics act by having a complex structural peculiarity which enables them to mimic a naturally occurring molecule essential to a metabolic process, and so block that process like a bent key in a lock, it is easy to see that a slight change in a bacterial molecule might prevent the bent key getting into the lock at all. For instance, both the tetracyclines and erythromycin interfere with the attachment of the amino-acid-tRNA complex to the mRNA-ribosome complex (Fig. 4); but the tetracyclines are highly effective against many Gram-negative bacilli unaffected by erythromycin. This suggests that the two drugs must interfere with the process in different ways, and that erythromycin does not fit this particular metabolic lock in the case of most Gram-negative organisms.

Sulphonamides act by interfering with an enzyme which synthesizes tetrahydroptericoic acid from para-aminobenzoic acid and another substrate. The action of the drug can be prevented by introducing an excess of para-aminobenzoic acid, which most sulphonamide-sensitive organisms require to obtain from their environment. A strain may be resistant to sulphonamides because it actually produces the para-aminobenzoic acid itself, or because it has a different enzyme less sensitive to sulphonamide, or because of a lesser permeability which reduces the penetration of the sulphonamide into the bacterial cell (Richmond, 1966). A single gene change, resulting in a change in one amino-acid in the enzyme, can render the enzyme resistant to the action of sulphonamides.

The absence of cell walls in mycoplasmata is responsible for their penicillin resistance. L-Forms, or protoplasts, which may develop from certain bacterial species, are also without walls. This may make them resistant to drugs active on the cell wall, though in fact such organisms are usually very sensitive to bacteriostatic drugs (Carter and McCarty, 1966). The possibility of L-forms being concerned with the phenomenon of persistence (see below) and relapse after treatment requires further investigation.

The production of antibiotic-destroying enzymes, of which penicillinase is the best known, may reflect a natural weapon in the ecological struggle between micro-organisms. The evolution of antibiotics by, for instance, fungi may tend to destroy species which are rivals for the supply of nourishment. The evolution of antibiotic-destroying enzymes would be a protective mechanism. In a hospital environment in which a particular antibiotic is intensively used the possession by a mutant strain of an enzyme destroying that antibiotic is clearly of survival value, and this largely accounts for the ecological success of the penicillin-resistant hospital staphylococcus. Bacterial strains or species resistant to the chemotherapeutic agents commonly used in a particular environment are naturally the ones which will tend to survive in this environment and to replace sensitive strains. Unfortunately there is a possibility that this process of natural selection may be accelerated by the transfer or transduction of resistance-conferring genetic material from resistant to sensitive strains or species (see below).

It is important, therefore, that certain drugs should be reserved for treating resistant strains. When drugs such as cloxacillin or methicillin are used for treating an infection with staphylococci known or suspected to be resistant to the usual drugs, it is our own custom to treat the patient in isolation with barrier nursing so as to minimize the chances of the emergence of strains resistant to the reserve drugs.

If the production of penicillinase can be decreased by inhibiting bacterial metabolism with a penicillinase-resistant penicillin, enough benzylpenicillin may survive, if given at the same time, to affect the bacteria which, in the case of staphylococci, are resistant to it only by reason of penicillinase production. Weight for weight benzylpenicillin is much more effective than methicillin or cloxacillin. Moreover, there is also evidence that penicillinase may be bound by methicillin and so prevent its action against benzylpenicillin (Farrar *et al.*, 1967). Even against Gram-negative bacilli such combinations may be synergistic experimentally, but in such large doses that it is doubtful whether the results can be clinically applicable (Bach *et al.*, 1966). There is, however, much to be said for adding benzylpenicillin in large doses to cloxacillin or methicillin in the treatment of infections with penicillin-resistant staphylococci.

Acquired Drug Resistance

This term may be used to indicate that a strain of bacteria infecting a patient, and originally sensitive to a particular drug, has become resistant to that drug during treatment. At one time there was some controversy over whether acquired resistance was due to the differential growth of naturally occurring resistant mutants or to adaptation of all members of the strain to the drug (Mitchison, 1962). To some extent the difference may be semantic in so far as "triggering" by the drug may be required to develop the full potential of resistance (De Courcy and Sevag, 1966), and this might be interpreted as adaptation. Nevertheless it is a genetically determined capacity for adaptation, and the genetic basis of acquired resistance is now generally accepted.

Chemotherapeutic agents may be conveniently classified into two groups; to one resistance may be very rapidly acquired, to the other only slowly. In the case of the "slow" group, if organisms are repeatedly cultured in increasing concentrations of one of these drugs, subculturing on each occasion from the highest tube in which growth has occurred, the degree of resistance may be slowly pushed up. This small-step increase is probably due to mutations occurring in a number of genes, each of which is responsible for a slight increase in resistance. In at least one instance, that of chloramphenicol resistance, there is evidence that the genes may be distributed right along the chromosome. In experiments in which organisms made artificially resistant to varying degrees were cross-mated with sensitive organisms it was shown that the degree of resistance in the progeny depended on the amount of chromosome transferred (Cavalli and Maccacaro, 1952).

In the case of slowly acquired penicillin resistance certain organisms such as pneumococci may lose their pathogenicity, but this does not occur with all species. Because of the difficulty with which this type of resistance is acquired, and perhaps because the organisms tend to lose their pathogenicity and are then rapidly destroyed by the patient's defences, this type of acquired resistance is rare in the individual patient. Nevertheless, if the drug is used extensively in a community naturally resistant strains may become prevalent, owing to selection, as already mentioned.

In the "rapid resistance" group, if the in-vitro experiment of growing organisms in ascending concentrations of the drug is repeated, high degrees of resistance may be obtained even on the first subculture. This is probably due to mutations occurring in more powerful genes which confer a considerable degree of resistance. In the case of streptomycin high degrees of resistance may be controlled by a single gene locus, but there is evidence that lower degrees may in fact be controlled by multiple loci (Mitchison, 1962). Streptomycin resistance appears to consist in a structural change in the 30S subunit of the ribosome (Cox *et al.*, 1964; Davies, 1964), and to be controlled by genes in the bacterial chromosome rather than by cytoplasmic genetic material (Richmond, 1966).

Clinical Significance of Acquired Resistance

It is important that the clinician should be familiar with the drugs to which resistance may be readily acquired, as, for reasons which are outlined below, such drugs should always be used in combination. In the chemotherapy of infections other than tuberculosis commonly used antibiotics may be classified as follows:

Risk of Acquired Resistance	
High Risk	Low Risk
Streptomycin	Penicillin
Erythromycin	Tetracyclines
Novobiocin	Chloramphenicol
Lincomycin	Colistin
	Polymyxin B
	Gentamicin
	Cycloserine

Resistance is rapidly acquired to almost all antituberculosis drugs if used alone, with the possible exception of cycloserine.

Rapidly acquired drug resistance is of great clinical importance and always a potential danger in the individual patient if one of these drugs is used alone. In general, strains rendered resistant in this way do not easily revert to sensitivity, though this is said to be commoner with erythromycin than with other drugs. The mechanism may be illustrated by pulmonary tuberculosis treated with a single drug. In any large population of "isoniazid-sensitive" tubercle bacilli about one in a million is a naturally occurring resistant mutant (Fig. 6). Under natural conditions the proportion remains small probably because the resistant mutants are at a slight biological disadvantage compared with sensitive bacilli. If isoniazid is given alone the sensitive bacilli diminish in numbers and the isoniazid-resistant mutants are now at a biological advantage. If the population is large and the patient's defences are poor, the resistant mutants may increase and ultimately replace the sensitive population. These resistant bacilli usually persist for the rest of the patient's life, and may indeed infect other people.

On the other hand, if both streptomycin and isoniazid are given to the patient (Fig. 7), streptomycin affects not only the isoniazid-sensitive population but also the isoniazid-resistant mutants. There are also, of course, a small number of strepto-

FIG. 6.—Emergence of isoniazid (INH) resistance with single-drug therapy. SM=streptomycin. FIG. 7.—Preventing resistance by combined chemotherapy.

mycin-resistant mutants (about 1 in 10^7), but these are inhibited by the isoniazid. In this way the emergence of resistant organisms is prevented. In clinical tuberculosis if a drug is given alone, resistant organisms may take from two weeks to two months to appear, but if streptomycin is used alone to treat pneumonia due to *Klebsiella pneumoniae* resistant organisms may emerge within a day or two. Therefore whenever one of these high-risk drugs is used to treat an infection it should always be used in combination with another drug to which the organisms are sensitive, and with which there is no cross-resistance.

[The conclusion of this lecture, together with a list of references, will be published next week.]

Active Uptake of Copper and Zinc during Haemodialysis

JEANETTE BLOMFIELD,* M.SC., F.A.A.C.B.; JEAN McPHERSON,† M.B., B.S.; C. R. P. GEORGE,† M.B., B.S.

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Summary: The uptake of copper and zinc by patients undergoing regular haemodialysis has been assessed by measuring the dialysis fluid for copper and zinc concentration, and the blood entering and leaving the dialysis coil for red cell copper, plasma free copper, and plasma zinc levels during priming of the coil and subsequent haemodialysis, and by in-vitro studies.

Red cells avidly removed copper from dialysis fluid when mixed with saline during priming, but did not take up copper during the haemodialysis. At both these stages plasma actively took up both copper and zinc from dialysis fluid, even against a concentration gradient, so that loss of these metals from plasma to dialysis fluid did not occur.

In the dialysis systems investigated the sources of the copper in the dialysis fluid were the copper plumbing of the tap-water and the dialysis coil, and the major source of zinc was the zinc oxide of the adhesive plaster around the dialysis coil.

Introduction

Lyle (1967) demonstrated loss of copper from dialysis fluid during haemodialysis and suggested that uptake of copper by the patient may be contributing to the haemolytic anaemia of these patients.

We have investigated both copper and zinc during haemodialysis by measuring their concentrations in tap-water and dialysis fluid, in blood taken before entering the dialysing coil, and in blood returning to the patient from the coil after dialysis.

The aim was to determine whether copper and zinc are taken into the blood and whether this mechanism could lead to toxic amounts of these metals entering the blood. A further aim was to see whether the body could be depleted of these minerals by dialysing against low concentrations, in the same way that

* Senior Research Officer, Children's Medical Research Foundation, Royal Alexandra Hospital for Children, Camperdown, Sydney, Australia.
† Registrar, Dialysis Unit and Medical Research Department, Sydney Hospital, Sydney, Australia.