"BACTERIAL RESISTANCE TO CHEMOTHERAPY"

by

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The anti-spirochaetal action of arsenic had been known since the middle ages. Ehrlich, however, attempted to incorporate this metal in an organic compound in a form in which it would retain its anti-spirochaetal action, yet be sufficiently non-toxic to be of practical use as a drug. His efforts culminated not only in the discovery of the famous compound 606, Salvarsan - the 606th compound which was tested and which proved successful in the treatment of syphilis - but also in the foundation of modern antimicrobial chemotherapy.

Further, while making fundamental contributions to the progress of histology with the discovery of differential staining, Ehrlich observed that many of the aniline dyes which he was then using had strong anti-bacterial activity. This led him to hope that in the same way as there existed dyes which specially stained certain cells or parts of cells, without affecting others, so one day an anti-bacterial dye would be discovered which exerted a specific toxic effect on the bacterium but which did no harm to the cells of the host. Although this conception is too simple to be completely true and it is now realised that the co-operation of the tissues of the host is usually necessary in successful chemotherapy, it stimulated much successful research. Success came from the direct empirical trial of large numbers of substances on experimentally infected animals.

Ehrlich's
Ehrlich's work on chemotherapy was closely linked to and much stimulated by the rapid advances in organic chemistry which were occurring at that time in Germany and his work was greatly facilitated by the concomitant extraordinary expansion of the German dyestuffs industry. Ehrlich, and later Domagk, tested dyestuffs for chemotherapeutic activity and in 1935 Domagk announced that prontosil (red) would cure streptococcal infections in mice. This discovery was a most important milestone in the development of chemotherapy, for it was shown for the first time that anti-bacterial chemotherapy was a practical possibility. This observation was immediately confirmed in France. Contrary to Ehrlich's concepts, however, the chemotherapeutic effectiveness of prontosil was not due to its dye nature, but resided in the non-coloured sulphonamide moiety of the prontosil molecule. It was soon shown that numerous sulphonamide compounds were effective not only against streptococci but had a chemotherapeutic effect over a considerable range of bacterial infections.

A second great advance in chemotherapy was initiated by the introduction into medicine of the antibiotics. Intensive research on antibiotics began in 1940 when Chain and Florey announced that penicillin, a metabolic product of Penicillium notatum and whose existence had been observed eleven years previously by Fleming (1928), possessed remarkable curative properties in experimental animal infections. From that time on the development of the literature on the antibiotics has shown/
shown the same logarithmic curve that is characteristic of bacterial growth. Penicillin, the most active and least toxic anti-bacterial substance known, is active against gram-positive bacteria and against some gram-negative organisms such as the meningococcus and the gonococcus. It is also very active against the spirochaete of syphilis. For a time penicillin was the only antibiotic known to medical practice and its use was confined to battlefield casualties; little was available for civilian patients. Its spectacular success, however, gave a great stimulus to the study of antibiotics and hundreds of active substances have since been described. Although most of these failed to pass a chemotherapeutic test, streptomycin, chloramphenicol, the tetracyclines, erythromycin and others have since been added to the armoury of effective antibiotics. Streptomycin has an antibacterial activity fifty to a hundred times lower than that of penicillin, but it is active in vitro and in vivo against bacterial species on which penicillin has no effect. Among these are many gram-negative organisms including Pasteurella, Salmonella, B. coli and, of particular importance, both the human and bovine type of tubercle bacillus. Chloramphenicol was the first of the "wide range" antibiotics and was the first substance found to be active in Rickettsial diseases. The tetracyclines have a similar wide-range and for the first time certain virus infections - atypical virus pneumonia, lymphogranuloma, and psittacosis - became amenable to chemotherapeutic treatment.
The past fifteen years may very well go down in medical
history as the "antibiotic era" - based upon what these drugs
have done to minimise human disease. Primary syphilis has
been so markedly reduced that it is now becoming very difficult
to find cases for clinical teaching and mass therapeutic
measures under the auspices of the World Health Organisation
threatens eventually to completely eradicate spirochaetal
diseases in tropical countries where these diseases are endemic.
Pneumococcal pneumonia is today regarded by clinicians as a
relatively minor ailment, the majority of cases no longer
requiring admission to hospital. Prior to the introduction of
serum the fatality rate was about twenty to thirty per cent.
The serums cut it to fifteen per cent., the sulphonamides to
around ten per cent., and penicillin to less than five per cent.
and these mainly at the extremes of life. Subacute bacterial
endocarditis was practically one hundred per cent. fatal before
antibiotics, but today the infection can probably be eliminated
in more than ninety per cent. of cases by giving adequate doses
of the appropriate antibiotic for a sufficient length of time.
Delay in treatment is the principal cause of failure. Operations
for acute mastoiditis are almost a thing of the past.

These are but a few of the outstanding examples of the
extensive and dramatic contributions made by modern chemotherapy
and one has only to reflect for a moment to recall some friend
or relative who has been spared some serious illness or whose
life has been saved on account of these chemotherapeutic drugs.
It /
It would indeed be difficult to overemphasise how in the past decade chemotherapy has so completely revolutionised the practice of medicine.

**BACTERIAL RESISTANCE TO CHEMOTHERAPY: THE CLINICAL PICTURE.**

Nevertheless, it would be unrealistic to say that these wonderful chemotherapeutic agents have not also brought their problems. Soon after the first flush of unqualified success, many and especially the newer of the antibiotics were found to be capable on occasion of exerting serious toxic effect, such as hypersensitivity reactions and blood dyscrasias. But that was not all. By far the most important problem was soon realised to be the alarming rate at which strains of pathogenic organisms can become resistant to these chemotherapeutic agents. The development of bacterial resistance to chemotherapy strikes a serious blow to the very foundations of one of our most valuable methods of medical treatment of the present day.

Resistance is by no means a unique property possessed only by bacteria; the capacity to develop tolerance or resistance to certain types of noxious agents appears to be a general property of living matter. It is seen in man in habituation to a drug such as morphia; it is appearing in insects which are becoming resistant to D.D.T. (B.M.J. 1951:2, 900); and it was known since the time of Ehrlich's work that protozoa are capable of developing resistance to chemotherapeutic substances. Treatment of gonorrhoea with sulphanilamide, which /
which at first threatened to remove this branch of therapeutics from the realm of the specialist and to make prostitution safe for posterity, was soon found to be not invariably effective. A small minority of cases always failed to respond. The reason for this was first demonstrated by Felke (1938), who showed that strains of gonococcus from cases resisting treatment were able to grow on media containing the drug, whereas those isolated before successful treatment could not. He was also able to render the gonococci artificially sulphonamide-resistant in vitro by cultivation on media containing gradually increasing concentrations of the drug. That the sensitivity or resistance of the organisms, as demonstrated in vitro, corresponds with success or failure in treatment, has since been repeatedly confirmed. Mahoney and Van Slyke (1945) artificially infected incarcerated male volunteers with two different cultures. One culture was sensitive and the disease produced by it in twenty-five volunteers was in every case cured by sulphathiazole. The other cultures were a strain from a prostitute whose infection had persisted despite repeated courses of sulphonamide treatment. It produced equally sulphonamide resistant disease in ten volunteers.

Although laboratory studies show that resistance can be acquired in vitro, the acquisition of resistance in vivo by an initially sensitive organism during treatment has not to my knowledge been reported. It would therefore appear that the presence or absence of natural resistance in the particular gonococcus/
gonococcus is the major factor in determining the effectiveness or otherwise of sulphonamide treatment.

For a time it was hoped that gonococci, although resistant to sulphanilamide, might remain sensitive to the more potent newer sulphonamides. However, it has been shown by Kirby (1943) that organisms that have rendered artificially resistant to one member of the sulphonamides were also resistant to all the other members of that group. This principle applies not only to the gonococcus but to all other organisms.

Clinical reports since the start of sulphonamide therapy have indicated a steadily increasing proportion of drug-resistant gonococcal infections until in 1943 they reached the figure of 50% in Britain (Grudale & Schwab 1944). At the London Lock Hospital the incidence reached 85.8% in 1946-47 (Dunlop 1949). Since we have seen that resistance is unlikely to arise during treatment, the survival and inevitably increasing prevalence of naturally resistant strains must be largely responsible for what has happened. It is indeed fortunate that penicillin became available to replace a treatment which was fast loosing the best of its efficacy.

The above example of the gonococcus, however, is clinically the most striking of all examples of bacterial resistance to the sulphonamides; no other species of organism has developed resistance on an equal scale or with such universally disastrous effects. In marked contrast it is surprising and very fortunate that the closely related meningococcus has behaved so /
so differently. The meningococcus has remained susceptible to the sulphonamides in spite of mass sulphonamide prophylaxis directed against other infections. Indeed, meningococcal meningitis is the only acute bacterial infection in which penicillin is still generally superfluous as an adjunct to sulphonamide treatment or as a substitute for it.

The two most important sulphonamide-sensitive species - the pneumococcus and the haemolytic streptococcus - have behaved in a way intermediate between these extremes. Resistant strains of each have appeared but have not been common. How common they might have been today but for the advent of penicillin however, cannot be guessed. Resistance was observed in the pneumococcus comparatively early, and it seems that of these two organisms it has much the greater tendency to undergo this change. That pneumococci are in fact capable of becoming resistant to sulphapyridine without loss of virulence was first shown by MacLean, Rogers and Fleming (1939). Hamburger, Schmidt and Ruegsegger (1942) measured the in vitro sensitivity of microorganisms isolated from patients before and at various times during and after treatment. Out of sixty-two cases of pneumococcal pneumonia treated with 15-50 G. of a sulphonamide drug in only three was a moderate increase in resistance detectable. A further three cases were studied in which the total sulphonamide exceeded 240 G.; without exception the organisms recovered after treatment showed a striking increase in their resistance to sulphonamides. For example, in a case of unresolved pneumonia,
pneumonia, the type II pneumococci obtained prior to treatment were unable to grow in media containing 0.6 mgm. % sulphathiazole, whereas organisms isolated after five weeks treatment multiplied in 20 mgms.% of this drug. In another case, that of a patient with subacute bacterial endocarditis due to pneumococcus type VII, organisms isolated prior to therapy failed to grow in media containing more than 2.5 mgms. % sulphapyrazine, but organisms isolated after four months of treatment grew in media containing 80 mgms. % of this sulphonamide.

These findings indicate that there is little hazard of producing sulphonamide-resistant organisms during the short intensive treatment required for pneumococcal pneumonia. However, if treatment is prolonged, this hazard is considerable. As with the gonococcus, what increase in sulphonamide resistance there has been is apparently due to the survival and selection of naturally resistant strains. It has been shown by Julianele & Sigel (1945) that mass sulphadiazine prophylaxis can produce a high prevalence of strains of pneumococci of progressively increasing resistance. Indeed, it is quite possible that, but for the antibiotics, our main resource in the treatment of pneumonia would again be serum.

Although the haemolytic streptococci were the earliest to be subjected to the attack of sulphonamides, sulphonamide-resistance in these organisms was a late development. It has only been observed in a few of the Griffiths types of Strept. pyogenes, notably XII, XVII, & XIX, and their occurrence suggests dissemination /
dissemination from a few original foci rather than the development of widespread resistance during treatment. They were first identified as causing cross infection in wards where wounds and burns were under treatment (Francis 1942). They reached their greatest prevalence when hundreds of thousands of men of the American armed forces were given 1 G. of sulphathiazole daily, mainly with the object of preventing haemolytic streptococcal infections. This treatment at first succeeded, but later highly resistant strains appeared and became widely prevalent so that the final state of the units concerned was considerably worse than the first (Damrosch 1946).

In general, with regard to the sulphonamides it may be said that the development of resistance during short term treatment of an infection due to a sensitive organism is unlikely; that the increasing prevalence of naturally resistant organisms and their selection due to the widespread use of the drug is responsible for the observed rise in the incidence of sulphonamide-resistant organisms; that this increase in resistance is clinically serious in only one organism, the gonococcus; and, finally, that resistance to one of the sulphonamides is accompanied by resistance to all the members of the sulphonamide group of drugs.
Bacterial resistance to penicillin closely resembles sulphonamide resistance in that the major difficulty occasioned by each is infection by a single species. In the case of sulphonamide resistance this species has been shown to be the gonococcus, while in the case of penicillin resistance it is *Staphylococcus pyogenes*. The incidence of penicillin resistant staphylococci in the large hospitals has been gradually rising until today it is the order of 50%. On this account the view has become widely accepted that micro-organisms rapidly build up resistance to penicillin. Indeed, in the first full length paper on penicillin as a chemotherapeutic agent (Abraham et al., 1941) it was reported that staphylococci could readily be trained in vitro to withstand relatively high concentrations of the antibiotic. Garrod (1950), however, states that he is not aware of a single authentic case which reports the genuine development of resistance to penicillin during clinical treatment.

There is little doubt that the answer to this paradox is that in hospital wards, where the apparent development of penicillin resistance by staphylococci was first observed, patients with infections caused by both penicillin-sensitive and naturally penicillin-resistant staphylococci were present. What happened during penicillin treatment was simply that penicillin eliminated all the sensitive staphylococcal strains but left untouched those naturally resistant (Barber 1949). Owing to cross-infection and the frequency of the carrier-state /
carrier-state in nurses the resistant strains gradually spread throughout the hospital. In particular, Barber has shown that a single resistant strain of a particular phage type, often 52A, may in this manner achieve overwhelming predominance. The importance of cross-infection in hospitals has been emphasised by Forbes (1949) who found from an analysis of 78 strains of *Staph. pyogenes* a resistance rate of those derived from out-patients of 12.5% as opposed to 68.4% from in-patients. Although it is quite well established that from 50 - 70% of normal subjects may be carriers of staphylococci, the important consideration is the number of individuals under hospital care and among the general population who are carriers of the naturally resistant strains of the organisms. A few studies have been made of out-patients and normal subjects and in all instances the incidence of penicillin-resistant staphylococci in hospital personnel has been considerably greater than that found in non-hospitalised individuals.

Gould & Allan (1954) carried out a survey of *Staph. pyogenes* infections in a general hospital from the beginning of January 1953 to the end of January 1954. In all there were 299 patients with *Staph. pyogenes* infections and 138 of these were classified as "hospital infections," since they occurred in operation wounds, varicose ulcers and the skin and eyes of children. The monthly incidence of hospital infections was found to be roughly related to the nasal carriage rate of the medical and nursing personnel. Nearly 90% of the hospital carrier strains were resistant to penicillin.
penicillin and produced penicillinase. At first more than 50% were resistant to streptomycin, but later this figure fell to 30%. No strains resistant to the other antibiotics in general use were encountered. The strains isolated from the patients with hospital staphylococcal infections were similar in bacteriophage type and antibiotic sensitivity to those isolated from the hospital carriers. The strains isolated from the patients with non-hospital infections were quite different in these respects (see fig.) Nasal carriage was considerably reduced after three months by the intranasal application of oxytetracycline cream for /

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<th>BACTERIOPHAGE GROUP</th>
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Pan sensitivity
Streptomycin-sensitive
Streptomycin-resistant
Pan-sensitive
Streptomycin-resistant
Pan-resistant
Streptomycin-resistant
Pan-resistant

Fig 1. Bacteriophage group + antibiotic resistance of strains of Staphylococcus aureus
Gould + Allen (1954): Lennert 3, 788
for ten days, and this resulted in a fall in the number of cases of hospital infection due to the staphylococcus. The relationship between the hospital carriers and the cases of hospital infection was thus established with reasonable certainty.

Furthermore, it has been shown that the artificial penicillin resistance which can readily be induced in vitro is quite distinct from the natural form of penicillin resistance. As will be discussed later, strains of staphylococci which are naturally penicillin resistant produce an enzyme, penicillinase, that destroys penicillin and is the cause of their resistance. On the other hand, strains made artificially resistant to penicillin do not produce this enzyme. Now, in all cases in which a careful bacteriological study was undertaken the penicillin-resistant strains of *Staph. aureus* isolated from infective processes are resistant because they produce penicillinase (Barber 1949). It may be concluded, therefore, that these organisms were naturally resistant to penicillin. Moreover, since they are less virulent it is unlikely that many of the artificially induced penicillin resistant organisms would be of any importance in clinical practice as they would be readily overgrown by typical penicillin-sensitive strains.

In this way, therefore, penicillin resistance also resembles sulphonamide resistance; in each case selection and not adaptation is the mechanism whereby the resistant strains have become prevalent. The resistance shown by other organisms to penicillin is
is surprisingly slight.

Other antibiotics are not so well favoured. For example, the facility with which bacteria acquire resistance to streptomycin is unequaled in the whole field of chemotherapy and in spite of the marked toxicity of that drug the development of resistance forms the chief obstacle to its successful use. Here there is no doubt that the change can occur during treatment; resistance can develop rapidly and often be of extreme degree far exceeding that required to preclude further therapeutic effect. Moreover, the resistance, which is usually permanent, is unaccompanied by any diminution in virulence. It differs from sulphonamide and penicillin resistance in that all species of bacteria are affected similarly. Thus, although the rate at which resistance develops varies with the growth rate and other factors, there is no difference in the capacity of different bacteria to develop resistance to streptomycin.

Isoniazide and isonicotinic acid, which with streptomycin are used in the treatment of tuberculosis, induce resistance with a similar rapidity. On account of this major disadvantage there would appear to be no ultimate future for these antibiotics.

Although it is possible to induce resistance in vitro to chloramphenicol and the tetracyclines (the "broad spectrum" antibiotics), so far no case of development of resistance during clinical treatment has been reported. The incidence among bacteria of natural resistance to these antibiotics is however /
however rising at a significant and distressing rate.

Erythromycin, discovered in 1952 at a time when the magnitude of the problem of bacterial resistance to penicillin and the "broad spectrum" antibiotics was being generally realised, was hailed as a possible answer to the drug resistant Staph. aureus. These early hopes were soon dismissed when the comparative ease with which organisms, and especially Staph. aureus, became resistant to it. It has been observed that within one month of the adaptation of erythromycin for general use in a hospital (Chicago) strains of staphylococci resistant to it began to appear in the nose and the throat of the staff; after five months the carrier-rate of highly resistant strains reached 75%. The use of the drug was then discontinued, and the carrier-rate fell to 37% in two months.

In summary of the clinical findings it may be said that bacterial resistance to chemotherapy is a change that threatens ultimately to extinguish the usefulness of all the present antibiotics with the exception of penicillin. Although resistance to streptomycin, PAS, isoniazid and erythromycin may develop to a marked and disabling degree during clinical treatment, the main problem is the ever increasing number of organisms which are becoming naturally resistant to the "wonder" drugs of the "antibiotic era". Today the curious and paradoxical situation exists in that it is not uncommon for a case of septicaemia to terminate fatally simply because the infecting organism is resistant /
resistant to all the chemotherapeutic agents used in current medical practice.

To investigate this problem rationally it is necessary to enquire into the mode of action of chemotherapeutic agents before we can hope to fully comprehend how bacteria develop the property of being resistant.

**MODE OF ACTION OF CHEMOTHERAPEUTIC AGENTS**

Ehrlich (1913) recognised that the efficacy of a chemotherapeutic agent depends largely on the differences in its toxicity for parasite and host (the chemotherapeutic ratio). He showed that the affinity of a drug for an organism is closely related to its chemical structure and that its potency may sometimes be destroyed by changing a single atom – a specificity that he explained by his side-chain theory. At that time it was characteristic of all the compounds used in chemotherapy – the metals, phenols and dyes, for instance – that though they had some selective action mainly on the gram-positive bacteria, their power to kill micro-organisms varied with their concentration, and doses which were lethal to the invading organism were usually toxic to the patient. The notable fact about the first sulphonamide, prontosil, was not so much that it inhibited bacterial growth as that it could be tolerated by the body in effective concentrations over a long period. Consequently, not /
not only is it desirable to know the vulnerable point or points which the chemotherapeutic agent attacks in the biochemistry of the bacterial cell, but also the reasons for the specificity of these drugs and especially why it is possible to use them in the living person.

The growth and multiplication of bacteria (and no doubt of all cells) involves the synthesis of new bacterial substance from simpler compounds. In the case of protein the simplest compound is amonia and from this and a suitable source of carbon some bacteria can synthesise protein, the energy required in the process being liberated from the oxidation of some such material as glucose. Although little is known about the mechanism of this synthesis, it is inferred that it is achieved in steps by a series of enzymes operating on each stage as it is completed.

The main evidence in support of this view is that whereas *B. coli* can grow (i.e. synthesise all its nitrogenous compounds) from amonia, *B. typhosum* cannot, except when certain amino acids which are constituents of protein are added. The amino acids which must be supplied to *B. typhosum* are demonstrably synthesised by *B. coli*, and it is concluded that the failure of *B. typhosum* to grow from amonia is due to a failure to synthesise the amino acids. These amino acids which must be added to the nutrients are "growth factors" for *B. typhosum*. They represent stages in the synthesis of protein which *B. typhosum* cannot achieve, but it will be understood that these stages are equally present in the syntheses carried out by *B. coli*, though in this case
they are not growth factors since the organism can synthesise them. In general, each stage in any synthesis necessary for growth is referred to as an "essential metabolite" without which, either synthesised or supplied from outside, growth cannot occur. A growth factor is therefore simply an essential metabolite that cannot be synthesised.

Now there is a considerable body of evidence to suggest that bacterial inhibition may be associated with interference with essential metabolites. Thus Fildes (1929) and Knight & Fildes (1930) showed that anaerobes would not grow when a certain degree of reduction potential (positive oxidation-reduction potential) is present in the medium. If the degree of reduction potential is diminished by oxygen or by an oxidising agent such as ferricyanide, thinmine or methylene-blue no growth took place until they had been reduced. A similar inhibiting effect of thiamine or methylene-blue was also observed with aerobes, for the reason that conditions within an aerobic cell are anaerobic and a sufficient concentration of these diffusible dyes imposes aerobic conditions within the cell. This inhibition was only observed with diffusible dyes and not with non-diffusible dyes of a similar oxidising capacity. It was therefore concluded that the inhibition was due to an interference of oxidation with an essential metabolite which required to be reduced in order that the enzyme associated with it might function normally as in growth.
A number of essential metabolites were shortly isolated and in particular the group -SH was shown to be necessary for staphylococci (Fildes & Richardson 1937) and moulds (Volansky 1932). In view of the fact that H-SH combines with mercury and H-SH reverses the antibacterial action of mercury it seemed possible that the antibacterial action of mercury was due to combination with essential -SH groups in the cell (Fildes 1940).

With the introduction of the sulphonamide drugs the question arose whether the same argument could be applied. Prolonged experiments were carried out to determine whether any known essential metabolite could be associated with the action of sulphanilamide in the sense that a quantitative relationship might be shown to exist between the inhibitory concentration of sulphanilamide and the counter-action of the essential metabolite. These experiments were unsuccessful. However, Stamp (1939) found that the action of sulphanilamide could be reversed by an extract obtained from streptococci and it was concluded that this extract contained an essential metabolite which was not a growth factor for streptococci, since at that time all these were known. Woods (1940) showed that extracts of yeast had similar properties and by fractionation of such extracts he obtained a material with well-defined chemical characters that reversed the action of sulphanilamide. This material could not for quantitative reasons combine with sulphanilamide on a molecular basis as does -SH with mercury. The possibility was therefore investigated that it might /
might act by competing with sulphanilamide for an enzyme for which it was the natural substrate. Substances in the yeast extract which had some structural similarity with sulphanilamide were tried and in this way p-amino-benzoic acid (PABA) was found to antagonise sulphanilamide quantitatively and in very high dilutions. Now, but for one radical, the chemical structure of the sulphanilamide molecule, or indeed of any sulphonamide molecule, is identical with that of PABA. The hypothesis (the Woods-Pildes hypothesis) was therefore proposed that PABA is an essential metabolite usually associated with an enzyme and that sulphonamides are capable of competing with PABA for this enzyme. If in sufficient concentration the sulphonamide molecule may succeed in displacing PABA and so stop an essential line of metabolism. In modern phraseology there is a competitive substrate inhibition between the sulphonamides and PABA.

More recently, the role of PABA as an essential metabolite for growth has been confirmed by the discovery that it is used in the bacterial synthesis of folic acid. The amino-group in PABA combines with the aldehyde group in reductone (CHOH:C(OH).CHO) and the substance so formed unites with a diamino-pyrimidine derivative to form pteroic acid, which is folic acid minus glutamic acid. The molecules of the sulphonamides undergo some of these changes too, but do not form folic acid, so that the metabolic path which leads to this substance is blocked. Moreover, it has been shown (Lampen & Jones 1946) that microorganisms /
organisms which have no need of folic acid are resistant to sulphonamides, and so are those which must have ready-made folic acid as a growth factor. On the other hand, organisms which make their own folic acid from PABA were found to be sulphonamide-sensitive. The effect of sulphonamides on the latter group of bacteria is reversed by the addition of either PABA or folic acid.

In several species of bacteria there is no doubt that PABA is an essential metabolite, for it is demonstrably a growth factor. Both *Cl. acetobutylicum* (Rubbo et al 1941; Rubbo & Gillespie 1942; Lampen & Peterson 1941) and *Aerobacter suboxylans* (Lempen et al 1942) require it for growth, and both are susceptible to an inhibiting action of sulphonamides that is reversed by excess of the acid. In other species susceptible to the action of sulphonamides, however, the evidence for PABA as an essential metabolite is indirect. The acid can be recognised in extracts of bacteria either chemically or biologically but in both cases the specificity of the test is open to question. One cannot therefore exclude the possibility of alternative hypotheses. Moreover, there are certain observations which have not been adequately explained on the Woods-Fildes hypothesis.

For example, sulphonamides may attack metabolic processes remote from those centring round PABA. Oakberg & Luria (1947) trained *Staph. aureus* to grow in increasing concentrations of sulphathiazole /
sulphathiazole and they observed that the resistance of the culture increased not continuously but in steps. This was interpreted as being due to successive mutations of the coccus. However, only one of these mutations was definitely associated with increased synthesis of PABA. It would therefore appear that the other mutant steps involved changes in other metabolic properties that were also susceptible to the sulphonamide. Johnson et al (1945) showed that sulphanilamide inhibited not only the growth but also the luminescence in *Phosphobacterium phosphoreum*, whereas PABA antagonised only the growth inhibition. Various compounds unrelated to folic acid - e.g. methionine, xanthine and serine - have been found to oppose the action of sulphonamides. Furthermore, it has been established that the respiration of certain bacteria, yeast and tissues is inhibited by sulphonamides. Henry (1943) and Sevag (1946) contend that PABA displaces sulphonamide non-specifically from combination with respiratory enzymes and that in both aerobic and anaerobic bacteria sulphonamides act through respiration by depriving the cell of energy necessary for its synthetic activities. Inhibition of respiration was first observed on intact bacteria by Baron & Jacobs (1937) and it seems to occur simultaneously with the cessation of growth in the presence of the sulphonamide. On the basis of numerous investigations, it can be assumed that certain relationships exist between sulphonamide inhibition of growth and of respiration.
Sulphonamides may affect these processes by interfering with the synthesis of the co-enzymes necessary for respiration (Dorfman & Koser 1942). However, it seems probable that the effect of sulphonamides on bacterial respiration is only indirectly linked to their effect on growth (Sevag & Shelbourne 1942). The inhibitory effect of sulphonamides on respiration has not yet been completely explained, although a number of studies point in the direction in which further progress may be made. It has been shown that sulphonamides form dissociating complexes with enzymes and other proteins (e.g. Davis 1943) and in several such cases loss of enzyme activity was found to result (e.g. Davenport 1945). The work of Dorfman, Rice, Koser and Saunders (1940) and Berkman & Koser (1943) indicates that phosphopyridine nucleotides participate in bacterial respiration and it explains the restoration of normal bacterial growth upon the addition of pyridine nucleotides to the culture medium, in the presence of sulphonamides (West & Coburn 1940). It has also been reported that sulphonamides inhibit the respiration of Plasmodium knowlesi in the presence of glucose (Coggeshall 1940), which may be indicative of interference with oxidation of carbohydrates.

In order to establish the point at which the drug interferes with respiration, attempts have been made to determine the effects of various sulphonamide drugs on isolated respiratory enzymes and co-enzymes. Among such investigations are those of /
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<th>Temperature</th>
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<th>Inhibition per cent</th>
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<tr>
<td>Cytochrome oxidase</td>
<td>25</td>
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<tr>
<td>&quot;c&quot; reductase</td>
<td>25</td>
<td>$1 \times 10^{-1}$</td>
<td>30</td>
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<tr>
<td>Triphosphopyridine nucleotide</td>
<td>25</td>
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<tr>
<td>Zwischenferment</td>
<td>(37)</td>
<td>(0.014)</td>
<td>(30)</td>
</tr>
<tr>
<td>Escherichia coli</td>
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<td>0.08</td>
<td>30</td>
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<tr>
<td>Staphylococcus albus</td>
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<tr>
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<tr>
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<td>37</td>
<td>3.4</td>
<td>53</td>
</tr>
<tr>
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The figures in parentheses are extrapolated from experimental data obtained at different temperatures.


Table I: The effect of sulfanilamide on Respirating Enzyme Activity and on Oxygen Consumption of Microorganisms and Tissues.
of Altman (1946), who observed that the sulphonamide greatly inhibited the activity of Zwischenferment but had relatively little effect on cytochromes, cytochrome c reductase, and lactic dehydrogenase (Table 1). Triphosphopyridine nucleotide and cytochrome oxidase were not affected at all. Addition of the prosthetic group to the protein moiety of the dehydrogenase was shown to protect the protein from the effect of sulphonamides, but did not reverse the sulphonamide effect. Therefore it would appear that sulphonamides react irreversibly with Zwischenferment and compete with the prosthetic group for the protein moiety of the enzyme.

Thus, although a primary action of sulphonamides on bacterial metabolism cannot be excluded, the evidence is greatly in favour of the Woods-Fildes explanation of their main effect. This evidence, however, is as yet largely indirect, but considerable weight must be attached to the demonstration that PABA is an essential nutrient of bacteria. Moreover, as will be discussed later, there is good evidence that PABA is produced in excess by some sulphonamide-resistant strains. It must also be remembered that many of the early anomalies of antagonism have largely been resolved by the discovery of the link between PABA metabolism and the various metabolic processes centred around folic acid, another essential metabolite. Certain of the present anomalies may cease to exist when bacterial metabolism becomes further elucidated.

At
At this juncture it is appropriate to discuss some of the experimental difficulties which have already been encountered with regard to the supposed action of sulphonamides on respiratory enzymes and which will be met with to an even more crippling degree in connection with the antibiotics.

Morphologically some chemotherapeutic agents are bacteriostatic while others are bactericidal. If the drug is bactericidal its action must of necessity be investigated in dying cells; that is, in cells whose metabolism must be grossly abnormal. The problem, moreover, is much the same with the bacteriostatic agents and bactericidal agents in bacteriostatic concentrations, because, although by definition they inhibit growth without killing the cell, they are slowly bactericidal. The second major difficulty is that, since the normal metabolic processes are so closely interconnected, interference with one process may affect so many others that the positive effect obtained may depend largely on the particular biochemical activity chosen for observation. For example, if the reaction inhibited by the smallest dose of the drug were the conversion of A to B, the organism may fail to grow from lack of B or from the toxic action due to excess of A. Or the presence of increased A in the cell may result in a useless reaction of A with X thus lowering the concentration of X and preventing its usual and necessary /
necessary reaction with $Y$. These considerations can be expanded almost indefinitely so that the observations of the modified metabolism may be a result that is many enzymic steps removed from the primary site of inhibition. In view of these experimental difficulties, it will be realised that to distinguish between the primary and the secondary positive effects of a given therapeutic agent may be impossible. The situation is not aided by the fact that interest in the normal metabolism of the bacterial cell is only of recent origin. Consequently, it is with some trepidation that an attempt is made to briefly review the present state of knowledge concerning the mode of action of several of the better known antibiotics. Most of the recent knowledge on this topic has been reviewed in a symposium arranged by the Society of American Bacteriologists (Bact. Rev. 1953).

Penicillin excels in its lack of toxicity, even when given in massive doses and over a long period. It has been possible to synthesise the penicillin molecule (du Vigneud et al 1946), which consists of a curious ring-condensation of two amino acids, $\beta$-dimethyl-cystiene and alanine. The various penicillins are distinguished by the different acids coupled to the alanine. Penicillin is bacteriostatic in higher concentrations and it has a preferential affinity for gram-positive organisms. According to Gale (1948) in their normal metabolism gram-positive organisms in /
in contrast to gram-negative ones - actively take up free glutamic acid and lysine from their environment. This process is accelerated by the magnesium ribonucleic acid which is present in the cell walls, and which is incidentally responsible for the gram-positive staining. Glutamic acid and lysine are concentrated in the interior of the cell wall, whence they are mobilised for protein synthesis. The sensitivity of gram-positive organisms and the immunity of gram-negative ones is explained by the fact that in the latter glutamic acid is synthesised inside the cell and does not have to be taken in from outside. Organisms which become penicillin-resistant are supposed to acquire the ability to synthesise glutamic acid and so need no longer assimilate it from their environment. Gale therefore suggests that penicillin acts by disrupting the metabolism of ribonucleic acid in the cell wall and thus preventing the normal assimilation of glutamic acid by sensitive organisms. This attractive theory, however, does not explain all the facts since *B. subtilis* remains sensitive to penicillin even when making all its own amino acids.

Umbreit (1953), on the other hand considers that penicillin acts by inhibiting an early stage of nucleic acid synthesis. Pratt (1953) has drawn attention to the similarity between the changes in the morphology and staining reactions of penicillin-treated organisms and those described by Stearn & Stearn (1930) as being characteristic of "starving bacteria". Ribonuclease exerts similar effects. Almost an equal number of reports can be /
be found in the literature to show that in sensitive bacteria penicillin retards, accelerates or has no effect on respiration. Probably all three statements are correct, each for different experimental conditions. Another hypothesis is the rather vague one that penicillin combines irreversibly with a cellular constituent essential for division.

Streptomycin is a glycoside containing streptidine (a base which is related to inositol), streptobiose (which is methyl pentose), and glucosamine (a derivative of dextrose). The gram-negative and acid-fast organisms are particularly sensitive to it. Unlike the sulphonamides and penicillin, it affects not only growing organisms but also those in the resting phase. Its bactericidal effect increases with higher concentration (Garrod 1948). The action of streptomycin seems to be more complex and even less well understood than that of penicillin. It has been reported, however, that it interferes with the oxidation of threonine and serine in gram-negative organisms, as well as with the oxidation of fumarates and pyruvates (Umbreit 1949; Oginsky et al 1949). Umbreit & Toulazy (1949) have described a process which they call the "oxaloacetate-pyruvate" reaction, which consists in the condensation of oxaloacetate and pyruvate molecules with the probable oxidation of many unknown intermediate compounds. This reaction is apparently as important to the animal as it is to the bacterium and it is inhibited by streptomycin. However, the enzymes involved in the /
the oxaloacetate-pyruvate reaction in animal tissues are mainly localised in the mitochondria. The explanation of its selective action seems to be that it is prevented from reaching the sensitive enzyme systems in the animal cell, presumably by permeability barriers at the cell wall and at the mitochondria (Umbreit 1951), whereas no such barriers exist in the bacterium. Oginsky (1953) drew attention to the inhibition by streptomycin of aspartate and amino acid oxidation by B. coli, and to other respiratory effects causing increased acetate formation. In bacterial cells capable of oxidising acetate, no streptomycin inhibition of the oxidation of pyruvate and oxaloacetate could be demonstrated, but the oxidation of these substances was much inhibited if acetate oxidation was slight or absent. The effect of streptomycin on the acid-fast bacteria is even more complex. It has been impossible to demonstrate an oxaloacetate-pyruvate reaction in the tubercule bacillus, but streptomycin has been shown to inhibit the oxidation of the higher fatty acids with twelve or more carbons (Oginsky et al. 1950). Very little is known about the metabolic pathways in acid-fast organisms, but everything discovered so far suggests a process comparable to the oxaloacetate-pyruvate reaction (Umbreit 1950).

Isoniazide has not yet been so intensively studied as the other antibiotics, but an explanation of its mode of action might be particularly interesting since it is active only against mycobacteria.

Smith (1953) named forty-nine bacterial metabolic processes and /
and enzyme systems which have been studied for their sensitivity to chloramphenicol, but he considered that only two of these were clearly significant as causes of growth inhibition - namely, the interference with utilisation of amino acids and with the metabolism of fats and esters. He cited work by Gale & Paine (1951) which suggests that chloramphenicol may possibly act by inhibiting protein synthesis because of its action in preventing the formation of combined glutamate and the utilisation of ammonia. An important point about chloramphenicol inhibition of esterase activity is that this action is found with esterases isolated from tissue cells as well as with bacterial esterases, but that intact tissue cells - of horse liver, for example - possess a mechanism which prevents the antibiotic from inhibiting the esterase activity of host cells.

Hobby (1953), reviewing work on aureomycin and terramycin, mentioned evidence for their action on the phosphorylation system, on the metabolism of tricarboxylic acids and α-ketoglutaric acid and on the oxidation of glycine, glutamic acid and aspartic acid. It is possible that the site of action of both these antibiotics may involve the initial step in acetic acid oxidation.

It is therefore quite evident that studies on the mode of action of antibiotics are as yet not sufficiently advanced to permit any generalisations as to their actions or to formulate with any precision a positive statement of any common features of their action. The assumption that the antibiotic specifically interferes /
interferes with some important metabolic reaction in the cell receives some support from studies of penicillin and streptomycin but little from studies on any of the other antibiotics. Even in the case of penicillin and streptomycin we are a very long way from localising the site of action, although hypotheses are abundant. It does seem clear, however, that as a group the antibiotics are not competitive analogues of essential metabolites as the sulphonamides are thought to be.
MECHANISMS OF ACQUIRED RESISTANCE

There are theoretically several ways whereby bacteria may be naturally resistant or acquire resistance to the action of a chemotherapeutic agent. Two of these follow directly from the study of the mode of action of chemotherapeutic agents that has just been completed.

In the first instance an organism may become resistant because of the excessive formation of a metabolite whose action is blocked by the drug. As previously discussed the observations of Wood (1940) first suggested that PABA was synthesised by certain species of organisms and that PABA, an essential metabolite, competed with the chemically related sulphonamides for a position in a bacterial enzyme system. It is conceivable, therefore, that acquired resistance may in some cases at least be due to an acquired ability to make large amounts of PABA. Thus, MacLeod (1940) observed that cultures of a sulphonamide-resistant strain of Type I pneumococcus yielded a filtrate which inhibited the antibacterial action of sulphapyridine for E. coli to a greater degree than a filtrate prepared from the parent sulphonamide-sensitive strain. Mirick (1941) utilised a suspension of a soil bacillus, which could be specifically adapted to oxidise PABA, and found that this suspension rapidly destroyed the diazotizable sulphonamide-inhibiting substance present in the filtrate prepared from a sulphonamide-resistant strain of pneumococcus. Landy and his colleagues (1943), using Acetobacter suboxyxylans /
suboxylane for microbiological assay and checking their results with chemical methods found that strains of *Staph. aureus* which had been made resistant to sulphathiazole by training in sulphathiazole-broth contained seventy times as much PABA as the normal strains. Spink et al (1941) obtained evidence that the resistance of staphylococci to the action of sulphonamides was similarly closely related quantitatively to the production of PABA by the bacteria. Thus strains of staphylococci produced diazotizable materials which could be converted to a dye and the intensity of the colour reaction could be quantitative in the same manner as PABA. The sulphonamide-resistant strain which they studied produced more diazotizable substance than non-resistant strains. They found that the development of a colour by the diazotizable substance could be inhibited by exposing the substance to Mirick's soil bacillus. Furthermore, the diazotizable substance produced by staphyococci inhibits the anti-staphylococcal action of sodium sulphathiazole to approximately the same degree as equivalent amounts of pure PABA. Two microbiological methods for assaying PABA were employed for quantitating the amount of this material produced by staphylococci and in general the sulphonamide-resistant strains produced more PABA than the sulphonamide-sensitive strains. However, the results obtained with the two different methods of assaying were somewhat inconstant and although the discrepancy might be explained by several sources of error which are inherent in any biological assay it is possible that another mechanism or mechanisms /
mechanisms may be involved in the development of such resistance. Moreover, certain criticisms can be made of the methods used in these estimations. For example, the danger of assuming that the diazotizable substances produced by sulphonamide-resistant bacteria are in fact PABA has been emphasised by reports that bacteria can produce diazotizable aromatic amines which apparently are not PABA. Miller (1941) found that filtrates of both susceptible and resistant staphylococcal cultures, although containing a primary aromatic amine as shown by diazotisation and coupling with dimethyl-$\beta$-naphthylamine, had no antagonistic effect on sulphanilamide action. As regards the microbiological tests, although Landy & Dicken (1942) observed that of fourteen related compounds none possessed more than one tenth the growth-factor activity of PABA for Acetobacter suboxytans, one can still question their specificity. Indeed, Mirick (1943) has pointed out several possible sources of error. Furthermore, there is evidence that sulphonamide-antagonism and growth-factor activity cannot be assumed to be associated phenomena (Henry 1943).

The answer to certain of these criticisms is perhaps given by the work of Leskowity et al (1952). A diazotizable amine was isolated from sulphonamide-resistant staphylococci and for purposes of characterisation ultra-voilet spectra were taken of the impure amine in buffered solutions at two different pH's. The differences in the wave length of maximum absorption bands was
was characteristic of various substituted benzenes, in particular PABA. For further comparison of the amine resort was made to paper chromatography; this revealed an amine resembling PABA and another contaminating diazotizable amine present in small amounts. The PABA-like amine was further separated by chromatography; its Rf values and its spectrum in the ultra-violet then equalled those obtained with PABA. A 2-4 dinitrophenol derivative of the amine was prepared and the m.p. was similar to that of the derivative made from PABA. Thus, on the basis of physical and chemical properties described, it would appear unequivocal that this bacterial amine is in fact PABA.
The second conceivable mechanism of acquired bacterial resistance which follows from the previous study of the mode of action of chemotherapeutic agents would be the development of metabolic pathways which avoid that blocked by the drug. Thus Gale has suggested that when gram positive organisms become resistant to penicillin they acquire the ability to synthesise the amino acide glutamine and lysine for themselves rather than depend upon expending energy in collecting them from the surrounding medium. Another example might be an ability on the part of organisms which have become resistant to streptomycin to dispense with the oxaloacetate-pyruvate reaction or its equivalent from their metabolism. Unfortunately, these examples and many others that could be made are purely speculative, for the simple reason that until we are certain of the primary positive effects which an antibiotic has on susceptible cells it is unlikely that we will be able to detect significant differences in the metabolism of resistant cells.

Organisms could also become resistant, however, if they developed a way of destroying the drug. Thus one of the major problems related to penicillin-resistance has been whether penicillinase plays a role in the resistance so notoriously displayed by staphylococci. Abraham & Chain (1940) first showed that \( \text{E. coli} \) produced an enzyme, designated as penicillinase on account of its ability to destroy penicillin. A later observation by Abraham et al (1941) recorded that a strain of Staph. aureus adapted by...
in vitro methods to grow in the presence of high concentrations of penicillin did not produce penicillinase. McKee, Rake & Honck (1944), however, encountered a strain of Staph. aureus which was resistant to penicillin and which formed a filter-passing enzyme capable of destroying penicillin.

The problem was more thoroughly investigated by Spink & Ferris (1947) and these workers repeatedly observed two fundamentally different types of staphylococcal resistance. First there is the type of resistance that is acquired in vitro by adapting the organisms to grow in increasing concentrations of penicillin. This type of resistance is only a temporary property of the bacteria and is not associated with the formation of penicillinase. The second type of resistance appears to be a permanent characteristic of the strains and is always associated with the production of penicillinase. This type of resistance is an inherent property of some naturally resistant strains and of strains that have acquired resistance in the human body as a result of treatment. It is not definitely known whether the temporary type of resistance occurs in the human body following therapy with penicillin. If the phenomenon does occur, it is probably of little clinical significance, since such strains have been shown to be less virulent than the sensitive parent strains (Spink & Ferris 1944; Blair, Carr & Buchanan 1946; North & Christie 1946). The underlying mechanism whereby penicillin-sensitive strains acquire a temporary resistance to the action of penicillin is not clear.
The fundamental question is not whether a given strain produces penicillinase but rather how quickly and how potent is the penicillinase produced by a strain in relation to the concentration of penicillin and the time necessary for the antibiotic to destroy the cells. Abraham & Chain (1940), for example, found that *M. lysodeiktiens* produced penicillinase and yet this strain was sensitive to penicillin. Spink & Ferris (1947) showed that strains of staphylococci with a relatively high degree of resistance produce a potent inactivator of penicillin; the greater the resistance the more potent the penicillinase.

This relationship of penicillin production to the resistance of penicillin has also been demonstrated for other species of bacteria. Thus Woodruff & Foster (1944) have described an aerobic spore-bearing bacillus belonging to *B. subtilis* group which at a pH of 6.0 or above was not inhibited in its growth by several hundred units of penicillin because of the penicillinase produced by that strain. However, when the same strain was grown at pH 5.5, no penicillinase was produced and growth was inhibited by 10 units/ml. of penicillin. McQuarrie et al (1944) working with three strains of spore-forming gram negative rods noted a quantitative relationship between penicillin resistance and the production of penicillinase. It is well established, however, that other mechanisms are responsible for resistance with other species of bacteria since many gram negative bacteria are highly resistant to penicillin and yet do not produce penicillinase (Bondi & Deitz 1944).
The penicillinases from different bacteria are not identical but possess different physico-chemical properties, though they all have the common property of inactivating penicillin. It is of interest that Perlatien and Liebmann (1945) produced an anti-penicillinase immune serum by injecting rabbits with purified penicillinase. The significance of this observation must await further investigations.

The final mechanism of acquired resistance that will be considered is the possibility that for some reason or other the organism may seem to combine with the drug. It is of interest in this connection that trypanosomes which have become resistant to certain arsenicals no longer bind with the specific compound to which they are resistant (Eagle & Dvak 1951; Eagle & Magnusson 1944). Thus if normal and resistant strains of trypanosomes are studied in vitro it is found that the normal strains remove the drug from the solution and the resistant strains do not do so. The same finding has been reported in the case of mycobacteria which have become resistant to isoniazid (Barclay 1953). In both instances the failure of the resistant organisms to bind the drug has been tentatively explained on the basis of specific permeability changes. However, using isotopically labelled penicillin it has been shown in several laboratories that, contrary to earlier reports, penicillin is bound and concentrated by bacterial suspensions (Rowley et al. 1948; Mass & Mass).
Mass & Johnson 1949; Pollock & Perrett 1951; Few, Cooper & Rowley 1958). Although the significance of this in relation to the mode of action of penicillin is not yet clear, Rowley et al (1950) made the interesting observation that four penicillin-sensitive strains of \textit{Staph. aureus} bound considerably more penicillin than did two resistant strains of the same species, \textit{E. coli} or \textit{Klebsiella pneumoniae}. Mass & Johnson (1949) however, could find no regular difference in the penicillin uptake of penicillin-sensitive and -resistant strains of \textit{Staph. aureus}.

The problem has since been reinvestigated more fully by Eagle (1954), using \textit{Strept. pyogenes}, \textit{Staph. aureus}, \textit{Pneumococcus}, and \textit{Strept. faecalis}. When these organisms were exposed to $^{14}$C- or $^{35}$S-labelled penicillin they bound and concentrated the antibiotic and the amount bound from low concentrations ($0.001 - 0.01 \mu g /ml.$) was found to be related to the penicillin sensitivity of the strain. Highly sensitive organisms such as \textit{Strept. pyogenes} concentrated the antibiotic as much as 2000-fold, while strains with a high degree of natural resistance scarcely bound any at all. The bound antibiotic could only be removed to a minor extent by washing. Penicillin inactivated with penicillinase, by hot sulphuric acid or by cold hydrochloric acid was not similarly concentrated. Of great interest is the observation that cell-free sonic extracts of these organisms were found to differ in their combining affinity with penicillin in the same order as the intact cells, and again in relation to the penicillin sensitivity of
the organism from which they had been derived. This infers that the differences in the amount of penicillin bound by the bacteria of varying sensitivity do not depend on differences in the permeability of the cell, but rather on the reactivity of these organisms with the antibiotics.

This relationship, however, did not hold for artificially induced penicillin-resistance (Eagle 1954). Some resistant bacterial variants bound more penicillin than the originally sensitive strains from which they were derived; some bound a great deal less; and with one strain there was a progressive fall in reactivity with penicillin associated with increasing resistance. None of the resistant strains showed an increased power to inactivate penicillin existing in an unbound state within the bacterial cells.

It is also of interest that mammalian cells in tissue culture (mouse fibroblasts and malignant human epithelium) did not concentrate penicillin from the culture medium. Even at low concentrations, the cellular accumulation was usually less than that in the surrounding fluid and most of it was removed by washing. The radioactive material in such eluates was actively bactericidal and was presumably in large part unchanged penicillin.

Penicilloic acid, produced by the action of penicillinase, was bound to the same (limited) extent as the active antibiotics. Cell-free sonic extracts had the same limited reactivity with penicillin as the intact cells. The relatively minute amounts /
amounts bound by the cells are therefore not due to their impermeability, but instead reflect the inherently low inactivity of the cellular constituents with penicillin.

It would appear that the relative non-toxicity of penicillin for the mammalian cells in tissue culture, may be related to this low order of reactivity with the antibiotic. In these respects mammalian cells seem to behave like naturally-resistant bacteria, and unlike such penicillin-sensitive bacteria as Strept. pyogenes or Diplococcus pneumonia. It would be of interest to study the combining affinity of cell-free extracts of arsenical-resistant trypanosomes and isoniazid-resistant mycobacteria in order to determine whether the primary determinant of their enhanced resistance is in fact an altered permeability as originally thought, or whether, as with naturally penicillin-resistant bacteria, the vulnerable cellular components may have become less reactive with the chemotherapeutic agent.

It may well be that decreased reactivity of a vulnerable cell component (e.g. enzyme protein) with a drug may be a fairly common basis for resistance to its cytotoxic action. Thus, Davis & Mass (1952) studied resistant strains of E.coli resistant to β-nitrobenzoic acid, to sulphamidine, and to a number of sulphamidine derivatives. In a cogent analysis of the cross-resistance relationships in those mutants, of the competitive ratios of the drug to metabolite, and of the PAB requirement of the resistant strains, the only explanation of resistance uniformly consistent with the experimental data was that an enzyme /
enzyme protein of the resistant cells had a decreased affinity for the inhibitor as compared with the normal substrate.
MODE OF ORIGIN OF BACTERIAL RESISTANCE

Such knowledge as we have of the metabolic changes responsible for acquired resistance has been discussed. This knowledge is substantial and satisfying in relation to the sulphonamides, but in relation to penicillin incomplete, and to streptomycin, chloramphenicol, the tetracyclines and others almost nonexistent. The question now arises as to the mode of origin of the acquired resistance. Two conflicting hypotheses have been advanced. The first assumes that the particular environment produces the observed change in some of the bacteria exposed to it, whereas the second assumes that the resistant variants arise spontaneous during growth under normal conditions, the part played by the antibiotic being purely selective. These are known respectively as the "adaptative" and the "spontaneous mutation" hypotheses.

A variety of evidence is adduced in favour of the hypothesis of adaptation. Among the most interesting is that obtained by Abraham et al. (1946) in a study of acquired resistance to helvolic acid, polystictin and an antibiotic derived from a strain of B. subtilis. Resistance to each of these is specific, leaving susceptibility to the others unchanged. When an organism had been made resistant to one of them, it would then be rendered resistant to a second with the same facility as the original sensitive culture. If the first acquisition of resistance had been due to selection, it must have involved the /
the suppression of the vast majority of the original cells, which should diminish the likelihood that cells resistant to the second antibiotic would persist.

In studies of the acquisition of resistance by *Sh. flexneri* to sulphonamides, Stewart (1947, 1948) showed that papillae developing on colonies after about five days' incubation on a drug-containing medium were composed of resistant cells; he regards their appearance as a response to a chemical stimulus, and describes the change as adaptive variation. Mills (1949) studying the habituation of species of Neisseria to penicillin, shows that the degree of increase in resistance bears an exponential relationship to the number of transfers in the drug-containing medium producing it. Interpreting this in the light of conclusions of Dubos (1940) and Hinshelwood (1944) on the adaptive production of enzymes by bacteria, he regards the change as likely to be due to the expansion of a single enzyme rather than the replacement of one partially blocked enzyme by another. Baskett & Hinshelwood (1951) pronounce explicitly in favour of the adaptation hypothesis. They assume an autosynthesis of cell material and the occurrence of changes in enzyme ratios under the influence of the chemotherapeutic agent. The lag observed prior to the detection of the cells with altered properties is attributed by them to the time required for the reorganisation of the cell. Furthermore, they state that before the new steady state in enzyme ratios has been attained reversion
reversion occurs readily on subculture in the original medium because there is no influence to maintain the existing unstable enzyme ratios. Perhaps the most cogent evidence in favour of this argument is the fact that in some instances the degree of adaption has been reported to correspond exactly with the concentration of the drug producing it. This is obviously better explained by a simple alteration of enzyme balance than by selection between resistant and sensitive cells. Other phenomena which have been advanced in support of the adaptation hypothesis are the appearance of variants in cultures derived from a single cell, the lack of any explanation for the loss of resistance which may occur in a normal medium, and the phenomenon of multiple variation in which altered characters develop at different rates. The adaptation hypothesis runs into serious difficulty, however, when the stability of acquired resistance is considered. For example, Schmidt, Leden and Deltweiter (1942) induced sulphonamide resistance in vivo by infecting sulphapyridine-treated mice with virulent pneumococci, pooled the culture from the blood of the mice that died, and with the pooled blood infected another group of sulphapyridine-treated mice. Two to three repetitions of this process caused an appreciable increase of in vitro resistance and three to nine repetitions produced a maximal degree of the resistance. The acquired resistance was sufficiently established to withstand 215 passages through normal mice. Penicillin resistance is believed to be a permanent character once...
once acquired.

Baskett and Hinshelwood (1951) attempt to explain this stability by stating that once a new steady state in enzyme ratios has been maintained for many generations in the presence of the drug, it becomes fixed and stable. That is to say the whole pattern of enzyme pattern may become structurally so stabilised that it becomes a repeating unit which will not be disturbed unless important fresh adaptive changes are imposed upon the cell. Such argument by analogy, however, never appeals to the true scientist for it merely distracts attention from the basic scientific principles. To accept the adaptation hypothesis would be to accept that an acquired character may be transmitted to subsequent generations. Consequently this field of study has been referred to as "the last stronghold of Lamarckian heresy".

Those who favour the adaptation hypothesis contend that perhaps the laws of heredity may not be fully applicable to unicellular organisms reproducing by simple fission. To them it seems reasonable that a new individual which is merely one half of its parent should retain the character of the latter however acquired.

It becomes imperative therefore to decide whether or not cellular structures and cytological processes comparable to those in higher organisms are also present in bacteria. It is difficult to answer this question wholly in the affirmative since bacterial genetics has not yet advanced to the stage where
where it is possible to correlate experimental and cytological information to the extent that has been achieved in higher organisms. Moreover, many of the cytological observations on bacteria relevant to problems of variation are still controversial.

Since studies with higher organisms demonstrated that most of the determinants of hereditary characteristics are transmitted through nuclear components, it is important to ascertain whether similar organised structures exist in bacteria. Early cytological observations with bacteria were controversial in this respect but more recent improvements in technique have verified the existence of nuclei in bacterial cells. An organised nucleus in bacterial cells was described by Meyer as early as 1897 and was recognised repeatedly thereafter. Light microscopy has been the major tool and it has only been within the last twenty years that sufficient differential staining methods have been devised so that inherent details within the bacterial cell have been made out. Following the development of the Feulgen technique in 1924, Piekarski (1937) developed a method which utilised the same basic procedures but substituted the Giesma mixture in place of the Schiff reagent. This addition to the use of acid hydrolysis was necessary in the Piekarski and Feulgen techniques, enzymic removal of RNA from the cytoplasm has also been utilised by Tulasne and Vendrely (1947). This was a remarkable advance since the RNA of the basophilic bacterial cytoplasm had previously done much to observe the staining reactions of the nucleus. With the help of these techniques Robinow (1942), Bisset (1951) and...
and Delaporte (1950) and others demonstrated deeply stained, often vesicular, bodies within the bacterial cell. Delamater (1951) has recently developed a freezing-dehydration technique which is consistently capable of producing cytologic preparations of bacteria and other micro-organisms comparable to those utilised by cytologists in other fields.

The phase-contrast microscope has been applied to the study of the nucleus by Tulasne (1949) and by Stempen (1950). It has possible to visualise the nuclear sites or loci in the bacterial cells as irregular areas of less density by means of this instrument. The picture is essentially the same as that observed by means of the electron microscope (Mudd & Smith 1950). Such areas of decreased density probably represent vesiculate nuclei containing so-called nuclear sap within which the chromosomes lie. The decreased density also probably indicates a relatively high water content of the nucleus in comparison with the surrounding cytoplasm, and a relative lack of density of the chromosomes themselves. Delamater (1950) has observed that starvation or ageing with a concomitant reduction of cytoplasmic RNA produces cells in which the chromosomal structures become clearly visible with the phase contrast microscope.

Although theoretically the electron microscope should provide greatly improved definition, the density and thickness of the bacterial cell wall has tended to limit the usefulness of this instrument to the peripheral structures, such as cell/
cell wall, plasma membrane, flagella etc. Further cytoplasmic RNA tends to mask the nuclear components. Use of acid and enzymatic hydrolysis has not apparently reduced the electron scattering power of the cytoplasm, and it is found that what appear to be nuclear elements are visualised as irregular areas of increased density. The density and depth of the bacterial cell and the coagulation dependent upon desication of preparations for use with the electron microscope are the chief factors which limit the use of this instrument in the elucidation of nuclear structures. The cytological observations indicating the existence of true chromosomes in bacteria are also supported by the finding that nucleic acid-containing elements with a coiled structure and characteristic ultra violet light absorption spectra, typical of the chromosome of higher organisms, can be separated from broken E. coli cells following differential centrifugation (Marshak 1951).

In general it may be said that structures within the bacterial cell have been demonstrated which, both by the Feulgen reaction and by ultra violet light absorption estimation, contain DNA and probably represent true bacterial nuclei. In the older cytologic literature it is usually stated that the bacterial nucleus divides longitudinally without revealing any special internal structure. However, the newer techniques mentioned above have uncovered organised chromatinic structures which indicate that a true mitotic process occurs similar to that observed in higher organisms. Thus Piekarski (1949), Robinow (1945), Bisset (1951) have shown that the nuclear structures undergo cyclic divisional /
divisional states. Dumbell shaped chromatinic bodies are described as lying transverse to the axis of the cell, and to divide longitudinally perpendicular to that axis. They then separate, and in the process may form various V-shaped configurations. After they have migrated, they subsequently repeat the divisional process as described. Bisset (1951) obtained evidence for true mitosis in six organisms representing four groups of bacteria. For example in *Bacillus megathrium* the chromosomes in prophase contract to short dense rods, which may be counted. Three such chromosomal rods or granules have been consistently observed in the vegetative cells of this organism. Subsequently the chromosomes become condensed into a compact mass, opposite which a centriole appears, the exact origin of which remains obscure. The centriole subsequently divides and the two parts migrate to the opposite sides of the condensed chromosomes to form a typical metaphase spindle. A polar view of such a metaphase spindle demonstrates again the three chromosome components. In anaphase the chromosomes divide and draw towards the poles in telophase. As the chromosomes forming the two daughter nuclei migrate apart the centriole disappears; a new nuclear membrane appears to reform; and the chromosomes begin to elongate into delicate threads.

It is felt that the evidence now available for the occurrence of mitosis in bacteria clearly demonstrates that (1) the structure of the nucleus is comparable to that which is known in /
in larger organisms, and (2) that the divisional process follows essentially the same mitotic pattern as has already been established in these larger forms. The only essential difference appears to be one of size.

Since it has been long established that the biochemistry of the bacterial cell is essentially similar to other living forms, the establishment of a mitotic process helps further to bring these organisms into the general biologic picture to which they belong. They can no longer be considered as separate and distinct on the basis of their nuclear structure or lack of it. In particular, a material basis for the hereditary determinants in bacteria similar to that established for higher organisms would appear to exist. Nevertheless one could accept the above evidence that a mechanism exists in bacteria for the transmission of hereditary characters without necessarily believing that bacterial resistance arises by spontaneous mutation. Thus induced and heritable variations involving a major proportion of the progeny of treated populations have been described by Sonneborn (1950, 1951) in the killer and serotype character of paramecia, by Speigelman et al (1950), in yeast adapting to galactose, by Ephrussi et al (1949) in yeast exposed to acriflavine, and by L'Heritier (1948) in Drosophila treated with extracts of carbon-oxide-resistant strains. In all these cases the induced character has been shown to be cytoplasmically inherited, although in instances it has been demonstrated that this cytoplasmic inheritance is not autonomous but is in turn genetically controlled.
Further evidence in favour of the spontaneous mutation hypothesis is, however, not lacking. The results of in vitro experiments will first be considered. The first clear experimental demonstration of the occurrence of spontaneous, undirected mutations in bacteria was advanced in 1943 when Luria & Delbrück (1943) reported their fluctuation test. In this test a series of tubes, usually containing 0.2 or 0.5 mL of liquid medium, is inoculated with a small number of phage-sensitive cells. These cultures are incubated, without phage, until a certain population size is reached. The number of phage-resistant mutants in each tube is then determined following exposure of the cultures to phage, usually by pouring the contents of each tube containing phage into an agar plate containing phage. The results obtained from such a series of similar cultures can then be compared with the results of a series of samples taken from one culture started with a similar concentration of cells per mL and permitted to attain a similar population size/mL. The principle of the test is that if resistant variants arise because of contact with the phage (i.e., by adaptation) it should not make any difference whether populations exposed to phage come from a series of similar cultures or from one culture. At the times of exposure to phage, all cells would presumably be sensitive and the number of resistant bacteria obtained from a series of similar samples should show only the fluctuations due to sampling error (Poisson distribution) regardless of whether they came from a series of/
of parallel cultures or from replicate sampling of a single culture. On the other hand, if resistant bacteria arise spontaneously prior to the exposure to phage, a series of similar cultures will yield results different from those obtained with a series of samples from one culture. Parallel cultures may differ not only in the number of mutations which have occurred in each culture but in the time at which these took place during the growth of the culture. Thus, if one mutation occurred just prior to exposure to the phage, a culture would display only one resistant cell; however, if a similar mutation occurred several generations earlier it would have given rise to a number of resistant descendants. Thus, even if equal numbers of mutations occurred in parallel cultures the number of phage-resistant cells at the time of exposure to phage would be expected to differ considerably among the cultures. Numerous tests have confirmed that this is in fact the case; the fluctuations in the number of phage-resistant variants obtained in tests on a series of parallel cultures is significantly greater than that obtained in tests on a series of samples from one culture. These results clearly support the hypothesis that bacteria are capable of undergoing spontaneous mutation. The fluctuation test has been widely adopted in the study of bacterial resistance to chemotherapy, as the adjacent table shows. In all these instances the result has been such as to support the operation of spontaneous mutations rather than adaptation in the acquisition of bacterial resistance.
resistance. To my knowledge no case has been reported in which the fluctuation test has yielded a result in favour of the adaptation hypothesis or, indeed, a result which has been equivocal. For example, Demerec (1945) prepared experiments with staphylococci in which resistant cells were enumerated in multiple samples of the same culture. When thirty of the former and twenty of the latter were plated on agar containing 0.064 unit/ml. the number of colonies obtained from the independent cultures (in which mutation may be supposed to have occurred) varied from 9 to 839, whereas the variation in samples from the same culture was only between 16 and 38 colonies, (a difference evidently representing the sampling error). In a later paper Demerec (1948) applied this test to streptomycin with similar results, Alexander & Leidy likewise obtained conclusive results as regards streptomycin resistance by Ps. aeruginosa, Shigellae, H. pertussis and S. typhosa.

Although the validity of the fluctuation test has not been challenged, it gained only limited recognition probably in part due to the statistical and essentially indirect nature of the argument on which it is based. Newcombe (1949) procured more direct experimental evidence in favour of the mutation hypothesis. Bacteria of E. coli strain B/r susceptible to phage T1 were plated on agar and incubated until a limited population increase had taken place. On alternate plates the bacteria were redistributed over the surface of the agar by spreading with 0.1ml. sterile saline.
saline. All were then sprayed with phage Ti, and counts made of the colonies of resistant survivors which developed after further incubation.

On the adaptation hypothesis, the bacteria present at the end of the initial growth-period would all be phage susceptible, and spreading would serve only to redistribute the members of a homogeneous population. No striking differences in colony count between spread and unspread plates would therefore be expected. On the alternative hypothesis (spontaneous mutation), both susceptible and resistant cells would be present at the end of the initial growth-period wherever a sufficient end-population had been reached. Further, mutations taking place a generation or more before the cessation of growth would each be represented by a minute cluster of resistant cells all descended from one original mutant. Where the arrangement of bacteria is left undisturbed, a cluster would give rise to a single resistant colony after the application of phage; but when the bacteria are redistributed over the surface of the agar by spreading, a colony would develop from each resistant cell. Higher counts would therefore be expected from the spread than from the unspread plated. This is, in fact, what was observed, the difference being as much as fifty times when the end-population was highest. This experiment, therefore, confirms the conclusion drawn from the fluctuation test that phage resistant variants arise by spontaneous change prior to contact with phage. The phage acts merely as a selective agent permitting the detection of the few phage-resistant mutants present in the population.
population.

Similar results were obtained when Newcombe's spreading test was used in a study with streptomycin-resistant variants. (Bornschein, Dittrich & Hohne 1951)

Nevertheless, one of the important features of the above techniques is the use of selective conditions (e.g. phage, antibiotic) which prevent the multiplication of the parent cells but allow the growth of the mutant, thus permitting the destruction of a few mutants among the many parent cells. Yet the possibility remains that the selective environment may not only select but also direct adaptive hereditary changes. Therefore the most direct method of demonstrating the undirected, spontaneous origin of bacterial mutants would consist of the isolation of mutants in the complete absence of the environmental conditions that favour this establishment. This ideal situation has been achieved in tests employing the replica plating technique.

Lederberg & Lederberg (1952) described a very simple method that permits the transfer replica plating of bacterial growth from one initial plate to corresponding sites on a series of other plates. In place of an inoculating needle one might imagine a device consisting of many needle tips in a fixed array, so that one operation would substitute for repeated transfers with a single needle. The requirements of this design are met by pile fabrics such as velvet or velveteen. The pile provides space in a vertical plane for moisture that might otherwise cause lateral/
lateral smearing of any impression. A velveteen square is placed nap up on a cylindrical wood or cork support and held firmly in place with a metal flange or hoop pushed over the fabric and around the rim of the support. The agar plate carrying the initial colonies is inverted on to the fabric with slight digital pressure to transfer the growth. The imprinted fabric can now serve as a master pattern for the inoculation of other plates. Thus replica-inocula can be made from one imprinted velveteen sample on to numerous plates, containing different media if so desired, and the resulting growth on all these plates will be at corresponding sites. This has permitted a confirmation of previous observations regarding the clonal occurrence of bacterial variants.

A culture of streptomycin- and phage-susceptible E. coli was transferred several times in large volumes of broth. Concentrated inocula, about $3 \times 10^7$ cells, were then inoculated onto nutrient agar plates. The resultant growth was imprinted on velveteen and serial replicas were then transferred to several plates containing nutrient agar plus streptomycin. Colonies consisting of streptomycin-resistant cells were found to develop on identical sites on each replica plate. This indicated that the resistant cells transferred to the streptomycin plates were derived from small clones of resistant mutants already present at corresponding sites on the initial plain agar plate.

The hypothesis of spontaneous undirected mutation would, however, be further strengthened if the resistant mutants could/
Diagram illustrating the technique of infecting solution of mutants.

From Prusiner (1982): "Prion protein:"

Fig. 2.
could be isolated in pure culture without direct exposure of the bacteria to the selective agent. With the technique of replica plating this has been made possible. The area of growth on the initial plain agar plate corresponding to the site where a streptomycin-resistant colony developed on streptomycin agar was picked off and transferred to a nutrient agar broth and inoculated. An aliquot from this broth culture was then used for inoculating a second plain agar plate. The resulting growth, now containing a larger concentration of streptomycin resistant cells, was then used for a repetition of the described replica platings, and at each of their repetitions the location of the presumably streptomycin-resistant cells was determined by inspection of the site of growth on replica plates containing agar and streptomycin. After six stages of this enrichment process, a pure streptomycin-resistant colony was isolated.  

It is to be emphasised that by this method the streptomycin resistant variants were isolated without altering the media in which they grew; but were indirectly selected without being exposed to streptomycin at any time. There can be little doubt, therefore, that the resistance to streptomycin so obtained was a spontaneous mutation that occurred independently of the presence of the selective agent; that is, independently of the environment to which they are better adapted.

English & McCoy (1951) also convincingly demonstrated that mutants could be isolated in the absence of the environmental conditions that favour their establishment. They observed that/
Table 4. Examples of mutation rates.
All estimates, except those indicated by (), are based on the fluctuation test.

<table>
<thead>
<tr>
<th>variation involved</th>
<th>species</th>
<th>mutation rate per bacterium per generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>R _ S (colonial morphology)</td>
<td>Salmonella aertrycke</td>
<td>$5 \times 10^{-3}$</td>
</tr>
<tr>
<td>Flagellar antigenic phase;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&quot;group--- specific &quot;</td>
<td>Salmonella typhimurium</td>
<td>$3 \times 10^{-4}$</td>
</tr>
<tr>
<td>&quot;specific group&quot;</td>
<td>Salmonella typhimurium</td>
<td>$1 \times 10^{-3}$</td>
</tr>
<tr>
<td>Pigmentation</td>
<td>Serratia marcescens</td>
<td>$1 \times 10^{-4}$</td>
</tr>
<tr>
<td>Radiation resistance</td>
<td>E. coli</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Threonine resistance</td>
<td>Salmonella typhimurium</td>
<td>$4 \times 10^{-6}$</td>
</tr>
<tr>
<td>Resistance to isoniazid</td>
<td>M. ranac</td>
<td>$5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Colonial morphology</td>
<td>Phytomonas stewartii</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Penicillin resistance</td>
<td>Staph. aureus</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Resistance to phage T</td>
<td>E. coli</td>
<td>$1 \times 10^{-7}$</td>
</tr>
<tr>
<td>Resistance to phage T</td>
<td>E. coli</td>
<td>$3 \times 10^{-9}$</td>
</tr>
<tr>
<td>Tryptophan independence</td>
<td>Salmonella typhimurium</td>
<td>$5 \times 10^{-6}$</td>
</tr>
<tr>
<td>Histidine independence (h to h)</td>
<td>E. coli</td>
<td>$3 \times 10^{-8}$</td>
</tr>
<tr>
<td>Histidine dependence (h to h)</td>
<td>E. coli</td>
<td>$1 \times 10^{-9}$</td>
</tr>
<tr>
<td>Utilization of itaconate as C source</td>
<td>Pseudomonas fluorescens</td>
<td>$2 \times 10^{-4}$</td>
</tr>
<tr>
<td>Sulfathiazole resistance</td>
<td>Staph. aureus</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin dependence</td>
<td>E. coli</td>
<td>$1 \times 10^{-6}$</td>
</tr>
<tr>
<td>Streptomycin resistance (1000 g)</td>
<td>Ps. aeruginosa</td>
<td>$4 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (1000 g)</td>
<td>E. coli</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (1000 g)</td>
<td>Shigellas</td>
<td>$3 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (1000 g)</td>
<td>H. pertussis</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (1000 g)</td>
<td>Salmonella typhosa</td>
<td>$1 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (25 g)</td>
<td>Salmonella typhosa</td>
<td>$5 \times 10^{-10}$</td>
</tr>
<tr>
<td>Streptomycin resistance (25 g)</td>
<td>H. pertussis</td>
<td>$6 \times 10^{-10}$</td>
</tr>
</tbody>
</table>

types of variation so as to illustrate the generality of the phenomenon throughout the whole of bacteriology. It must be recognised, however, that phenotypically similar changes can be caused by mutations affecting any one of several genes. Under such conditions the mutation rates can be dependent upon the gene involved, and different mutation rates may be observed for similar phenotypic changes.

It would be of great interest to know whether organisms showed resistance to chemotherapy before these drugs were introduced into medical practice; for example, whether any strains of Staph. aureus were capable of producing penicillinase before penicillin was marketed. The difficulty here of course is that, the enzyme penicillinase was not described until well after the introduction of penicillin. However, although I cannot quote a reference, I am told (Gould: personal communication) that when old uncontaminated pre-penicillin cultures which had been maintained in the laboratory stocks for years were re-examined some were in fact found to produce penicillinase. More conclusive evidence on this important point seems to be lacking.

As mentioned previously, supposed correspondence of the resistance level with the concentration used in the "training" to an antibacterial agent has been considered as one proof of the active induction of resistance by adaptation of enzyme systems (Hinshelwood 1944). However, Hinshelwood's finding of a gradual and even development of resistance by Bact. lactis aerogenes to diaminocacidine has not been confirmed by others in regard to a variety /
variety of bacterial species and chemotherapeutic agents. Thus Demerec (1949) maintains that so far but two patterns of resistance have been established, the penicillin pattern and the streptomycin pattern. The pattern of resistance appears to be characteristic for the sulphonamides and for each type of antibiotic, and is not determined by the bacteria involved. Detailed analyses of the pattern of streptomycin resistance were made by Demerec with *E. coli* and *Staph. aureus*, and by Bryson with *Mycobact. ranae*. Quantitative studies of penicillin resistance have only been made by Demerec with *Staph. aureus* (1945), but the results of other investigators, who have studied various phases of the action of penicillin with a variety of species, do not suggest that the pattern of resistance to penicillin is different in other bacteria from that analysed with staphylococci.

Although, the "penicillin" and "streptomycin" patterns differ significantly in the manner in which high-level resistance is arrived at, a "stepwise" development of resistance is common to both. In connection with the present argument as to the mode of origin of resistant strains this "stepwise" development of resistance is of considerable theoretical importance. An even and gradual development of resistance which is exactly proportioned to the concentration of the drug producing it is what one would expect on the basis of an adaptation hypothesis, whereas sudden jumps in a stepwise fashion are what one would anticipate as the basis of mutation. Accordingly Demerec interprets these findings in/
in terms of the mutation hypothesis. 

Demerec observed that in the case of penicillin the first-step mutants are very uniform in their degree of resistance, which is only slightly higher than that of the original strain. Additional mutations occurring in such first-step mutants result in bacteria possessing a higher (second-step) degree of resistance, and in a similar manner third-step and still higher resistance develops. The variation in degree of resistance among mutants of the same step is always slight. This uniformity constitutes the most striking feature of the penicillin pattern; and it explains the stepwise increase in resistance. Because a significant degree of resistance can be obtained only by additional mutation in an already mutant bacterium, at least two mutations in the same individual are required to bring it about. Since the mutation rate is low, however, about $1 \times 10^{-5}$/bacterium/generation - the chances for simultaneous occurrences of such double mutations are extremely low ($1 \times 10^{-16}$; or practically nil), so that a large number of first-step resistant bacteria must first be present in order that a second-step mutant may occur. In other words, higher resistance may be attained only in successive steps, and no step may be skipped in the process. (See fig.3)

The streptomycin pattern of resistance observed by Demerec differs from the penicillin pattern in that the variation between the first-step mutants is very great. They range from individuals only /
only slightly more resistant than the original bacteria to those
having complete resistance to streptomycin. Thus, although
higher resistance may be attained in successive steps, as with
penicillin, it may also arise in a first-step mutant.

Attempts to analyse the resistance pattern for aureomycin
are complicated by the mode of action of that antibiotic on E. coli,
the organism used for the experiments. The results, however,
show definitely that the pattern of aureomycin resistance is
different from that of streptomycin resistance, in that highly
resistant mutants do not appear in one step. It may be similar
to the penicillin resistance pattern.

The genetic mechanism responsible for both the penicillin
and the streptomycin resistance patterns may be explained by the
assumption that several genes govern the reactions that determine
sensitivity or resistance, and that the set of genes affecting
penicillin resistance is different from the set of genes affecting
streptomycin resistance. If any one of these genes should mutate,
the bacterium in which such a mutation occurs and the strain
developed from such a bacterium will be more resistant to the
respective antibiotic than was the parent strain. Such a strain
is what Demerec has called a "first-step resistant strain."

The fact that first-step penicillin-resistant strains are
fairly uniform in degree of resistance is consistent with the
assumption that all genes affecting resistance to penicillin have
similar potency, so that the effect of mutation is the same
regardless of which of the genes happen to mutate. According
to /
to this hypothesis, there is still present in a first-step resistant strain a number of unmutated genes that affect resistance. Mutations of any one of these produces a second-step resistant strain, which possess a higher degree of resistance than the first-step strain. Similarly, by mutation of another gene in a second-step resistant strain, a still higher degree of resistance is attained, characteristic of the third-step resistant strain; and by

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**Fig 5.** Comparison of survival curves, showing the penicillin and streptomycin types of resistance. Bottom:

by repetition of the process a very high degree of resistance may be reached.

The observed behaviour of resistance to streptomycin also can be explained by assuming the existence of several genes determining such resistance. Unlike the genes for penicillin resistance, however, these are assumed to differ greatly from one another in potency. If a gene of low potency mutates, the first-step resistant strain will have a low degree of resistance; but if mutation occurs in a highly potent gene, the first-step resistant strain will be highly resistant. Consequently, considerable variation in degree of resistance is to be expected between first-step strains, and for the same reason a highly resistant strain may be obtained either in one step, by mutation of a highly resistant first step mutant, or in several steps, by selection of mutants of low resistance value.

It is perhaps worth noting that the assumption that several genes are responsible for resistance is not a new one. A similar assumption, involving the presence of about twenty genes, is necessary to explain the complex situation observed in a study of resistance of E. coli to several bacteriophages.

The weight of available evidence, therefore, would appear to indicate that bacterial resistance to chemotherapy arises not by a modification of the bacterial metabolism caused by contact with the drug (adaptation hypothesis), but rather as a result of undirected spontaneous mutations. Nevertheless, some evidence exists indicating that resistance to low levels of antibiotics may /
Fig. 4. Development of Resistance to Di-hydroxystyptogercyl G.T. rat
may also occur by a process akin to long-term adaptation. For example, Gibson & Gibson (1951) have performed experiments which indicated that with regard to dihydrostreptomycin both adaptation and selection may be involved in the development of resistance. Their experiments were of two types (a) "progressive training" by using the organism growing in the highest concentration of the antibiotic and (b) "repetitive training" by subculturing repeatedly from a low concentration of the antibiotic and determining the level of resistance in each subculture. (Fig 4)

End-points of growth, i.e. concentrations up to and including these at which growth has occurred without exception were observed at all dihydrostreptomycin levels from 2.5 - 50 μg/ml; but no end-points were obtained between 50 - 5,000 μg/ml. Thus if an organism was resistant to more than 50 μg/ml it was also resistant at least 5,000 μg/ml. It appears reasonable to postulate, therefore, that a relatively slight increase in resistance (up to 10x) is due to adaptation initiated by the exposure of the cells to low concentrations of the drug, and that the degree of adaptation of the drug can exceed that required for growth at the concentrations to which the cells are exposed. Highly resistant cells appear in sufficient numbers to give rise to growth in all concentrations of dihydrostreptomycin tested. These cells also appear in the populations obtained by repetitive training but are not favoured by a selective process.

Again, it has been shown by Eagle et al (1952), using penicillin, chloramphenicol and streptomycin, that at low (threshold)
(threshold) concentrations of antibiotic, a large proportion of the organisms in a single colony culture may grow out to form slightly resistant clones. That slight increase in resistance was shown to be definite, reproducible and capable of persisting for more than 200 generations on subcultures on antibiotic-free media. It would appear unlikely that in a single colony culture consisting of $10^6$ to $10^8$ cells, as many as 10 to 90% of the organisms could have been preformed resistant mutants for this would imply an improbably high mutation rate. Moreover, if bacterial cultures contained so large a population of preformed mutants then individual colonies grown in antibiotic-free medium, each derived from one or two cells, should differ frequently and significantly in their resistance spectrum. This was not found to be the case. There is either an initial multiplication in the presence of the antibiotic with the appearance of a resistant cell as a spontaneous mutant which then grows out selectively (Demerec et al 1950) or there is an adaptive change to the drug.

These results show that a high level of resistance to dihydrostreptomycin can be reached from a very low one in a single "step", indicating a single mutation, either occurring spontaneously or induced by the drug itself. The results of repetitive training, however, excludes the possibility of selection of a rare mutant by the suppression of the sensitive cells. Future studies on the selective growth-rates of resistant and sensitive cells at low concentrations of dihydrostreptomycin may make such an explanation unnecessary.
unnecessary.

Finally, it is pertinent to point out that the spontaneous origin of mutants, independent of a specific environment, does not necessarily imply that the reactions of such mutants are identical in the absence and presence of a specific environment. For example, streptomycin-resistant mutants may display different metabolic reactions depending on whether they are growing in the absence or in the presence of streptomycin. These reactions which are capable of performing in the presence of streptomycin can be held responsible for their ability to grow under these normally inhibitory conditions. A mutation, therefore, may merely endow a cell and its progeny with the capacity to perform certain functions, or to display a certain phenotype, under appropriate environmental conditions.
THE PREVENTION OF BACTERIAL RESISTANCE TO CHEMOTHERAPY.

In this final section an attempt will be made to apply the knowledge discussed in previous sections to the practical clinical problem of what measures should be taken to prevent or delay the emergence of bacterial resistance to chemotherapy.

In the first place it is clear that the emergency of resistant strains correlated with the degree of abandon with which the chemotherapeutic agent used. At one time there was only one antibiotic and that in short supply. It was reserved for selected cases and administered with such care and circumspection that clinicians gradually became expert in assessing its value and limitations. Soon, however, research and synthesis in this new field devised fresh products with such rapidity that the harried clinicians could no longer keep pace with them. The effect of these developments has grown much more serious since oral antibiotics became fashionable. Potentially dangerous capsules are distributed by general practitioners as freely as aperient pills and with a much less specific end in view. The common cold or sore throat, the mild attack of enteritis or influenza is seldom allowed to subside until one antibiotic or other has been empirically prescribed. Even the hospitals are not immune.

Hassar (1955) has estimated that 25% of the patients in the medical wards of general hospitals would probably benefit from antibiotics. This figure was very close to that observed /
observed in three hospitals where strict indications had been set up for the administration of antibiotics. But in three other hospitals the proportions of general medical patients treated with antibiotics were 32%, 38% and 41%. Perhaps a major factor responsible for this indiscriminate use of these drugs is that since antibiotics are so dramatically effective against grave illnesses they will be at least equally useful for the treatment of various minor disabilities. The so-called prophylactic use of antibiotics is also often quite unwarranted. It would be a bad thing if it comes to be regarded as a token of eccentricity or over confidence to do a deliberate surgical operation without "antibiotic cover." Court et al (1955) reversed their earlier policy in treating with penicillin all staphylococcal infections in the new born; and have had no cause to regret their decision: no serious infection, such as pneumonia or osteitis, developed in any of the 633 babies with a superficial infection who were treated on conservative lines which precluded the use of antibiotics in the absence of some good clinical indication, such as evidence of a systemic infection. Many clinicians today appear to have forgotten that the tissues of the body have their own and usually very adequate defense mechanisms. If a wound infection or an abscess in the breast is localised and shows no sign of spreading, it is no less than maltreatment and an insult to the natural defenses of the body to interfere with chemotherapeutic agents. Yet I have seen this done repeatedly in one of the leading teaching hospitals.
hospitals in this country.

Hopps, Wisseman, and Whelan (1954) present a different approach to the demonstration of increased resistance in microorganisms attendant with the excessive and prolonged use of antibiotics. It depends upon a comparison of antibiotic sensitivities of strains of *Staph. aureus*, collected more or less simultaneously from widely separated geographic areas which differ markedly in the intensity and duration of use of antibiotics. The sensivity of the staphylococci from the different areas was determined for penicillin, (the most widely employed antibiotic) and for carbomycin (an antibiotic which had not yet received clinical application at the time the strains were collected), where the use of antibiotics had been minimal the distribution of penicillin-sensitive staphylococci closely resembled the distribution which was obtained in this country before penicillin had been in widespread use for any length of time (Bondi & Dietz 1945). Where antibiotic usage had been maximal, penicillin-resistant strains were common. In contrast a uniform sensitivity of these same strains to carbomycin was observed.

The effect of mass prophylaxis with sulphonamides on drug resistance has already been referred to (Damrosch 1946).

Since the conclusion has been reached that the majority of resistant strains of bacteria arise from spontaneous mutations rather than by adaptation, the importance of more discriminate and restricted use of chemotherapeutic agents is obvious. The mutation /
mutation rate for resistance is not high, but with the indiscriminate use of the sulphonamides and antibiotics these few resistant mutants will be selected so that the proportion of resistant to sensitive strains will rise. Consequently, any particular infection is more likely to be due to a resistant organism than to a sensitive one. Furthermore, it is easy to forget when treating a patient that chemotherapeutic drugs not only affect the causative pathogenic organism but also the whole bacterial population of the patient. Economy in the use of chemotherapeutic agents is therefore called for.

When the diagnosis has not been fully established clinically, treatment should not be started until steps have been taken to confirm the diagnosis by bacteriological methods. Thus, pyrexia is not a diagnosis and there is no justification for the indiscriminate use of potent chemotherapeutic substance to any /
any patient with fever. On the other hand, in many cases such as erysipelas and pneumonia, a correct clinical diagnosis implicates a limited range of organisms and demands the use of some form of chemotherapy.

The next question that should be asked is whether the particular infecting organism or organisms is susceptible to chemotherapy. Most virus infections are not susceptible and it therefore follows that in the great majority, especially those of a mild character, chemotherapy is unjustified. Although most bacterial infections are susceptible to one or other agent, the possible acquisition of resistance to chemotherapy must be remembered. Sensitivity tests would prevent the continued use of a sulphonamide or antibiotic in the treatment of a resistant infection. Theoretically, it would be desirable to withhold chemotherapy until the results of "culture and sensitivity" are at hand, but in practice this is not always desirable. Nevertheless, when facilities are available, a specimen of sputum, blood, pus or cerebro spinal fluid should be obtained before treatment is begun, so that if the initial empirical choice of drug is not successful the bacteriological report will then indicate a more rational therapy. An example would be a case of penicillin-resistant staphylococal pneumonia.

Because of the readiness with which bacteria acquire resistance to certain antibiotics, such antibiotics should only be used when other chemotherapeutic agents are ineffective. Thus
Thus, although the tetracyclines are the drugs of choice in the treatment of rickettsial infections, psittacosis, lymphogranuloma group of virus infections and undulant fever, they should not be used in other infections unless these infections fail to respond to sulphononides, penicillin or streptomycin.

The problem of dosage should be given serious thought. Gould, Bowie & Cameron (1953) attempted to correlate the therapeutic doses of the antibiotics and the resulting in vivo concentrations with the results of in vitro sensitivity estimations. In their study with urinary tract infections they concluded that if the dosage of an antibiotic is based on the in vitro sensitivity of the infecting micro-organism, it will often be much lower than the "standard - dosage". According to them this "rational" dose, except for chloramphenicol, may be very low indeed. When the dosage was comparable to the minimum effective in vitro concentration these workers were impressed that in no case did antibiotic resistance develop in any of the original species either during therapy or after therapy, even when this was prolonged. They suggest that it may be possible to prevent the development of resistance of antibiotics by exposing the organisms to in vivo concentrations closely related to their in vitro sensitivities.

The explanation for this clinical observation is based on the observation that all the individuals in a bacterial population are sensitive not to one particular concentration of a drug but rather to a range of concentrations, which may be narrow or
or wide according to the antibiotic and species concerned. If this range is X-Y, only 1% of the bacterial population may be killed at X (bacteriostatic level) but 99.9% at Y (bactericidal level. It is the latter value which is generally given in sensitivity reports. Probably the potential resistant variants are to be found among the individuals which persist at this concentration, but these cells will not become resistant or dominant until a higher and critical concentration of antibiotic is reached. The aim in therapy should therefore be to subject the organism to a concentration that is related as closely as possible to Y, which is the in vitro bactericidal level.

However, there is no direct experimental evidence to support such an explanation. On the spontaneous mutation hypothesis it would be quite untenable, although that does not necessarily destroy the argument. Further work is required to confirm or refute this important question of the "rational dosage" which has so far only been investigated in Gould's et al. (1953) seventeen patients.

A more widely accepted view today is based on the patterns of resistance described by Demerec and subsequent workers. It is argued that since low in vivo levels of chemotherapeutic drugs would favour the establishment of first-step mutants with these drugs that give the "stair-case" pattern (e.g. sulphonomides aureomycin), these drugs should be employed at high in vivo levels. In this way conditions would prevail in the body under which first-step mutants would be unable to survive. As we have seen/
seen, however, organisms are capable of becoming fully resistant to streptomycin, isonicotinic acid, hydrazide and penicillin (penicillinase - producing) in one mutational step. By giving high doses of these antibiotics as early as possible in the course of the infection the total number of organisms and therefore the number of such mutations would be reduced to a minimum. On the other hand, since there is evidence that such mutations are spontaneous the original inoculum will no doubt contain highly resistant organisms. By giving high levels of antibiotic all the sensitive strains will be eliminated and the resistant strains will be left unharmed to flourish unimpeded. It is immaterial to the defences of the body, however, whether the infecting organism is or is not resistant to a particular antibiotic unless there is an associated change in virulence; what does matter is the total number of organisms. It would therefore appear on present knowledge that economy and efficiency can best be combined by giving the chemotherapeutic agent in sufficient amount to achieve a complete effect in the shortest possible time - an approach to the *therapia sterilans magna* of Ehrlich.

In the event of failure of the first chemotherapeutic drug, a second is tried either empirically or on the basis of a bacteriological report on a specimen taken before treatment commenced. It is therefore important to know whether the first drug can induce resistance to the second one; that is, if any cross-resistance occurs among the antibiotics as occurs so markedly among the sulphonomides. Szybalski & Bryson (1952) tested isolated /
isolated resistant strains for cross resistance by the gradient plate technique, a method permitting the rapid and accurate development and quantitative comparison of resistance. *E. coli* were used on account of their rapid growth. As shown in the adjacent table (Table III), bacteria resistant to one antibiotic may display highly increased resistance to another with which they had no previous contact. The cross resistance patterns fall into four internally related major groups, as indicated by the shading in Table.

Several interpretations of the cause of cross resistance may be advanced, with the reservation that an explanation valid for one example may not apply to another. The simplest and most probable cause of cross resistance would depend on identity or close chemical similarity of toxic agents, resulting in parallel biological effects. Reciprocal cross resistance would indicate similar configuration of the biologically active portion of the drug molecule. A second interpretation would be that chemically dissimilar agents may interfere with the same metabolic pathway. For example, an agent may block an enzyme, whereas another may form a complex with intermediary products in the same chain of biochemical events. Obviously, bacterial mutants or adapted cells resistant through use of an alternative pathway would be immune to the effects of toxic agents related by intracellular site of action, rather than by common structure. A similar interpretation is that bacteria may become cross-resistant to certain substances by non-specific biological change. For/
Table IV  Cross resistance relationship of certain antibiotics as tested with strain B of E. coli.
(From Szybalski, W. and Bryson, V., J. Bact., 64:489, 1952.)

<table>
<thead>
<tr>
<th>Antibiotic polypeptides</th>
<th>BACTERIA</th>
<th>STREPTOMYCES</th>
<th>FUNGI</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polypeptide antibiotics</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bacitracin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>1/20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Circulin</td>
<td>1/100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptothricin</td>
<td>1/1.5</td>
<td>1/1.1</td>
<td>1/1.5</td>
</tr>
<tr>
<td>Neomycin</td>
<td>1/1.1</td>
<td>1/1.1</td>
<td>1/1.1</td>
</tr>
<tr>
<td>Catenol</td>
<td>1/1.1</td>
<td>1/1.1</td>
<td>1/1.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>1/1.5</td>
<td>1/1.5</td>
<td>1/1.5</td>
</tr>
<tr>
<td>Penicillin</td>
<td>1/1.5</td>
<td>1/1.5</td>
<td>1/1.5</td>
</tr>
<tr>
<td>Lupin</td>
<td>1/1.5</td>
<td>1/1.5</td>
<td>1/1.5</td>
</tr>
<tr>
<td>Polypeptide sensitivity of B(mcg/ml)</td>
<td>700</td>
<td>20</td>
<td>60</td>
</tr>
</tbody>
</table>

Figures in the table columns represent the factor of resistance (fold increase) compared with the parent strain set as unity (first row). Figure 1.1 designates slightly increased resistance; other figures are rough estimates. Increased sensitivity (collateral sensitivity) is shown by a fraction. The bottom row indicates the concentration of antibiotic, heading each vertical column, required to inhibit the parent strain. Framed and cross-hatched areas emphasize the high degree of cross resistance within groups of similar antibiotics. Unidirectional hatching indicates non-reciprocal cross resistance. Non-hatched frames point out a high degree of collateral sensitivity. Heavily outlined boxes on the diagonal show the resistance factor of each experimental strain to the antibiotic used in its selection. Resistant derivatives of strain B are listed in the first vertical column, in the same sequence as the antibiotics heading the table. Resistance to radiation (r) or to a specific antibiotic (abbreviation in the first column) is conventionally represented by a bar.
Fig 5. Diagram of divergent pathways in one area of gene-controlled reactions.

For example, selection by the toxic agent for decreased permeability would afford some degree of simultaneous protection (cross-resistance) against antibacterial agents dependent upon free diffusion into the cell. Another explanation is that the development of resistance in a specific case would be governed by mutation at one or a few loci and that these mutations always result in several phenotypic changes related in origin but diverse in effect. Thus a change that affects a precursor reaction could affect several subsequent diverse reactions (see fig. 5).

In practice cross-resistance is only important between closely related antibiotics, for example, between the various members of the tetracycline group. A patient whose infection has been found to be resistant to aureomycin is therefore not likely to be benefited by the subsequent treatment with oxytetracycline.

Another problem of interest, from both a theoretical and a practical point of view, is collateral sensitivity in which a strain made resistant to an antibiotic becomes considerably more sensitive to another. This phenomenon may be regarded as the result of a selective process. Reference to the Table (III) shows that in the isolation of strains possessing resistance to one antibacterial substance (e.g. chloramphenicol), we may at the same time select associated characters producing higher sensitivity to another agent (e.g. polymixin B). If these characters are associated in an obligatory manner, it will enable/
enable the facile selection of back mutations from resistance to sensitivity, at present a formidable task. Preliminary experiments have shown that it is impossible to have a strain of *E. coli* simultaneously more resistant to both chloramphenicol and polymixin B than is the parent strain. Whenever an antibiotic can be found that is particularly effective against bacteria resistant to another antibiotic, the combination of the two in a rational sequence might be found useful.

Monnier & Schoenbach (1951) induced resistance to five different antibiotics by serial transfer in media containing one of these antibiotics—penicillin, aureomycin, chloramphenicol, oxytetracycline and streptomycin. Micro-organisms which had been exposed to aureomycin, chloramphenicol, oxytetracycline or streptomycin showed an increased sensitivity to the antibiotic effects of penicillin. Moreover, the phenomenon of acquisition of penicillin sensitivity was most marked among strains found initially capable of forming penicillinase. The production of penicillinase appeared to be suppressed by exposure of micro-organisms to other antibiotics, but whether this is a result of a specific effect on organisms capable of forming penicillinase, or whether it represents a suppression of function which is maintained through successive generations, cannot at present be answered.

From a practical point of view an infection which is caused /
caused by a penicillinase-producing strain which is resistant to penicillin and does not respond to adequate penicillin therapy may possibly become susceptible to treatment by another antibiotic such as aureomycin. In this way the exact study of both collateral sensitivity and cross-resistance may help in designing a proper programme of multiple chemotherapy.

Not only can different antibacterial drugs be given consecutively but they can also be given simultaneously in combination. This latter practice has recently grown commoner until today much combined chemotherapy is being carried out with no more cogent logic than that "if one drug is good, two should be better!" Such facile reasoning cannot be too strongly condemned. Nevertheless, on the basis of the mutation hypothesis, since the mutation rate to antibiotic resistance is relatively low and since resistance to one antibiotic can be entirely independent of resistance to other antibiotics, the chance of occurrence of mutants simultaneously resistant to both antibiotics is as good as nil. Enquiry soon reveals, however, that the subject of combined chemotherapy is one of great complexity and that for various reasons these combinations may often be useful, sometimes may be no better than one of the pair alone, and at least occasionally may be contra-indicated because a diminished instead of an enhanced effect results. When the net effect is greater than the sum of each synergism is said to occur, and when the net effect is less, antagonism.
antagonism.

It is strongly emphasised by Jawetz & Gunnison (1952) that no general rules about synergism and antagonism can be laid down. The same pair of antibiotics may exhibit either effect against different organisms, according to their degree of sensitivity. Other authors have found that the same pair may be either synergic or antagonistic according to the concentrations used. The need for laboratory studies to provide a rational basis for combined treatment thus becomes obvious. Such studies, however, are time consuming and at present one prohibition on a large scale. Indeed, Garrod (1953) goes as far as to say that the bacteriologist who tackles individual problems of this kind seriously is undertaking the most complicated task in the whole of routine laboratory medicine.

The practice of combined chemotherapy has been fully vindicated in the treatment of tuberculosis. Streptomycin, as the antibiotic to which high level resistance is most readily acquired, stands in most need of a partner to prevent this, particularly when given over long periods. The combinations of streptomycin with para-aminosalicylic acid (PAS) or isoniazid, now regarded as obligatory in the treatment of tuberculosis, has been clearly shown to prevent or delay the development of resistance to either of the pair of antibiotics provided that the dose of PAS or isoniazid is adequate (Report 1949; Daniels & Hill 1952; Report 1953) In addition an improved therapeutic response is obtained.
obtained. Since giving a combination of two of these drugs to a patient whose organisms are already resistant to one of the two appears to be tantamount to giving the other alone, it is essential that sensitivity tests be done.

Precisely how the presence of a second drug may not only exert its own independent effect but also prevent the acquisition of resistance to the first cannot be claimed to be understood. However, it is fairly clear that the sulphonomides act by blocking a stage in an essential synthesis, and the antibiotics probably act similarly although the block may be effected in a different way. Now in some cases it has been postulated that the acquisition of resistance may result from the circumvention of this chemical process, another being substituted for it having the same ultimate effect and with which the antibiotic cannot interfere. If the second drug of a combined therapy blocks this second process then the alternative metabolic pathway cannot be established and the organisms will remain sensitive. Should this explanation be true it seems remarkable that so many combinations of drugs seem to act in this way and by this means it may be possible for more to be discovered about the points of attack of individual drugs.

With some hesitation it is suggested that in practice it may even prove advantageous to use more than two drugs together; Carpenter et al (1945) found that gonococci could be entirely prevented from acquiring resistance to sulphathiazole, rivanol, /
rivanol, promenade and penicillin only by exposing them to all four together, a combination of the first three not being fully effective. Similar in vitro studies have been made by Kaipainen (1951, 1952), who has shown that a mutual resistance-preventing relationship exists between streptomycin on the one hand and aureomycin, chloramphenicol or terramycin on the other. However, it does not follow that these combinations will be effective therapeutically.

A final method of reducing the incidence of drug resistant bacterial infections is directed against the spread of resistant organisms. The importance of cross-infection in determining the high incidence of penicillin-resistant staphylococci was discussed at some length at an early stage in the review. It is common knowledge that the classical methods for controlling cross-infection — barrier nursing, cubicle isolation, aseptic techniques — are quite inadequate for the exclusion of resistant staphylococci from susceptible sites of hospital patients. If no answer can be found to the growing problem of bacterial resistance to chemotherapy more elaborate, expensive and troublesome procedures to prevent cross infection within hospitals will have to be adopted.

The experiments of Gould & Allan (1954) are of much interest in this connection. Their statistics have already been referred to. The nasal carriage rate of medical and nursing personnel was considerably reduced for three months by the intra nasal application of oxytetracycline cream for ten days with a corresponding/
corresponding reduction in the number of cases of hospital infection due to the staphylococcus. Whether or not it would be feasible to use repeated administration of the antibiotic at intervals as a means of maintaining reduced infectivity of the carriers remains to be seen. The obvious dangers are the emergence and spread of strains of staphylococci which are not only resistant to penicillin but also to the tetracyclines, the upset of the normal commensal flora of the nose and adjacent passages, and the sensitisation of the carriers to the antibacterial agent. Observations on the repeated use of the nasal cream at well spread intervals are obviously necessary before this promising method could be widely adopted.

SUMMARY

The last decade has witnessed an unprecedented development in the therapy of infectious diseases and a significant change in the practice of medicine. The variety of antibiotic substances discovered and made freely available during this short space of time permitted the ready control of many microbial infections and thus prevented many deaths. The developments were exceedingly rapid. As one drug speedily followed another, pressure from the public, industry and physicians continuously urged the achievement of practical objectives. As a result, fundamental investigations of the properties and behaviour of these antimicrobial /
antimicrobial substances lagged far behind their widespread clinical use.

It was fortuitous (and possibly unfortunate) that the first of the antibiotics to become widely used, penicillin, possessed characteristics closely approaching that of an ideal chemotherapeutic agent; virtual lack of toxicity for the host; satisfactory absorption, distribution and action in the host tissues and fluids; highly lethal action against susceptible parasites; and, in particular, only very limited development of bacterial resistance. Physicians soon developed the habit of administering penicillin quite indiscriminately because "it might help and could do no harm." As other antibiotics became available this same attitude was applied. Unfortunately, the "newer" antibiotics were not as close to the chemotherapeutic ideal as was penicillin and their widespread use and abuse were followed by a surge of reports on treatment failure due to drug toxicity and the development of bacterial resistance.

In this essay an attempt has been made to review the present state of knowledge concerning the fundamental properties of the sulphonamides and antibiotics and the manner whereby bacteria can acquire resistance to their action. It is only by such a rational approach to the problem that measures which will be permanently effective in counteracting bacterial resistance will be achieved. So far the supply of new antibiotics has in
in most instances more than matched the capacity of bacteria to resist them, but if this supply should cease - and presumably the number yet to be discovered is limited - the time may come when a few of the more enterprising bacterial species will flourish more or less unhindered.
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