TWENTY YEARS OF ANTIBIOTICS—A REVIEW

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> " ... a man who has done a ... job in any special field should stop his paltry items and make a monographic study of the special territory once in a life time or better once in ten years. His fellows are entitled not to the printing of his notebooks, not to the rehash of his contributions over a period, but to his critical judgement of what all its means when brought together with the whole literature of that field of thought. Such would be of larger value to his fellows than a few more petty contributions."
>
> —Charles Thom (1952)—"Man, Mutants and Monograps"

The above advice of a distinguished mycologist is the guiding principle of this review. The literature on the modern era of antibiotics which started with the systematic search of Dubos "based on the scientific philosophy inherited from Pasteur", and the work of the Oxford school, is a veritable jungle of information contained in thousands of publications with a large number of reviews, monographs and books written in various languages all over the world. Presenting one's "critical jugment of what all it means" is to epitomise one's impression of the wood forgetting the trees, after the initial not-too-critical wave of enthusiasm has subsided. The subject of antibiotics is a "broad spectrum" one, ranging from engineering at one end to clinical medicine on the other; but here, only those aspects which are of interest to the physiologists and pharmacologists are dealt with.

CHARACTERISTICS OF AN ANTIBIOTIC

The term 'antibiotic' was introduced by Waksman in 1941 to categorise the chemical agents exhibiting the property of 'antibiosis'. A struggle for survival was visualized among the microbes with some surviving by the elaboration of chemical agents which can inhibit the growth of, or destory, the others (Waksman, 1945). Whether this mechanism actually works or not in nature is debatable but the antibiotic property is not different in general principle from the chemotherapeutic activity. So, what is novel about the antibiotics is just their source; previously, scientists searched for drugs among

the inorganic agents, dyes, plant products, and syntheticals, but now they have turned to the microbes and their metabolic products.

By subjecting some antibiotic producing microorganisms to artificial treatment, or feeding them with special nutrients, a variety of agents have been produced. Some antibiotics have been synthesised, many new derivatives prepared, and, in addition, analogues have also been prepared having the antibiotics as model compounds. Search for therapeutic agents is being made in the plant kingdom These force us to extend the scope of the definition of Waksman. While the concept of antibiosis need not be the overriding consideration, the other two - the inhibition of growth or destruction of other microbes in high dilutions, and the microbial source - should be given due weight The three characteristics of an antibiotic could be put down as follows. First, it should be the product of a living organism; in addition to the microbial source, we can include the plant products if they satisfy the other criteria. Secondly, the antibiotics should inhibit the growth of pathogenic microorganisms in concentrations of the order of a few micrograms per ml, the concentration likely to be reached in the human system after routine therapeutic dosage. Thirdly, if the analogues on the model of antibiotics are produced synthetically, they can also be classified as antibiotics provided their actions are comparable to the original model. Such a characterisation of the antibiotic would be operationally useful and will avoid futile logic-chopping about definitions.

SEARCH FOR ANTIBIOTICS

The concept of antibiosis, visualising a sort of Darwinian struggle in the microbial world, has been of great heuristic value in initiating a search for the antibiotics among the microbes colonizing the soil (Waksman, 1945). Samples of soil from every part of the world have been examined in a very intense hit-or-miss project where a chance of 1 in 10,000 is considered to be worthwhile. This means that only a small fraction of the microbes examined produce the antibiotics. The actinomycetes, bacteria and fungi are the microorganisms screened, the actinomycetes being the major source. As a result of an extensive search all over the world during the last two decades, about 600 antibiotics have been discovered. Of these, more than a half have been obtained chemically pure, and the chemical structure of about ten percent of the total completely unravelled. As a result of passing through the mill of further elaborate pharmacological and clinical testing, about thirty have emerged as clinically useful. The range of their usefulness is presented in a very concise form Welch, (1959). Penicillin, streptomycin, chlortetracycline, oxytetracycline, tetracycline, chloramphenicol are of major importance while the rest are of restricted application.

The question is asked whether those still engaged in antibiotic prospecting are not flogging a jaded horse. While it is true that the rich veins have been exhausted, the immense good even a chance discovery could do to suffering humanity justifies the effort expended, inspite of the "boredom of rediscovering endlessly and uselessly substances already known". We still want safe and effective agents against intestinal infections, tuberculosis, leprosy, amoebiasis, etc. The chemotherapy of viral infections remain a "tilled field with no harvest". A vigorous search is going on for agents against neoplastic conditions. While many of the discarded antibiotics are much better than the agents used some thirty years ago because of lack of anything better, today our standards are high because of plenty of agents to choose from.

A SURVEY OF ANTIBIOTICS

The structures of some of the antibiotics worked out are given here. We see the enormous variety ranging from the apparently simple structure of azaserine and sarcomycin to very complex ones like carbomycin and erythromycin. It is interesting that such atoms or groups like chloro, nitro, azo,

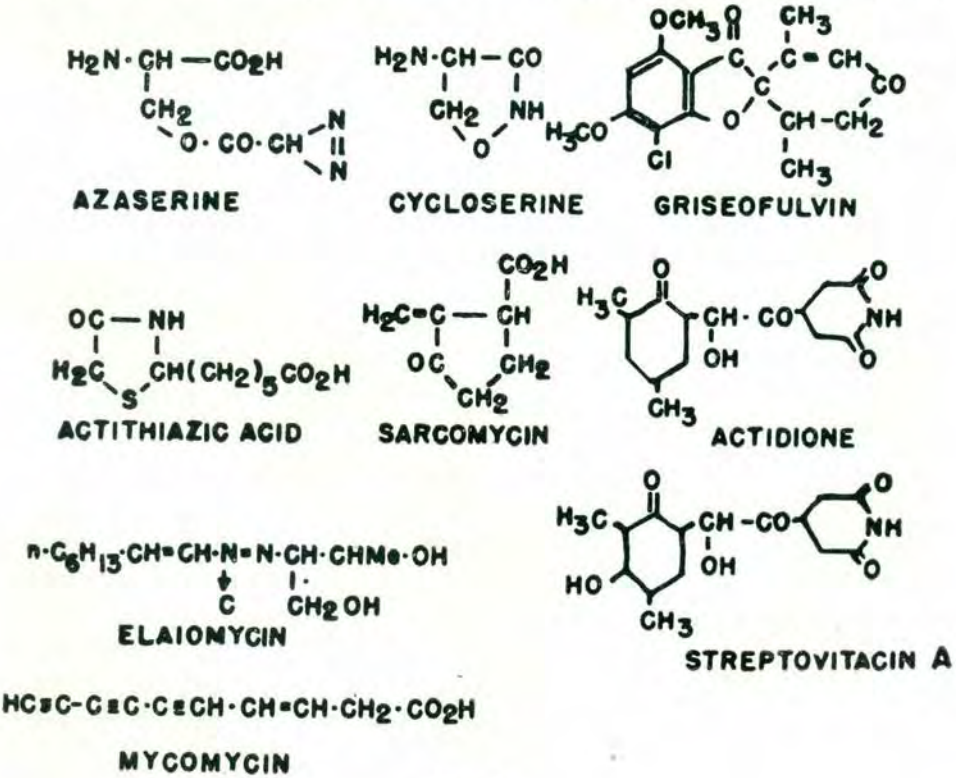


Fig. 1.

azoxy, dimethylamino occur in products of microorganisms. New sugar derivatives, high degrees of unsaturation as in mycomycin, nucleotides, and cyclic polypeptides have been met with. To devine the common denominator for the antibiotic property from out of the kaledoscopic array of structures requires intensive studies on the molecular basis of antibiotic effects. Some studies have been made around penicillin, chloramphenicol, tetracyclines, puromycin, etc. While the tremendous sums of money spent on the discovery of new agents have "resulted in a veritable deluge of new chemotherapeutic agents", the field of fundamental studies remains mostly parched. The science of antibiotics is suffering at the hands of their therapeutic usefulness.

The antibiotics do not appear to be of any use from the point of view of the economy of the microbes producing them. They are produced by the microbes from the surplus metabolites available after the synthesis of the essential cell constituents has been slowed down. In other words, they are products of the microbes out of their surplus materials and leisure time. They are also species specific in that they reflect the synthetic patterns of the microbes.

Antibiotics with simpler structures.—Azaserine, active against neoplastic conditions, is too fantastic to be a product of the microbe. Closely related to it is 6-diazo-5-oxonorleucine, and some more diazo compounds have been discovered. Elaiomycin, active against the tubercle bacilli, is an azoxy compound. Cycloserine has valuable antitubercular properties but produces peculiar toxic reactions. Sarcomycin, so simple in structure, is active against neoplastic conditions. 2-Nitroglyoxaline presents itself as the antibiotic azamycin. Actithiazic acid is active against tubercle bacillius *in vitro* and not *in vivo*, and has a formal resemblance to biotin. These antibiotics, though not in actual use, are of interest from the point of their biosynthesis and mode of action.

The penicillins.— Penicillin is a generic name for a group of antibiotics which possess a particular bicyclic ring system with the side chain R as the vari able. Benzylpenicillin, or penicillin G, is one of the oldest known, most important and also the most active of the antibiotics, its therapeutic activities being limited to the infections caused by the gram positive bacteria, gram negative cocci and spirocheates. Cephalosporin N or synnematin B has the side chain of an amino acid, is effective against gram negative bacteria and shows significant therapeutic activity against typhoid. This shows that by modification of the side chain R, penicillins with activities against different microbes could possibly be produced. In addition, this group R also influences the stability of the penicillins. So, penicillins with a variety of side

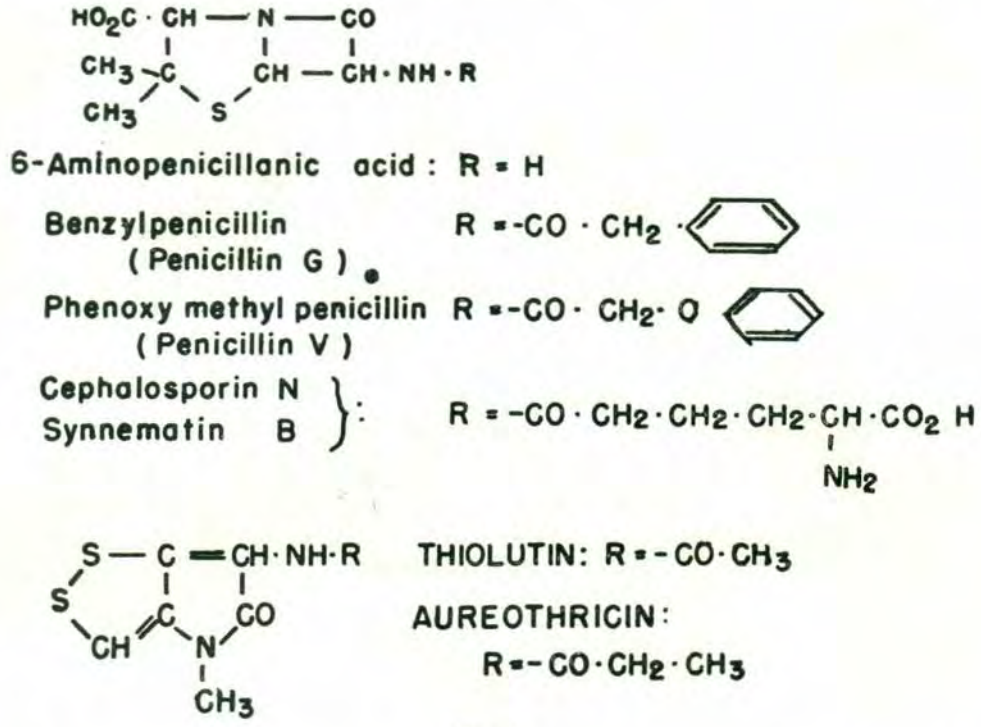


Fig. 2.

chains have been produced by two methods. In the first and older method, specific precursors of the formula R-COOH are introduced into the fermenting medium with *Penicillium chrysogenum*, so that penicillins with the particular side chain R are produced. Over a hundred such 'biosynthetic penicillins' were produced and studied during 1944-46. Of these, phenoxymethylpenicillin, or penicillin V, is of practical importance. While being as active as penicillin G, it is stable as the free acid and so can be administered orally.

The second method of producing new penicillins starts from 6-aminopenicillanic acid (which may be called 'protopenicillin') which can now be produced in quantities by fermentation methods (Batchelor *et al.*, 1959). By altering the side chain R, almost an endless variety of penicillins could be produced by synthetic methods. Already 500 compounds have been reported to have been synthesised. The objective of this study is to obtain penicillins which would be stable, could be administered by mouth, and show activity against a wide range of pathogens.

Streptomycin group.—Streptomycin, neomycin (Jawetz, 1956), kanamycin (St. Whitelock *et al.*, 1958; Welch and Finland, 1959), and paromomycin

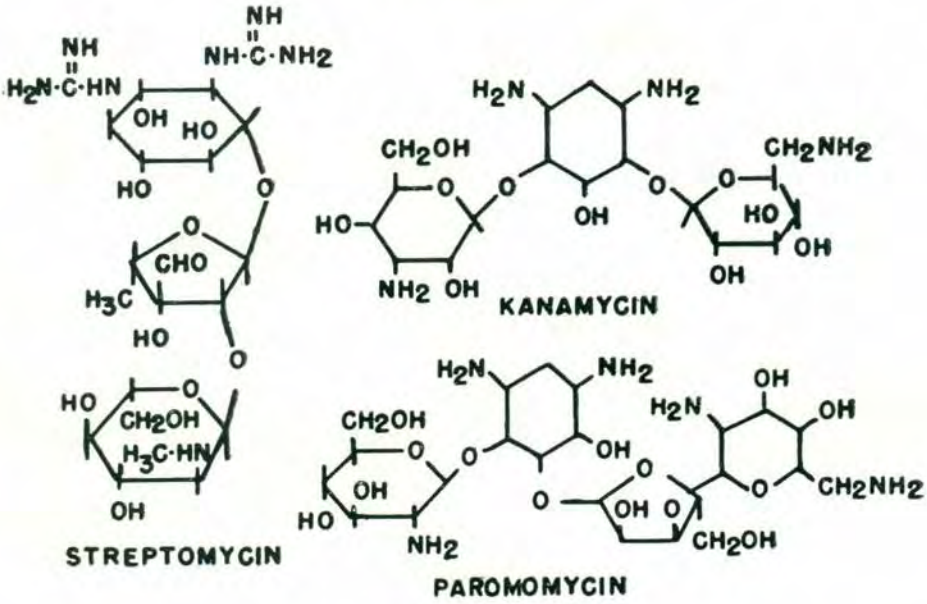


Fig. 3.

(Fisher *et al.*, 1959-60) form a group having many properties in common. Streptomycin is a 1:3-diguanidino derivative of inositol, while the other three are derivatives of 1:3-diamino-4:5:6-trihydroxycyclohexane. All are linked to two or three sugar residues of which at least one is an amino-sugar. The sugar residues in neomycin and paromomycin are probably the same. These possess activity against gram positive and gram negative bacteria and particularly against *Mycobacterium tuberculosis*. They are not absorbed from the gastrointestinal tract so that they cannot be administered by mouth to treat systemic infections. They are of use in producing intestinal asepsis but ineffective against typhoid. When administered intramuscularly, they all show nephrotoxicity, neurotoxicity and ototoxicity which greatly restrict their practical application. Streptomycin produces deafness after prolonged administration while dihydrostreptomycin does this in even small doses. Streptomycin is used mostly for treating tuberculosis combined with isonicotinic acid hydrazide or para aminosalicylic acid to avoid the quick development of resistant strains. Kanamycin is of value in treating cases where penicillin cannot be used for various reasons. Paromomycin is claimed to be exceptionally useful against intestinal amoebiasis (Courtney *et al.*, 1959-60), and appears to be the least toxic of the group while neomycin is the most toxic. Neomycin is of value in producing intestinal asepsis and for topical applications.

Tetracyclines.—The tetracyclines constitute the 'broad spectrum' antibiotics, showing activity against the gram positive and gram negative bacteria, spiro-

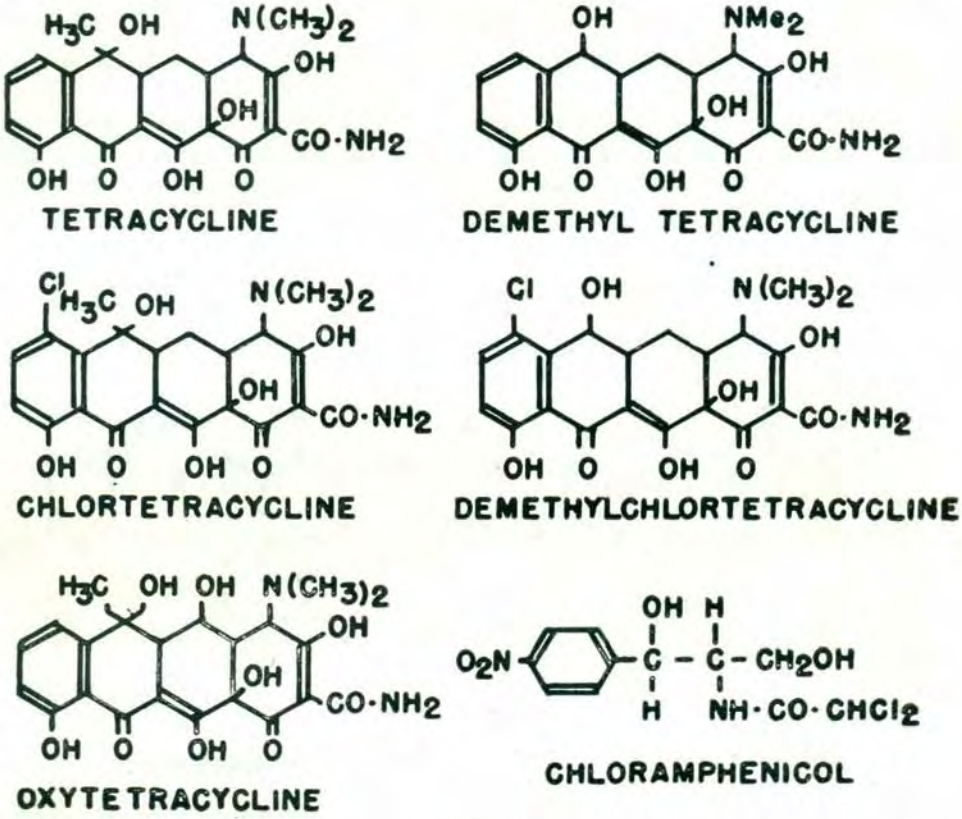


Fig. 4.

cheates, rickettsia and amoeba. By appropriate manipulation of the fermentation conditions, over a dozen new tetracyclines have been produced (McCormick *et al*, 1957), of which four, chlortetracycline (Lepper, 1956), oxytetracycline (Musselman, 1956), tetracycline (Dowling, 1956), and demethylchlortetracycline (Finland *et al*., 1959-60) are in the field. Tetracycline can be produced by fermentation and also by chemical methods from chlortetracycline and oxytetracycline. These show qualitatively the same type of activity, although there are quantitative differences due to their varying stablities (Garrod and Waterworth, 1959-60). The demethyl derivative seems to be more stable. There is cross resistance between them and so their modes of action are the same. They are well absorbed after oral administrations, are free from undue toxicity and are clinically very valuable. Since they sometimes produce nausea and vomitting on oral adminstration, many dosage forms are being tried to administer them by injection and also to prolong their effective blood levels. Since they possess many reactive groups, they lend themselves to the preparation of many derivatives and complexes.

Chloramphenicol.—This is another antibiotic of the 'broad spectrum' group. Though entirely different in structure from the tetracyclines, the range of activity of chloramphenicol is almost like the tetracyclines. This antibiotic is unique in possessing a dichloro and a nitro group, and also in being the only antibiotic produced synthetically on a commercial scale. A large number of analogues have been synthesised and studied but none has come out as better than chloramphenicol. It is used orally as the palmitate and is of special value against typhoid.

'*Macrolide*' *antibiotics.*—About twenty members of this group have been isolated; all contain a lactone ring with twelve, fourteen or seventeen atoms (hence the name 'macrolide'), along with one or more glycoside residues, one being a dimethylamino sugar residue. The chemical structure of six of

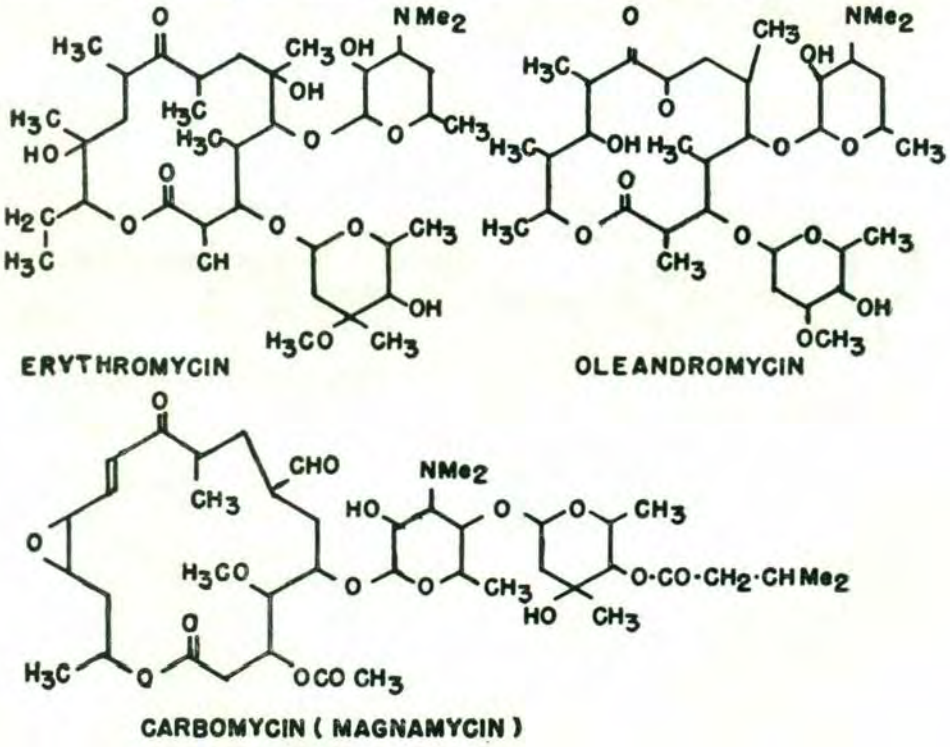
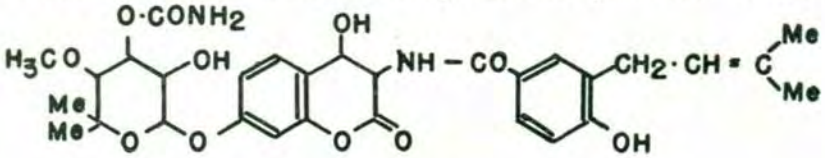


Fig. 5.

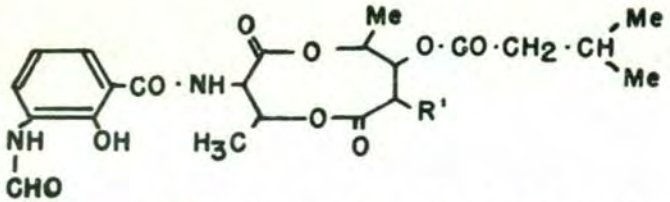
these, namely, erythromycin (Herrel, 1956), carbomycin (magnamycin), picromycin, methymycin, neomethymycin, and oleandromycin, have been unravelled in what may be called brilliant chemical investigations. Of these, erythromycin, carbomycin, spiramycin (formomacidin), and oleandromycin are in use or tried clinically. They all act almost like penicillin, are adminis-

tered orally and are free from undue toxicity. Resistance develops against them readily and they all show cross resistance against each other. Of these, erythromycin is the most active and carbomycin the least. Next in order come oleandromycin and spiramycin(Garrod, 1957). There is a case to use only erythromycin and that too very carefully where penicillin cannot be administered.

Other clinically useful antibiotics.—Novobiocin, the chemical structure of which is known, is another antibiotic acting almost like penicillin. It can be admi-

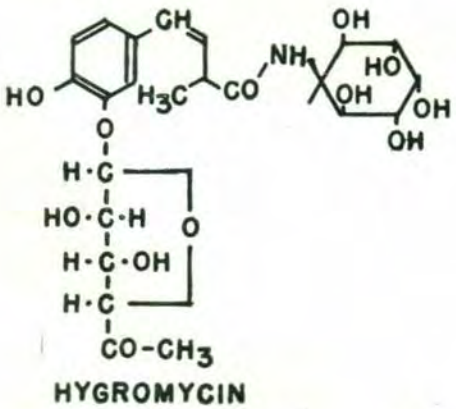


NOVOBIOCIN

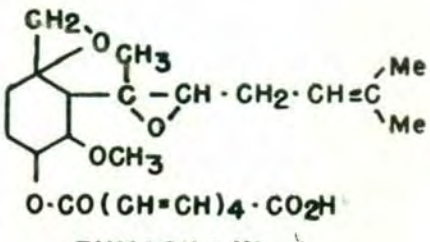


ANTIMYCIN A_1 : R = $-CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3$

ANTIMYCIN A_3 : R = $-CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH_3$
(BLASTAMYCIN)



HYGROMYCIN



FUMAGILLIN

Fig. 6.

nistered orally but it produces skin rashes in about twenty per cent of the patients. Resistance developes against this antibiotic which is at best used as an adjunct to penicillin (Welch and Finland, 1959). Vancomycin and ristocetin, the structures of which are not known, show powerful antistaphylococcal action. They are to be used only intravenously but they show renal toxicity and ototoxicity. They are limited to treat staphyloccoccal infections resistant to penicillin (Welch and Finland, 1959). Fumagillin came into prominence because of its action against amoebiasis; but there are antibiotics which show better effect.

Antifungal antibiotics.—The fungal infections, though not life threatening, are annoying to the patients and their incidence is also heavy. A vigorous scarch is being made to discover useful antifungel antibiotics. About fifty antifungal antibiotics have been reported so far.

Griseofulvin, discovered early but discarded, has turned out to be a very valuable antibiotic. Its antifungal properties were discovered later (Robinson, 1959-60) and it was found to be deposited after oral administration in the keratin of the skin, hair, and nails, an ideal condition for the action of an antifungal agent. It has been found to be extremely useful in controlling a variety of epidermaphytosis due to *Microsporum*, *Epidermophyton* and *Trichophyton* groups of fungi. Its effect is claimed to be dramatic against onchomycosis and tinea capis. It is not effective against histoplasmosis, candidiasis, actinomycosis, etc. Nystatin (mycostatin, fungicidin) which belongs to the tetraene group of antifungals, is clinically useful in treating candida infections, histoplasmosis, trichophyton and epidermophyte infections. Amphotericin B, a heptaene, is the third clinically very valuable antifungal antibiotic for treating deep mycotic infections and is of choice in the treatment of blastomycosis, histoplasmosis, and cryptococcosis. It is very insoluble and is injected. Soluble preparations are being made to make the administration easier. Other antibiotics of this group are candidin, candicidin, trichomycin, etc. Eulicin is another antifungal antibiotic acting like nystatin; it contains two guanidino groups and an aliphatic chain with eight carbon atoms. Pimaricin which is a highly unsaturated compound like the macrolides, and rimocidin are recent additions. Actidione (cycloheximide) is one of the earliest known antifungal antibiotics but because of its toxicity, its use is restricted to control fungal infections of the plants. Antimycin is a powerful antibiotic which consists of a nine membered dilactone ring and is a derivative of meta aminosalicyclic acid. This is not used clinically but is biochemically of great interest.

Miscellaneous group.—There are a number of antibiotics which are not used clinically but are of scientific interest. Hygromycin shows action against

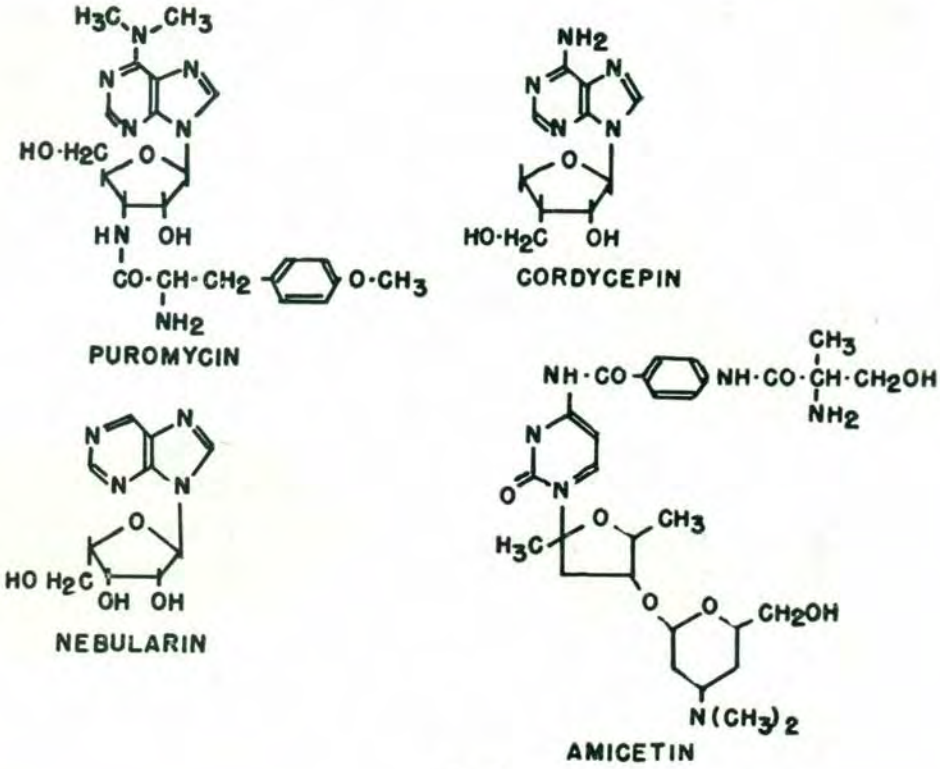
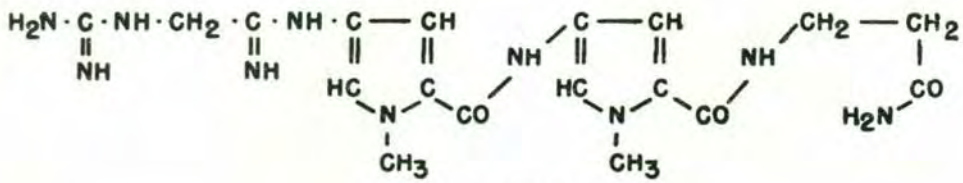
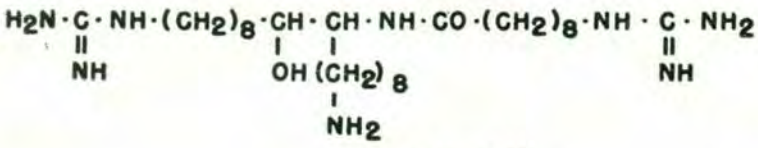


Fig. 7.

gram positive and gram negative bacteria and mycobacteria, and has an interesting structure. Netropsin contains a guanidino group and two pyrrole rings joined by a peptide linkage. Puromycin shows activity against the trypanosomes and tumours; it possesses an adenine radical with the amino group dimethylated and also a methylated tyrosine residue. Cordycepin, which has action against mycobacteria, is an isomer of adenosine with the sugar isomeric to ribose. Nebularin is a purine compound, actually adenosine without the amino group. Amicetin which shows action againt gram positive and acid fast bacteria, is a pyrimidine derivative with para aminobenzoic acid and threonine residues and a dimethylamino sugar. The two antibiotics, micrococcin and bottramycin, possess the thiazole ring so far met with only in thiamine.

Polypeptide antibiotics.—About a hundred polypeptide antibiotics are known, of which about a half are produced by bacteria and the rest by actinomycetes and fungi. In these almost all the amino acids are reported, though each antibiotic does not contain the whole lot. All these are probably cyclic in the





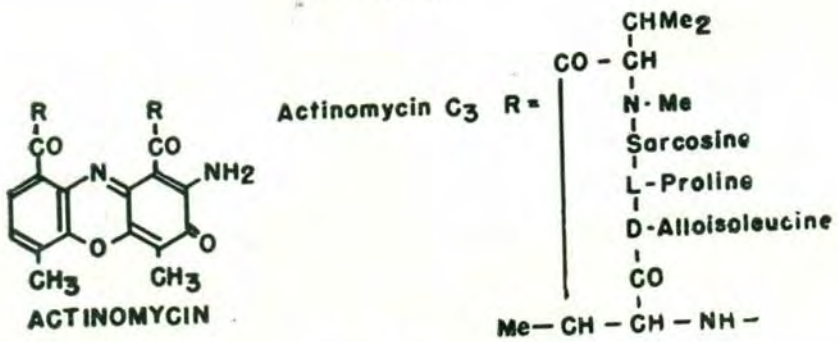


Fig. 8.

arrangement of the amino acids and in each antibiotic there is present atleast one amino acid of the unnatural D configuration. Their antimicrobial properties vary widely. As a rule they are not absorbed after oral administration and on injection show nephrotoxicity. Since the margin of safety is narrow with these, even though they show valuable effect against pathogenic microorganisms, their use is very much restricted. These antibiotics are usually resolved into a number of closely related members on careful fractionation. Tyrothricin originally isolated by Dubos has been resolved into two tyrocidins and many gramicidins which are decapeptides. Gramicidin S, isolated by the Soviet workers, is very crystalline and its structure has been fully worked out. It contains of two sets of the amino acids, L-valine, L-ornithine, L-leucine, D-phenylalanine, and L-proline, all arranged cyclically with two identical halves. One half is the same as in tyrocidin A where as the other half consists of L-phenylalanine, D-phenylalanine, L-aspartic acid, L-glutamic acid, and L-tyrosine arranged in the sequence. Bacitracin (Jawetz, 1956) has been resolved into a number of components, bacitracin A being

the one clinically used. This acts like penicillin but is too toxic for extensive use. It consists of the twelve amino acids, L-leucine, L-isoleucine, L-cystine, L-histidine, L-lysine, D-phenylalanine, D-ornithine, D-glutamic acid, D-aspartic acid and L-aspartic acid. Polymyxin consists of five members, A to E; polymyxin B is clinically used (Jawetz, 1956). It consists of D-leucine, D-phenylalanine, 2 threonines, six a: γ-diaminobutyric acid units and the aliphatic acid 6-methyloctanoic acid, which is present in all the polymyxins. Threonine is also present in all; polymyxin D contains serine and no phenylalanine. Circulin is a member of this group consisting of leucine, threonine and diaminobutyric acid (Welch *et. al.*, 1957-58). The latest addition to this group is colistin which consists of D-leucine, L-leucine, L-threonine, 5 units of diaminobutyric acid and 6-methyloctanoic acid. Polymyxin B is unique in possessing striking action against *B-pyocyanase* but it is too toxic to be used on a mass scale. Colistin is claimed to be less toxic (Wright and Welch, 1959-60; Schwatz *et al*, 1951 - 60). Viomycin shows valuable antitubercular properties but again is too toxic to be of extensive use. While it seems doubtful whether polypeptide antibiotics could be discovered with more than restricted use, they are of scientific interest from the point of view of their biosynthesis and modes of action.

Antitumour antibiotics.—A very extensive survey is being made to obtain antibiotics against a variety of tumours (Sugiura, 1959-60; Panel discussion, 1957 - 59). The best known among these is actinomycin. Discovered as early as 1940, it was found to be extremely active and also extremely toxic. Discovery of its action against the neoplastic conditions has touched off an intensive search. The actinomycins occur very widely and atleast 30 members are known. They all contain a phenoxazone ring to which are attached two peptide units. The amino acids and their arrangements make the difference among the actinomycins. The amino acids, L-threonine, sarcosine, L-proline, D-valine, L-methylvaline, D-alloisoleucine, have been reported. Actinomycins C and D are of value against neoplastic conditions. Of the fifty and odd compounds reported to possess action against tumours, those well known are azaserine, other aliphatic diazo derivatives, mitomycin C, sarcomycin, netropsin, actinobolin, streptovitacin, etc. The mechanism of their oncostatic action should be of interest to investigate.

FACTORS AFFECTING THE CLINICAL USEFULNESS OF THE ANTIBIOTICS

The isolation of the antibiotics on the basis of their activities on the microbes *in vitro* evaluates only their inherent antimicrobial action. Before passing them as fit for clinical use, their action on the host and the way the host deals with them, should also be investigated.

The antibiotic-host interaction decides the toxic properties of the antibiotic. Theoretically, every compound will have some effect on the host and this effect, except in the case of allergic reactions which are provoked even in minute quantities, will be proportional to the concentrations in the system *in vivo*. Since the antibiotics produce their therapeutic effects in concentrations of the order of a few micrograms per millilitre, the margin of safety in their use would be there if, in such concentrations, they do not produce any adverse effect on the host. The dosages are adjusted such that the therapeutically effective concentrations are maintained in the system of the host. In practice, the antibiotics do produce some reaction or other on the host but we have to examine the magnitude, seriousness and permanence of these in assessing their usefulness. Penicillin, the tetracyclines, chloramphenicol, and erythromycin, are the safest agents in the therapeutic dosages. Penicillin is, however, highly antigenic and gives rise to allergic reactions which cautions us against the indiscriminate use of this agent. Streptomycin, neomycin, kanamycin, bacitracin, polymyxin, viomycin, vancomycin, etc. produce nephrotoxicity and ototoxicity and they are used only if there is a specific need. About 95 percent of the antibiotics discovered are rejected as unfit for human use because of their undesirable effects on the host and treatment with them would be worse than the disease. It is essential to understand the mechanism of production of these toxic reactions, so that ways could be found for suppressing them and using the antibiotics with greater margin of safety (Dubos, 1959-60).

It is important that the antibiotic after adminstration should reach the site where it has to meet the pathogen in adequate concentrations. Such a site will vary depending upon the infection. So the absorption of the antibiotic, the distribution in the system, the rates of metabolisation and excretion, are of importance. The solubility of the antibiotic in the body fluids, its stability, rate of diffusion through the tissues, passage through the blood-brain barrier, the rate of metabolisation, excretion through the kidneys, are the factors which decide how the host deals with the antibiotic. Benzylpenicillin is destroyed in the stomach and the gastrointestinal tract but penicillin V is not and is absorbed well after oral administration. The tetracyclines, chloramphenicol, erythromycin, and novobiocin are well absorbed after oral administration, but the tetracyclines produce nausea and vomitting. Streptomycin, neomycin, kanamycin, paromomycin, bacitracin, polymyxin, vancomycin, and ristocetin are not absorbed after oral administration; they remain localised in the stomach and intestines. They have to be injected if they have to be distributed in the system. The mode of their injection is also of importance. Vancomycin and ristocetin are to be administered only intravenously. Novobiocin, polymyxin, vancomycin, ristocetin and bacitracin do not reach

the cerebrospinal fluid after administration and so are not useful for treating meningitis. Griseofulvin after oral administration gets deposited in the keratin of skin, hair and nails. The absorption, distribution and excretion could be modified to some extent by making suitable dissociable complexes altering the physical properties of the original antibiotic, so that we can have more useful dosage forms, such as, the phosphates, glucosamine complex, pyrrolidine derivatives and admixture with citric acid of tetracyclines, procaine salt of penicillin, palmitate of chloramphenicol, propionate of erythromycin, methansulfonate of colistin, etc. In all these cases, we must have a good picture of how the host deals with these antibiotics.

To get the maximum benefit, the ideal route of administration of every antibiotic should be found out. *Prima facie*, the oral route seems to be the method of choice, particularly to treat infections localised in the gastrointestinal tract. But this method of administration has its own complications. If the antibiotic is not absorbed after oral administration, or causes severe nausea or vomitting, then other methods have to be resorted to. Since the antibiotics are very powerful antimicrobial agents, in the concentrations present in the intestines, they kill the normal intestinal flora and cause the growth of undesirable microbes as *Candida albicans*. Where the distribution in the blood and tissues is required, it is better to inject the antibiotics since reaching these sites via the stomach and intestines by oral dose will require more of the drug and involve the risk of superposed infections in the gastrointestinal tract which are more difficult to tackle Finding out the best way of administration of an antibiotic is an important issue, since even a valuable antibiotic can give disappointing results by being used in the wrong way.

The therapeutic effect of an antibiotic is proportional, within limits, to its concentration at the site where the antibiotic meets the parasite. An issue of practical importance is whether it is necessary to keep the concentration continuosly at the site or only to exhibit it at periodic intervals. In other words, is there to be a prolonged trench warfare between the antibiotic and the parasite, or only a "blitzkreig"? This depends upon the nature of the pathogen involved, site of the infection and the local microenvironmental lesions, the mode of action of the antibiotic, and the duration of contact required for the complete annihilation of the pathogen. The antibiotics, by the way they have been selected, act on the microbes when they are multiplying and not when they are in the resting state. To deal with those which are not multiplying and the "persistors" (Bigger, 1944), it is better to withdraw the antibiotic and allow the resting organisms to multiply to be dealt by the antibiotic. The duration of exhibition of the antibiotic has to be decided by studying the individual cases, and we cannot take a general view that the longer the duration, the better the effect.

PROBLEM OF ANTIMICROBIAL RESISTANCE

Microbial resistance to the antibiotic after a course of treatment has now become a serious problem in the case of *Staphylococcus aureus* and *Mycobacterium tuberculosis*. The original view that resistance 'develops' as a result of insufficient dosage or too prolonged administration is too much of a simplification. All the antibiotics known which act by subtle interference with the vital metabolic patterns of the microbe rather than as a gross protoplasmic poison, give rise to resistant forms (Eagle, 1954; Eagle and Saz, 1955). Different mechanisms for the emergence of resistance have been visualised. According to the first one of adaptation, the resistance is actually caused by the gradual training of the pathogen to tolerate small sublethal doses of the antibiotic so that ultimately the level of toleration is much higher than the bacteriostatic or bactericidal dose; the resistant strain is assumed to have altered its metabolism and use a pathway which is not blocked by the antibiotic. The second view is that the antibiotic causes mutation in the population and the mutant which is not susceptible to the antibiotic survives and proliferates while the susceptible ones are killed off. The third explanation is that the original population of the microbe is heterogeneous as regards its sensitivity to the antibiotic which kills off those which are susceptible so that those not susceptible proliferate and become the sole members of the population. According to this view, resistance is not created but it is just an alteration in the pattern of the population created by the selective action of the antibiotic. It is difficult to decide between the alternatives by unequivocal experiments, since handling single individual members of a culture is beset with so many experimental difficulties. The opinion of the majority is now coming round to the third view as most probable, which is also consistent with the genetical principles. There are a few strong upholders of the first view which has a strong physicochemical flair.

If the antibiotics are used indiscriminately, we are sure to create populations of pathogenic microbes which would be resistant to the antibiotics used and which will render therapy with these useless. Paradoxical as it may seem, it is a good strategy to keep as the major population the pathogens which are susceptible to the antibiotics, rather than kill off those which are susceptible and be left with only those which are resistant to the antibiotics.

ANTIBIOTIC COMBINATIONS

From the commonsense point of view, it appears reasonable that the use of two antibiotics should be better than the use of one, but unfortunately the issue is not so simple as it looks. The antibiotic combinations have not been thoroughly studied but still the market is flooded with so many combinations for reasons of commercial interest and are being used under wrong impressions

and under a false sense of security. Many of these are needless, unwanted, and fraught with dangers (Finland, 1957 ; Dowling, 1957 ; Gold and Cattek, 1958).

The arguments for the use of the antibiotic combinations are five fold : possible synergism in effect, reduced toxicity, preventing or delaying the appearance of resistant organisms, broad cover for mixed infections, and suitable treatment for seriously ill patients before a bacteriological diagnosis can be made. Unfortunately, none of the claims can be substantiated for the vast majority of the combinations being tried.

It is fairly difficult to establish by unequivocal experiments *in vitro*, synergism; it is far more difficult to do so in human cases. Jawetz and Gunnison (1952, 1953) have divided the antibiotics into two groups :

Group I : primarily bactericidal as penicillin, streptomycin, bacitracin, and neomycin.

Group II : primarily bacteriostatic as chlortetracycline, oxytetracycline, tetracycline, chloramphenicol, erythromycin and sulfonamides.

Mixtures *in vitro* of members of group I frequently show synergism, some indifference but never antagonism. Members of group II are neither synergistic nor antagonistic but merely additive. When the antibiotic from group I is used with that in group II, the effect may be synergism, indifference, or antagonism Which of these will occur depends upon such complex factors as the relative amounts of the two antibiotics used in combination and susceptibility to resistance of the microorganism to the individual antibiotic. In no case is it possible to predict whether synergism or antagonism will occur with a combination. This can be determined only by actual experiment. Clinical experience has shown that undoubted synergism has been established only in three cases : (i) in enterococcic endocarditis with the use of a combination of a large dose of penicillin and moderate one of streptomycin, (ii) in brucellosis when streptomycin is administered with one of tetracyclines or chloramphenicol (though of late tetracycline alone is recommended as the drug of choice), and (iii) of streptomycin with para aminosalicylic acid or isonicotinic acid hydrazide in tuberculosis. In infections of the gastrointestinal tract, polymyxin with neomycin is used. The nystatin and tetracycline combination is not recommended. The combination of penicillin with streptomycin and dihydrostreptomycin, and of streptomycin with dihydrostreptomycin are fraught with more danger than usefulness. There is evidence to show that it is better to administer the antibiotics individually in proper doses rather than as fixed combinations.

The question of the combinations of antibiotics has to be carefully studied in the laboratory and clinically before any general recommendation is made for clinical use.

MECHANISM OF ACTION ON ANTIBIOTICS

Understanding the mechanism of action of the antibiotics is of great fundamental importance in elevating the subject of antibiotics from an empirical to an exact science. Unfortunately, the amount of attention paid to this subject is far less than what is devoted to the mechanical search for new antibiotics. This is dealt with in three levels, namely, (i) the gross level of the nature of the effect (bacteriostatic or bactercidal effect), the phase of the microbe involved, etc., (ii) how the antibiotic deals with the pathogen, the type of lesion involved, etc. and (iii) the precise details of the biochemical process involved. We are here dealing with the last type of action. In the studies conducted, particularly in *in vitro* systems, many types of effects may be observed but we have to differentiate between the primary and the secondary or tertiary effects, and also the mechanisms which are involved in producing the specific antibiotic effect from those which are nonspecific and are just other properties of the molecule (Umbreitt, 1954, 1955). Gross effects as the interference with protein synthesis or nucleic acid synthesis, do not convey any definite meaning unless the precise steps involved are pin pointed.

Attempts to correlate the structures of the antibiotics and the nature of the microbes affected only leave us confused. Penicillin, erythromycin, novobiocin, and bacitracin possess entirely different structures but act on the same types of microorganisms. Streptomycin, kanamycin, neomycin,and paromomycin act in a similar way but they possess some herd structural resemblance. The tetracyclines and chloramphenicol show the same type of effect but possess entirely different structures. Antibiotics which have been reported to show activity against a particular microbe, say tubercle bacilli, amoeba, fungi, etc. possess entirely different structures. All these go to show that subtle interference with the vital metabolic chains of the microbes in a specific way is possible at many loci and the antibiotics with different structures act at different loci.

Penicillin, bacitracin, novobiocin and cylcoserine (oxamycin) stop the synthesis of the cell wall at the cytoplasmic membrane. The mechanism of the synthesis of the cell wall of gram positive bacteria consists of the building up from uridinetriphosphate (UTP), the acetylmuramic acid derivative of uridinediphosphate (UDP. AGlac), and then the peptide chain, step by step, consisting serially of L-alanine, D-glutamic acid, L-lysine, D-alanine and D-alanine, and then splitting off of the acetylmuramic polypeptide from UDP and forming the cell wall. The antibiotics mentioned above interfere with specific steps in the chain of reactions, ultimately stopping the synthesis of the cell

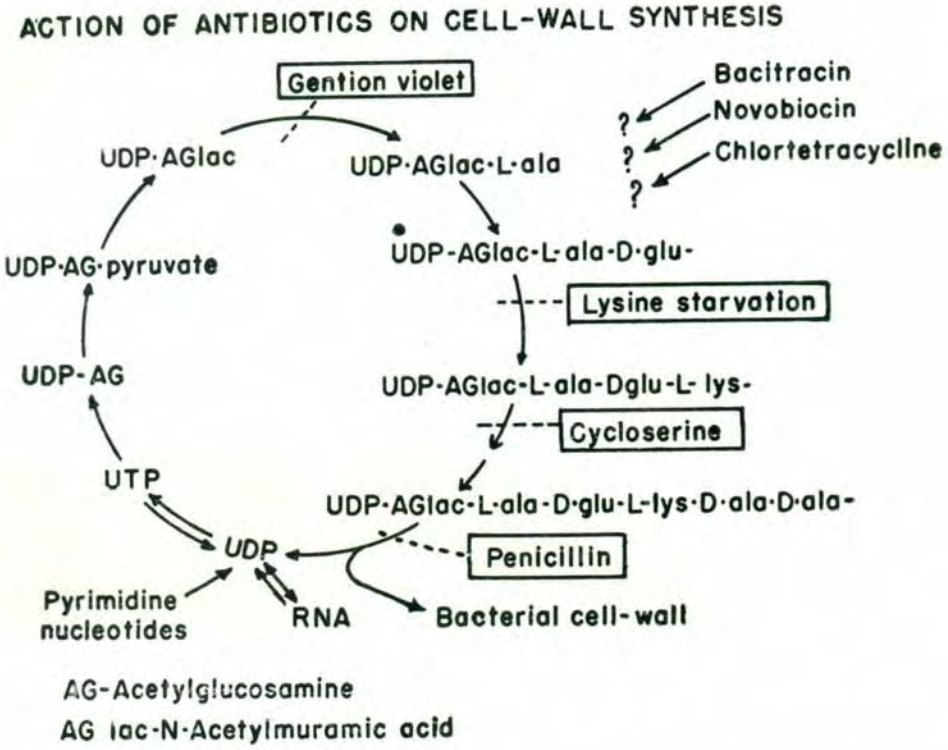


Fig. 9.

wall. Cycloserine inhibits the incorporation of D-alanine by competitive inhibition. Gentian violet affects the incorporation of L-alanine, and penicillin affects the last step. Bacitracin and chlortetracycline affect some step not yet identified (Park, 1958 ; Park and Strominger, 1957 ; Strominger *et al* , 1959 ; Gale, 1959).

Penicillin has no action on the synthesis of protein. Chloramphenicol is an inhibitor of protein synthesis by interfering with the transfer of aminoacyl residues from their primary acceptor sites to the peptide bond forming site (Gale, 1959). It does not affect the synthesis of RNA or DNA. The tetracyclines also interfere with the synthesis of proteins. They form complexes with magnesium and calcium and can affect the steps by the withdrawal of these elements in the reaction chains involving them.

Streptomycin is known to interfere with a variety of steps *in vitro* (Williams, 1957 ; Umbreitt, 1954, 1955) but we do not know what exactly is the primary inhibiting step. Actithiazic acid in view of its structural resemblance to biotin, inhibits the biosynthesis involving biotin.

Azaserine has a structural resemblance to glutamine and interferes with the steps where glutamine is involved, just as in many stages of aminations, and also in the biosynthesis of purine and pyrimidine nucleotides. So the antitumour effect of azaserine is understandable. Many antibiotics are known to interfere with the biosynthesis of nucleic acid even though the precise steps are not known. Mitomycin specifically blocks DNA synthesis without affecting RNA synthesis. Griseofulvin inhibits nucleic acid synthesis at or prior to the polymerisation stage, and also arrests mitosis in metaphase (Paget and Walpole, 1958). Actidione selectively inhibits protein and DNA synthesis.

The antifungal antibiotic, antimycin A, has been shown to affect oxidative phosphorylation blocking the electron transport chain at the cytochrome stage (Potter and Rief, 1952.)

Polymyxin, gramicidin S, tyrocidin, and circulin act like surface active agents by combining with the phospholipid groups in the cellular membrane (Hotchkiss, 1944 ; Newton 1954 ; Newton and Abraham, 1958).

It is clear how interesting would be the studies on the precise biochemical mechanisms involved in the action of the antibiotics.

IMPACT OF ANTIBIOTICS ON SCIENCE AND SOCIETY

The impact of the antibiotics on science and society is very marked in many areas. By their striking therapeutic effects, the antibiotics have brought under control many infections that afflict the old and the young. The antibiotics now constitute about 50 per cent of the total medical prescriptions. Though the physician can now manage much better many diseases, he has to think of the diseases in terms of the susceptibility of the microorganisms to the antibiotics and so is more dependant on the laboratory. The surgeon can now, under an umbrella of antibiotics, operate on any part of the body without fear of sepsis.

Antibiotics are used in animal husbandry to tackle many infections and to stimulate the growth of the animals. They are used for the preservation of meat from bacteriological spoilage. In the field of agriculture, antibiotics are used to control many plant pests.

The organic chemist and chemotherapeutist have compounds with new and interesting structures to think and work on as model compounds for elaboration of the series. The unravelling of the mechanism of action of these by specific interference with the metabolism of the microorganisms, will not only put chemotherapy on a higher level but will also provide biochemistry with powerful tools to probe into the metabolic patterns of living organisms.

The manufacture of the antibiotics has established a new powerful technology which is adaptable for the manufacture of many biologicals where the microbes beat the record of the synthetic organic chemists.

The antibiotics have also touched off a serious social problem The demographers who think of population in only numbers, and those given to the *laizzes fairre* mode of thinking, are somewhat distressed by the lowering of the death rate, creation of a 'pool of millions of people too old to work" (Smith, 1959-60), and the "population explosion" in the underdeveloped countries, all as a result of the use of the antibiotics. Even the eminent scientist who plaved a great role in ushering in the modern antibiotic era is worried that "the introduction of the antibiotics and other means by which death rate can be reduced may not be an unmixed blessing" (Florey, 1958-59). There is also appearing Malthusianism in many garbs in expressions as "the most difficult challenges confronting the drug industry are not unconquered diseases but the problems that are created as diseases are conquered", and "it costs much more to remain well than to be treated for all the ills" (Smith, 1959-60). The conclusions which these lead to by cold logic are revolting even to utter. The social organisations at present, it seems, cannot stomach the benefits of science. Humanity is now at the cross roads and has to choose between the two alternatives : whether it has to travel in the old road in which a falling death rate and long life would be big problems and will entangle the scientists in an "ethical dilemma" (Hill, 1952), or whether it has to choose one where every case of preventable death would be treated as murder, and low death rates are deliberatly sought after.

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