# 1. The Mode of Action of Chemotherapeutic Agents

**A Discussion held by the Biochemical Society on Saturday,
29 November, 1941, at the Courtauld Institute of Biochemistry,
Middlesex Hospital, Prof. E. C. Dodds in the Chair**

G. M. FINDLAY, in opening the discussion, said that chemotherapeutic action might be classified as direct or indirect. Except in the case of parasites present in the intestinal canal it was essential that the chemotherapeutic drug should be absorbed into the body, that it should penetrate to the site where the parasites were acting, and that it should not be excreted or converted into an inert form too rapidly. Time must be allowed for chemotherapeutic action, and in some cases for the conversion of the compound from an inactive into an active form.

When once the drug and the parasite had been brought face to face three stages might be distinguished: adsorption, interference with metabolism, and death or such injury to the parasite that it was destroyed by the phagocytes of the host. An adsorbed chemotherapeutic drug may prevent an essential food factor from being absorbed or it may cause a breakdown in metabolism by combining with a specific substrate or by competing with an essential cell metabolite for an enzyme or coenzyme. One break in the chain of metabolic reactions may rapidly give rise to others. Specific immune serum and sulphapyridine do not compete for the same receptor group in the *Pneumococcus* and may therefore enhance one another's effects.

Parasites may be killed in the body without the aid of phagocytes, but usually when a parasite has been damaged it is destroyed by the normal defence mechanism of the host.

Indirect action produces such changes in the environment that parasites can no longer grow. Physical changes may prevent growth, the temperature or the $pH$ may be altered, the formation of immune bodies may be stimulated or the character of the cells may be altered, as in the treatment of gonococcal vulvovaginitis in children with oestrin preparations. The highly specific action of certain drugs and the no less specific reactions of certain closely allied parasites could be explained by postulating that, after adsorption of the compound at the parasite/solution interface, the nature of the interference with the metabolism of the parasite depended on what groupings in the molecule of the compound came within the influence of other acceptor groups in the parasite: there was thus a multipoint action.

A. FLEMING. In 1929 I applied the name penicillin to an antibacterial substance of unknown constitution elaborated by *Penicillium notatum*. This substance is formed when the mould is grown in ordinary bacteriological media or in a modified Czapek-Dox medium described by Clutterbuck, Lovell & Raistrick. The action is mainly bacteriostatic and shows a marked degree of specificity. Pyogenic cocci, clostridia, and some other bacteria are sensitive, while the coli-typhoid, haemophilic chromogenic bacilli, and others, are insensitive. The pathogenic Gram-negative cocci (*Gonococcus, Meningococcus* and *M. catarrhalis*) are sensitive, while the saprophytic varieties, e.g. *M. flavus*, are insensitive. (In this respect penicillin differs from the sulphonamides.)

The activity can be assayed by noting the bacteriostatic concentration in a simple titration, or by confining the solution in holes or gutters cut in an agar plate—holes if many solutions have to be tested to the same microbe, and gutters if many microbes have to be tested to the same solution. *Staphylococcus* is a convenient test organism.

In concentrations which are much less than are necessary completely to inhibit growth it has been shown by Gardner to affect the morphology of bacteria and to interfere with cell division.

The action of penicillin is not interfered with by substances known to inhibit sulphon-amide action, such as large numbers of bacteria, bacterial extracts, pus fluids, tissue autolysates, peptone and $p$-aminobenzoic acid.

Penicillin is non-toxic to leucocytes and animals. The uses to which it has been put are: (1) The easy isolation of insensitive bacteria from sensitive bacteria. (2) The dramatic demonstration of many bacterial inhibitions. (3) The treatment of bacterial infections in man and animals.

Florey and his co-workers have extracted an impure principle which, *in vitro*, is much more active than any of the sulphonamides on staphylococci and streptococci. A total dosage of a few milligrams sufficed to cure mice of experimental infections with *Strepto-coccus*, *Staphylococcus* and *Vibrio septique*, and a later trial in man shows that it is probably the most powerful remedy known for streptococcal and staphylococcal infections.

As penicillin is apparently of a different constitution from the sulphonamides, the isolation of the pure active principle and its synthesis will open up a new chemothera-peutic field.

WARRINGTON YORKE described the investigations leading to the production of various aromatic diamidines which have proved very active in the treatment of animals experi-mentally infected with trypanosomiasis, leishmaniasis and babesiasis, respectively. During the past two years certain of these compounds, notably diamidinostilbene (stilbamidine), diamidinodiphenoxypropane (propamidine) and diamidinodiphenoxy-pentane (pentamidine), have been tested clinically in the field. All these compounds exert a rapid curative action in Indian kala-azar, and they all cure Mediterranean and Sudan leishmaniases of man, which are notoriously difficult to cure with antimonials. Recent reports from Kenya and from Uganda show that a single injection of stilbamidine or propamidine suffices to cure about 90 % of dogs naturally infected with *Babesia canis*. It is as yet too early to assess the value of these compounds in human trypanosomiasis, but reports so far received indicate that they produce rapid peripheral sterilization and cure early cases.

In Warrington Yorke's opinion our investigation of the phenomenon of drug resistance might throw light on the mechanism of the action of drugs. Certain observations on drug resistance in trypanosomes which had been made in his laboratory during the past twelve years were discussed in their bearing on various theories regarding the mode of action of drugs. In conclusion, attention was drawn to the fact that, although drug resistance in trypanosomiasis has been extensively studied, little is known of the phe-nomenon in other protozoal infections. Drug resistance had been recently demonstrated in *Babesia canis* infections of dogs and in *Plasmodium knowlesi* infections of *Macacus rhesus*.

F. HAWKING. According to the phenomenon of drug resistance, there are four kinds of receptors on the trypanosome, viz.: (*a*) for arsenicals, acriflavine etc., (*b*) for para-fuchsin, (*c*) for diamidine compounds, (*d*) for Bayer 205. Most study has been directed to the first of these. Trypanocidal action may be considered to occur in three phases: (*a*) fixation of the drug by the cell, (*b*) secondary chemical reaction inside the cell, (*c*) death. The second and third phases are mostly unknown, but considerable information is avail-able about the first. It occurs quickly, being completed in a few minutes; and it is re-versible. With arsenical compounds, fixation seems to depend upon the trivalent arsenic atom linked to a benzene ring. Most side-chains prevent fixation, but certain ones (—NH$_2$, —OH) prevent it for the animal cells but not for normal trypanosomes; it is

compounds with these side-chains which have chemotherapeutic value. The receptors of resistant trypanosomes are modified so that they resemble those of the animal's cells and thereby therapeutic action is lost. Over a wide range the amount of drug fixed is proportional to the concentration in the surrounding fluid. In the case of acriflavine the partition ratio, i.e. concentration inside the trypanosome/concentration outside, is 8000 for normal trypanosomes, and only 60 for resistant trypanosomes. This difference explains the mechanism of drug resistance against acriflavine, or arsenicals.

Some compounds which are fluorescent, e.g. acriflavine and diamidinostilbene, can be shown to be concentrated in the blepharoblast and in cytoplasmic granules.

With Bayer 205, the mode of action is quite different. It is not directly trypanocidal *in vitro*, and although it does seem to form some kind of direct combination with the trypanosome, the amount which is taken up is quite small. There is some evidence that it has an opsonin-like action.

H. H. DALE emphasized the importance of having a theory of chemotherapeutic action. It was one of Paul Ehrlich's great contributions to the subject to have produced a theory which, though it would probably not survive unmodified, had given a tremendous stimulus to research. His speculations had sometimes appeared more plausible than convincing; but it was remarkable in how many cases they had eventually proved to be well founded. An example was Ehrlich's explanation of the action of certain dyes on infection by trypanosomes, as due to injury of the reproductive power of the trypanosomes, without affecting their other vital functions. At the time this suggestion seemed artificial and unconvincing, but when Dobell & Laidlaw found a method for growing *Entamoeba histolytica* in permanent culture *in vitro*, it could be demonstrated that the action of emetine was just of this type. Another factor of chemotherapeutic activity which he believed to have importance was the 'factor of persistence', by which the curative agent was enabled to remain in the body, at a concentration harmful to the parasite but harmless for the host cells, for a sufficiently long period. Effective chemotherapy was usually, if not always, a war of attrition rather than a 'Blitzkrieg'. This seemed to be the reason why a quinquevalent arsenical on the one hand, or an arseno-compound on the other, was a better chemotherapeutic agent than the arsenoxide produced by reduction from the one or by oxidation from the other, although the arsenoxide was recognized as the directly parasiticidal agent. In the same way, sulphaguanidine seemed to owe its effectiveness in bacterial dysentery to its poor solubility, enabling it to remain in solid form in the intestinal contents and to keep up a steady, low concentration in contact with the infected mucous membrane. Aromatic diamidines, the brilliant promise of which had been made clear by the results which Prof. Yorke had reported, might similarly owe part of their superiority to their limited solubility. If a derivative of this series could be found, of low activity and poor solubility itself, but steadily liberating a soluble and active diamidine in subtoxic concentration, this might have even a greater curative value. He ventured to suggest, as problems, the solution of which might greatly accelerate advance in parts of the field of chemotherapy, the discovery of a method of keeping trypanosomes alive and reproductive indefinitely in artificial culture, and the discovery of a method of treating a strain of trypanosomes which had acquired a drug resistance, so as to restore the normal susceptibility.

D. D. WOODS. There is strong evidence to support the view that antibacterial agents act by virtue of their capacity to interfere in some way with essential metabolites (substances essential to the organism, including growth factors etc.). Such interference may occur in different ways: (*a*) by formation of a definite compound between antibacterial agent and essential metabolite (e.g. between Hg salts and —SH compounds); (*b*) by inhibition of an enzyme reaction involved in the synthesis or utilization of an essential

metabolite. An example of (b) is provided by the case of sulphanilamide. Evidence was obtained that this drug acts by competitive inhibition of an enzyme reaction involved in the further utilization of p-aminobenzoic acid, and that it inhibits by virtue of its chemical relationship to p-aminobenzoic acid. The latter is presumed to be an essential metabolite. Recent work has provided further strong support for this hypothesis, for p-aminobenzoic acid has been actually isolated from natural sources and has been demonstrated to be a growth factor both for *Cl. acetobutylicum* and higher organisms.

The application of the above hypothesis to the search for new substances with antibacterial properties has been fruitful. The following substances, all chemically related to a known essential metabolite (latter shown in brackets), have been shown to have antibacterial activity in varying degree: pyridine-3-sulphonic acid and amide (nicotinic acid and amide); aminosulphonic acids (analogous aminocarboxylic acid); sulphonic acid analogue of pantothenic acid (pantothenic acid); indole-3-acrylic acid (tryptophan); barbituric acid (uracil).

H. McIlwain. A chemotherapeutic agent is regarded as depriving the inhibited organism of the use of enzymes or metabolites by various types of interference. The organism thus becomes nutritionally more exacting than in its normal state, and its new demands can, with due consideration for extraneous effects, be analysed by the usual techniques of bacterial nutrition.

*Bact. coli* and *Streptococcus haemolyticus*, inhibited by acriflavine components, require for further growth two types of material not normally needed. Type I (so called from a previous nutritional study of inhibitory action: McIlwain, *Brit. J. exp. Path.* 1940, 21, 136) is best replaced by nucleotides, and type II by a concentrate of amino-acids but partly by phenylalanine. In the presence of type II compounds, but not without, artificial hydrogen carriers are further active against inhibition of *Bact. coli*. Type I compounds form complex salts with acriflavine components, and it is considered that the inhibitors inactivate enzyme systems of which type I compounds are essential parts, of which type II compounds are substrates or products, and of which some can be replaced by the hydrogen carriers.

E. Chain. The purest penicillin preparations have the following properties.

Penicillin is a strong acid, containing two, or a multiple of two, acid groups with approximate $pK$ values of $2 \cdot 1$ and $3 \cdot 7$. Elementary analysis shows the absence of elements other than C, H and O. Different preparations of the purified Ba salt gave (calculated for the free acid) C 55% and H $6 \cdot 3$%. Methoxyl groups cannot be detected, but two hydroxyl groups are present in the molecule. As the dried Ba salt, penicillin keeps its activity indefinitely. In watery solution it is most stable at $pH$ 5–7; at higher or lower $pH$ penicillin loses its antibacterial activity, the rate of inactivation depending on temperature and $pH$. Electrometric titration indicates that alkaline inactivation may be due to the opening of a lactone ring. Free penicillin is freely soluble in ether, acetone, esters and dioxan, moderately soluble in chloroform, slightly soluble in benzene and carbon tetrachloride. In water it is soluble to the extent of 5 mg./ml. Penicillin in the form of the free acid is stable only when absolutely dry, or in organic solvents; in the latter it is very much more stable than in dried form. The most suitable salt for general use is the Ba salt. Penicillin forms water-soluble salts with most heavy metals, except $Fe^{...}$. It is inactivated by a number of heavy metal ions, especially Cu, Pb, Zn and Cd. The Ba salt of penicillin is soluble in absolute methyl alcohol, but is insoluble in absolute ethyl alcohol. Penicillin is inactivated by primary alcohols. The mechanism of the inactivation of penicillin by acid, alkali, heavy metals and primary alcohols is not yet understood. Penicillin is stable towards atmospheric oxygen, but is oxidized by $H_2O_2$ and $KMnO_4$. The antibacterial activity is lost in these oxidations. Penicillin does not reduce Fehling's

solution; on oxidation with alkaline ferricyanide an amount of ferricyanide corresponding to two reducing equivalents per Ba atom is used up. No catalytic hydrogenation takes place under atmospheric pressure with various catalysts. $NH_2OH$ inactivates penicillin. In this reaction, carried out at $p$H 5, H˙ ions corresponding to one equivalent of $NH_2OH$ per Ba atom appear. $NH_2.NH_2$, $NaHSO_3$ and $Na_2S_2O_4$ also inactivate penicillin; the inactivation by $NH_2.NH_2$ cannot be due to reduction, as no nitrogen is liberated during the reaction. Semicarbazide and thiosemicarbazide do not inactivate penicillin, but no derivatives could be isolated. It has not been possible to obtain normal esters of penicillin by the action of various alkyl iodides on its silver salt.

A new antibacterial substance with chemical properties different from those of other natural antibacterial agents has been isolated from a strain of pro-actinomyces by Prof. A. D. Gardner and Dr E. Chain. It is a base very easily soluble in water in a form of its hydrochloride, and particularly effective against gram-positive pathogens. Strains of pneumococci insensitive to penicillin were affected by 'pro-actinomycin'. Its toxicity is much greater than that of penicillin.

E. P. ABRAHAM. The present activity of the penicillin-containing medium, on harvesting, is about four of the arbitrarily defined units per ml. The active fluid is no longer replaced directly by more medium, but the culture vessels are cleaned and used afresh.

The instability of penicillin has so far necessitated three methods of purification, which depend on distribution between solvents, adsorption and reduction. Owing to the difficulty of separating active from inactivated penicillin care must be taken not to inactivate any of the material during the processes involved. The crude barium salt, obtained from an amyl acetate extract of the medium, has an activity of 15–25 units per mg., and usually contains at least five different pigments. Distribution between water and ether at $p$H 2 and 6, adsorption of impurities by charcoal, and chromatographic analysis on alumina, yield a light yellow barium salt with an activity of about 150 units per mg. On reduction of this material in neutral solution with aluminium-mercury couple the remaining pigment is adsorbed by alumina, while the supernatant fluid contains about 80 % of the original activity. The white barium salt obtained from this solution has an activity of approximately 240 units per mg. It inhibits the growth of *Staphylococcus* completely at a dilution of one in 5 millions and partially at one in 16 millions.

W. H. LINNELL. The recognition of the outstanding properties of proflavine and the corresponding methylacridinium chloride (acriflavine), mainly due to Browning, does not appear to have stimulated research as highly as might be expected. Only two of the many possible, simple, isomeric diaminoacridines, 2:5- and 3:7-diaminoacridines, had been prepared and examined up to 1930, the former compound only appearing in patent literature. Since 1935 a sufficient number of these isomers have been prepared to determine the effect of the position of the amino groups on the properties of the compound and some correlation with bactericidal activity has been observed. A 1-amino group causes complete loss of bactericidal activity and reduces toxicity; a 2-amino group increases activity, and this is further enhanced by another amino group in the 2-, 3-, 4- (in the other ring) or 5-positions accompanied by increased toxicity in the case of a second 2-substituent. When two 3-amino groups (=3:7) are present activity is moderate, but the 3:8-diaminoacridine (=2:7) is as active as proflavine but of lower toxicity. Manifold has recently shown that this derivative is much less harmful to brain tissue than proflavine or acriflavine. A 4-amino substituent confers small activity, whilst position 5 is highly active but probably leads to increased toxicity. It is interesting to note that compounds containing 1-amino and 4-amino substituents appear to be the only amino-acridines that do not fluoresce. It may be significant, as Roab pointed out, that acridine was

toxic to paramoecia in daylight but innocuous in the dark. Albert has shown that similar differences in activity exist amongst the five isomeric monoaminoacridines and that their activities appear to run parallel with their strengths as bases and their partition coefficients between oil and water. The corresponding acridones are inactive as are certain amino derivatives of 5:10-dihydroacridine and of iminodihydroacridine. This suggests that the intact acridine molecular structure is necessary. An extension of this work to cover hydroxy- and chloroalkoxy-acridines showed that 3-, 3:7- and 3:9-hydroxyacridines were inactive in concentrations of 1 in 2000, whilst corresponding chloroalkoxy compounds possess some activity but present insuperable difficulties due to extremely small solubility.

L. G. GOODWIN. The uncertain action of antimony in protozoal diseases, of which the resistance of Sudanese kala-azar to antimony is an example, is an added difficulty in investigating its mode of action. The active form of antimony might be the stibinoxide grouping, but while this is probable in trypanosome and schistosome infections, it is unlikely in leishmaniasis, where quinquevalent compounds are the most effective and massive dose therapy is successful.

Excretion of antimony after doses of the quinquevalent compounds or of stibophen is much more rapid than with tartar emetic. There is some indirect evidence that the rapidly excreted fraction of the drug passed through the body unchanged. Both direct toxic action on parasites and stimulation of the host's defence mechanisms are produced by antimonials. Increased phagocytosis may be of primary importance in leishmaniasis, though histological work on the spleens of infected hamsters injected with a quinquevalent antimony compound suggests some degree of direct action.

F. R. BRADBURY and D. O. JORDAN. The behaviour of sulphanilamide, p-aminobenzoic acid and chemically related compounds such as aniline and sodium benzenesulphonate at the surface of *Bact. coli*, is being studied by electrokinetic methods. The shape of the curves relating variation of mobility with time of contact for sulphanilamide and p-aminobenzoic acid is distinctly different from that of the curves obtained for the chemically related compounds. Further, the curves for sulphanilamide and p-aminobenzoic acid show a close resemblance, indicating that these two substances behave in a like manner at the bacterial surface. These results are in agreement with the findings of Fildes, Rubbo et al. concerning the antagonism between the two compounds.

A. St G. HUGGETT. Certain azo-dyes, notably chlorazol sky blue FPS (Chicago blue) and chlorazol fast pink BKS, are excellent anticoagulants, only equalled by heparin. In their structure they resemble another azo-dye, afridol violet, from which was derived Bayer 205. Bayer 205 has an anticoagulant action which is, however, less effective than that of the azo-dyes. They are, on the other hand, excellent trypanocidal agents, only slightly less effective than Bayer 205, and with a somewhat higher toxicity. This was demonstrated by Huggett & Suffolk on mice infected with *T. equiperdum*. The following points, which have been determined as to their mode of action as anticoagulants, may throw light on their mode of action as trypanocidal agents: (i) They act at two points in the clotting mechanism, as antikinases and antithrombin. (ii) They do not destroy thrombokinase, thrombin, or fibrinogen, merely paralysing the action of these two enzymes. (iii) They are adsorbed on to the clot if formed. (iv) They disappear asymptotically from the blood passing into the urine and into the reticulo-endothelial system. This antienzyme action with blood clotting may bear an analogy with the mechanism as trypanocidal agents. The analogy cannot be pushed too far, for the dyes are better anticoagulants but worse trypanocidal agents than Bayer 205.

E. BOYLAND.   The problem of cancer therapy is possibly more difficult than treatment of diseases due to bacteria or trypanosomes, because the tumour tissue closely resembles the host's tissues and because of the great variation in malignant disease. It has been shown that sarcomata and carcinomata of mice differ in their response to treatment with bacterial filtrates, with colchicine or with heptaldehyde.

Two lines of approach have led to the conclusion that many diamines are able to reduce the rate of growth of tumours. The earliest work was concerned with the effect of extracts of normal plant and animal tissues.  Extracts of muscle have been examined, and it seems possible that inhibitory action is due to the simple bases which are present in such preparations. The active bases have either two amino groups (e.g. cadaverine) or a basic group and hydroxyl group (e.g. ethanolamine).

Examination of the effect of antibacterial compounds of the sulphanilamide type showed that many aromatic diamines were able to reduce the growth rate of tumours in mice. For this activity sulphur is not essential, and benzidine, 4:4'-diaminodiphenylether and diaminodiphenylmethane appear to be just as effective as diaminodiphenylsulphoxide in the small number of trials that have been carried out. In this type of substance two amino groups appear necessary for activity.

Investigation of the tumour growth-inhibitory actions of heptaldehyde and citral lead to the conclusion that some dibasic acids, such as malonic acid, have this same action.

All of the active substances described can be represented as $P - x - P'$, where $P$ and $P'$ represent polar groups.  None of the substances are sufficiently active to be of much practical value, but they indicate a group which might contain some really effective drug which might be useful in cancer treatment.