FIFTEEN YEARS OF SULPHA DRUGS—A PERSPECTIVE

K. GANAPATHI
(*Department of Chemotherapy, Haffkine Institute, Parel, Bombay*)

Introduction

It was in 1932 that the method of preparation of prontosil was patented and the therapeutic property also discovered, though this discovery, which "created a sensation and shook the world", was announced by Domagk only in 1935. Since then, a tremendous amount of work has been published with the accumulation of a great deal of knowledge. The lapse of fifteen years has tempered the initial enthusiasm about the efficacy of the drugs, and the new ideas and data have been subjected to critical examination, so that the time is now mature to reckon the advances made in this field. A number of critical reviews have been published dealing with the details of many aspects of the problem. As complementary to these, herein, is attempted, without focussing on the details or scanning isolated sections, a perspective of the whole field.

A New Era in Chemotherapy

It is not a mere platitude to reiterate that the discovery of prontosil and the sulpha drugs is an event of epochal importance in the history of chemotherapy. A great discovery distinguishes itself from others not merely by presenting results of immediate importance but by stimulating thought and action in many related fields thus acting as a lever to make great fundamental advances. The discovery and the progress in this field have satisfied these requirements. Ehrlich, as the father of chemotherapy, gets the credit for having recognised and enunciated the quintessence of the problem. In his time, and even subsequently, chemotherapy could not advance in practice beyond a hit-or-miss project. That the subject has been taken up seriously, in spite of this, is an eloquent testimony to its great practical importance and attractiveness even as a commercial venture akin to the extraction of gold from the rocks. There have been very useful discoveries in this field as a result of prodigious effort (such as germanin, plasmoquin, atebrin), but these have not resulted in any great fundamental advances in the field of chemotherapy. For many important questions relating to these drugs, the answers could be no better than guesses, speculations or new obscure phrases. The advent of the sulpha drugs has revolutionised the position. The physiological and bacteriological phenomena relating to chemotherapeutic action have come to be tackled on a chemical, physico-chemical and cytological level, and chemotherapy has, consequently, become the fertile intermixing ground for the physical and biological sciences. This has introduced a new outlook and logic in the subject, and has earned for chemotherapy the status of a science with a rational basis and a rational approach.

Evolution of Sulpha Drugs

The discovery by the French school that the therapeutic property of prontosil is due to sulphanilamide derived by reduction, simplified the problem and presented it in a clear-cut manner as regards the synthetic side. It did not take long to realise that the free amino-group in sulphanilamide is inextricably connected with the therapeutic activity and that the heterocyclic substituent at the sulphonamide radical actuates the intensity and spread of the spectrum of antibacterial activity, this concept being roughly analogous to the functions of the prosthetic groups and proteins of the enzyme systems. There was, consequently, a lively hunt in laboratories all over the world for all heterocyclic ring systems. The period, 1939 to 1942, was the most fruitful on the synthetic side, when almost all important possibilities were explored and the sequence of sulphapyridine, sulphathiazole, sulphadiazine, sulphamerazine, sulphapyrazine established. The period following which, frankly, is one of disintegration and decay, attracted the second batch of workers from many academic laboratories. Much of this work remains uncorrelated divorced from the biological testing and is, therefore, of little interest either from the chemotherapeutic or the chemical point of view.

Of the thousands of compounds synthesised and passed through the mill, only about a dozen came out as worth considering from the clinical point of view. Caustic comments have been made on this ratio, that the search for drugs in chemotherapy is a costly gamble for luck in the dark. There is justification for this. Because the discoverer of a drug gets a halo in the press and the public and even improves his financial position, the effort spent on the study of the more important fundamental problems connected with the chemotherapeutic action is not as much as that directed towards the discovery of new drugs by blind venture. However, in the present case, there is the consolation that we have gained some important knowledge. As a rough approximation, we can now scent how the chemotherapeutic activity runs through the structural section. As was not appreciated before, this activity transcends the purely organic linkage or radical level, and is governed by molecular geometry and configuration as reflected in the physicochemical properties. As a result of theoretical reasoning, it is concluded that as far as the intrinsic chemotherapeutic activity is concerned, the maximum appears to have been touched in the region, sulphathiazole, sulphadiazine and sulphamerazine. That nothing has been discovered to contradict this conclusion adds a great deal of prestige to this theoretical reasoning. From this point of view, the chemical work is now well nigh complete and the chances of discovering sulpha drugs with greater intrinsic activity than the above seem to be very remote.

When it comes to the question of using the drug clinically, the way the host deals with and affects the pathways of the drug in the physiological system assumes as much importance as

its intrinsic activity. The sulpha drugs are used for the treatment of a variety of infections wherein its action is required in such diverse sites as the blood stream, tissues, intestines, cerebrospinal fluid, urinary tract, surface of wounds, etc., depending upon the nature of the infection. The drug should reach these sites in sufficient concentrations and also be maintained therein sufficiently long. The properties of the drug responsible for the therapeutic activity will be different from those governing its transport and metabolisation in the physiological system. In addition, the acetylation of the drug and its rapid excretion from the system limit the activity and overall clinical result of the sulpha drugs. If these could be controlled, the intrinsic activity of the drug could be capitalised from the clinical point of view.

Animal Experiments

Two properties of the drug that are of importance from the clinical point of view are: the intrinsic level of chemotherapeutic activity and the degree of toxicity, *i.e.*, the effect of the drug on the parasite and the host respectively. While the first decides the degree of response we can expect from the patient to the drug, the latter governs the degree of safety in administering the drug. Methods of measuring these two properties have now been standardised and rationalised.

Testing the effect of the drugs in experimental streptococcal, pneumococcal, *P. pestis* and other infections in mice (which are convenient to handle and require only small quantities of the drug for the screening experiments) yields clear-cut results. Though the course of the disease in these experimental infections and in human cases (except in the case of *P. pestis*) do not even closely correspond, the results obtained are quite comparable. The results of the animal experiments signify that the drug retains its antibacterial activity within the system of the host (acting as a true chemotherapeutical) and, if given a chance to meet the bacteria, can successfully destroy them. If the testing is done on a strict quantitative basis, the results can be of clinical importance. in the case of the systemic infections. It is the concentration of the drug maintained in the blood and tissues that decides the degree of therapeutic activity; the oral dose as such cannot be used for this comparison, because the blood concentration is a function of the rate of absorption, excretion and metabolisation which vary from drug to drug. For accurate estimation of the intrinsic chemotherapeutic activity and also the level of this concentration at which this effect is maximum in experimental infections, the drug-diet method, which ensures definite uniform concentration of the drug in the blood of the experimental animal, is resorted to.

In his anxiety to keep an eye on the therapeutic activity and toxicity of the drugs simultaneously, Ehrlich developed the chemotherapeutic index, and this ratio he took into consideration to grade the compounds in the order of merit for clinical use. Though in this he shifted the locus of emphasis by giving undue consideration to the maximum tolerated dose, the results were not wide off the mark as far as the arsenicals were concerned. But in the case of the sulpha drugs, the disparity will be very glaring. So long as a drug is absolutely safe in therapeutic doses, the ceiling of toxicity is not at all of importance from the practical point of view. If *a la* Ehrlich we couple chemotherapeutic activity with the maximum tolerated dose to obtain the chemotherapeutic index and use this to grade the compounds with regard to their clinical utility, we stray away from our original aim. So, the determination of the chemotherapeutic index is now given up. When we know that the compounds are not lethal in therapeutic doses, we look in for the minor toxic symptoms produced by the drug. The chronic toxicity of the drugs in repeated doses over an extended period of time is, therefore, estimated.

Rationalisation of Clinical Use

The clinical value of the sulpha drugs for the treatment of many bacterial infections is now well recognised even by laymen. The extent of its use can be judged by the fact that in 1943, in U.S.A. alone, nearly ten million pounds of this drug were manufactured, an unprecedented record for any chemotherapeutic agent. There are misgivings that penicillin may supersede the sulpha drugs altogether. The mechanisms of action of sulpha drugs and penicillin are different; their antibacterial spectra also differ though they overlap in certain regions. The sulpha drugs, from the point of view of cost, ease of administration, stability, storage, etc., have great advantage over penicillin, and these will decide their infiltration even into the rural areas for clinical use. There is no possibility, therefore, that the sulpha drugs would go out of use.

From 1935 onwards, about two dozen compounds have been tried clinically. If we carefully scrutinise the data, keeping the two cardinal features, cheapness and effectiveness, of the drugs as the guiding principle for selection for clinical use, we arrive at the conclusion that sulphathiazole, sulphadiazine and sulphamerazine are the best of the lot. Of the last two, sulphamerazine is preferable because its rate of excretion is much slower and its acetyl derivative is more soluble than that of sulphadiazine. Sulphamerazine can, therefore, be administered at longer intervals, and the renal damage due to it is much less. Sulphaguanidine, succinylsulphathiazole and phthaloylsulphathiazole are popular as intestinal antiseptics, because their absorption from the intestines is very little. But it appears that the same overall result could be obtained by a judicious use of sulphadiazine or sulphamerazine. As the manufacture of these three drugs are patented, attempts are being made to fabricate all types of products as possible rivals.

The important advance made in the clinical field is in the rationalisation of the therapeutics. Till the advent of the sulpha drugs, the oral dose of the drugs was taken into consideration for clinical trials, and even this dose was arrived at arbitrarily by trial and error. Now, as a rational procedure, emphasis is put on the

blood concentration attained which we know governs the therapeutic effect and of which we have definite information. So the oral dose is adjusted to maintain the optimum concentration of the free drug (5 to 15 mg. per cent. as is required) in the blood. In view of this, we can appreciate the importance of the rate of excretion of the drug from the system from the therapeutic point of view, and this property now requires as much study as the chemotherapeutic activity itself. If the drug can circulate in the system, say for two or three days, the advantages are of a far-reaching nature. The total dose to be administered will be cut down to about a sixth and, consequently, the toxic symptoms will also become negligible.

A chemotherapeutic drug which can be administered orally has great advantages. The treatment becomes very popular, not at all annoying to the patient and also very cheap. But this oral administration has other side consequences if the drugs happen to possess strong bacteriostatic action. In the treatment of systemic infections wherein the drug is required in the blood stream or tissues, by oral administration we make them reach these sites by way of the stomach and the intestines. The intestines are populated by bacteria that supply by their synthesis man's requirements of some vitamins. The sulpha drugs, while they are in the intestines for a number of days, because of repeated administration prior to absorption, kill the intestinal flora and, as a consequence, give rise to symptoms of deficiency of these vitamins. So far, we have found that the vitamins thus involved are vitamin K and folic acid. The above deficiency is particularly noticeable in the case of drugs poorly absorbed from the intestines and so have a greater destructive effect on these bacteria. This side effect should be kept in mind whenever an intensive therapy with the sulpha drugs is undertaken. Advantage has been taken of this phenomenon to study the synthetic abilities of the intestinal flora and the part they play in the vitamin supply of the system.

Mechanism of Action of Sulpha Drugs

The unravelling of the mechanism by which the drugs act should not be considered to be of mere academic interest. The discovery of prontosil actually arose out of the wrong concept that those compounds which show activity *in vivo* should be inactive *in vitro*. This is probably derived as a converse of the observation that many compounds that show very good activity *in vitro* are inactive *in vivo* and also partly as a consequence of the lack of understanding of the difference in the mechanism of action of an antiseptic and a chemotherapeutical. Domagk vehemently held the view that the activity of prontosil is due to some action elicited by the drug from the host. As a result of subsequent extensive experiments it emerged that the bacteriostatic action of the sulpha drugs is produced by the same mechanism *in vivo* and *in vitro* by virtue of their inherent property and that they do not call upon the host for any part to play in this. The chemotherapeutic action of the sulpha drugs result from the interference with an enzyme system vitally connected with the proliferation of the bacteria. The inhibition of multiplication, rather than the instantaneous killing, appears in essence to be the mechanism of action of the sulpha drugs. Thus the interest shifted from the immuno-biological to the cytochemical and enzymic field, wherein the problem is capable of being tackled in a rational manner.

The most important advance resulted from the remarkable discovery of the specific reversal of the bacteriostatic effect of the sulpha drugs both *in vivo* and *in vitro* by *p*-aminobenzoic acid. This action is distinctly different from the reversal observed in the case of methionine, purines, peptone, etc. The theory developed by Fildes and Woods conceived *p*-aminobenzoic acid as an essential metabolite of the bacteria, the utilisation of which is prevented by the sulpha drugs by stopping the enzyme system concerned with it from functioning. The sulpha drugs show this property by displacing *p*-amino-benzoic acid from the enzyme system by virtue of the structural similarity of the two compounds concerned. Though this appeared to have solved the problem, a great deal of controversy and many issues were raised as regards *p*-aminobenzoic acid being an essential metabolite and on the mechanism of competitive inhibition. As a result of this, very fruitful work has been done.

It was found that *p*-aminobenzoic acid is a growth factor for *Clostridium acetobutylicum, Acetobacter suboxydans* and *Streptobacterium plantarum* and is even classed as a member of the vitamins of the B group. The exact part played by this acid in the life of the bacteria is not exactly known, though it appears to be connected with cell multiplication. We have also not identified the enzyme system involved, though it appears that the acid as such is not a prosthetic group of an enzyme or coenzyme as some conceived it. The discovery of the growth-promoting effect of *p*-aminobenzoylglutamic acid and the presence of this grouping in folic acid are significant pointers. Work in this direction is likely to throw much light on the mechanism of cell division and multiplication of the bacteria.

Chemotherapeutic and Antiseptic Action

The mechanism of competitive inhibition has greatly influenced the imagination of the chemotherapeutists, even to the extent of making them blind to other possibilities. This gives an insight into one of the important problems in chemotherapy, *viz.*, difference between the actions of an antiseptic and a true chemotherapeutic agent. A huge number of synthetic reactions, all mediated by specific enzymes, are going on simultaneously within the same cell *in vivo* and in the bacteria. That each one of these reactions can go on as if in isolation, without being interfered by any other, has been made possible by the extreme specificity of the enzyme reactions, *i.e.*, of the substrates and the coenzymes. If the parasite lodged in the system of the host is to be selectively destroyed, this can be achieved by putting out of action one of the enzyme systems which is vital for the bacteria but differs from that of the host. Since the bacteria are versatile in syn-

thetic activities and are equipped to meet all emergencies by adaptation, they should not be capable of developing alternative pathways or other shunts to meet the needs. The true chemotherapeutic agent shows the specific selective action by the above-mentioned mechanism. The antiseptic, on the other hand, not possessing this selective action, when put in a complicated system, gets entangled in the one it comes across first and thus, going astray, is not available where its action is required. This is how the antiseptic which is very active in the test-tube looses its activity *in vivo*.

One of the ways to stop an enzyme system from functioning in a selectively specific way is by the mechanism of competitive inhibition, taking advantage of the structural specificity of the substrate of any other participant in the enzyme system. A compound which is close enough in structure to this to get involved in the first stage but not identical enough to be actually utilised in the enzyme reaction can stop the enzyme system from functioning. If this enzyme system is itself vital or a vital link in an important chain, we have obtained the typical chemotherapeutic effect. . If this effect is to be of clinical value, the additional conditions to be satisfied are: (*i*) the inhibitor should not undergo metabolisation or have affinity for other compounds *in vivo* and (*ii*) the substrate or compound being displaced by the inhibitor should not be produced *in vivo* in sufficient concentrations to nullify the action of the inhibitor itself. Though we are able to chalk out these principles in concrete terms, no great advance has been made in discovering more chemotherapeuticals because we do not know enough about the chemistry of the enzyme systems involved in bacterial multiplication and proliferation. Strangely enough, we came to know of the role of *p*-aminobenzoic acid by the reverse process of working with a true chemotherapeutic agent. Thus, the mechanism of action of the chemotherapeuticals gives us a clue to the understanding of the chemistry of bacterial multiplication.

Then there is the question as to the exact phase of bacterial growth on which the drug should act to obtain striking chemotherapeutic action—whether it should affect the respiration, metabolic or catabolic reactions, the cell division, etc. This action will decide the nature of the antibacterial effect obtained. The sulpha drugs and penicillin show their effect only when the bacteria are rapidly multiplying and not when they are in the stationary phase. Their effect is not, therefore, observed at once. This is roughly taken as a bacteriostatic action as differentiated from the bactericidal effect in which the lethal action is immediate. If an immediate chemotherapeutic effect is desired, the action of the drug must be directed against even the stationary phase of bacterial growth. We do not as yet know enough about the bacterial enzyme systems to evolve anything useful in this direction.

Physicochemical Theory of Chemotherapeutic Action

The theory of action of the sulpha drugs, by displacing *p*-aminobenzoic acid from an enzyme system by competitive inhibition, has provided a solid base on which to build the physicochemical theory of the intensity of the chemotherapeutic effect. One fortunate fact helping us in this venture is the structural simplicity of the drugs in which the only variable is the substituent at the sulphonamide radical. So the problem is to find out how this substituent governs the degree of the intrinsic therapeutic activity. Since the mechanism involved is competitive inhibition, the more the sulpha drug resembles *p*-aminobenzoic acid the greater the degree of activity. Though the *p*-aminobenzoic acid ion and the *p*-aminobenzenesulphone radical resemble each other in geometric configuration, the only distinct feature about the former is the negative charge. So the more negative the sulphone group, the greater the activity of the sulpha-radical. The only way of gauging the negativity of the sulphone group (governed by the attached amino or substituted amino group) is by the acid dissociation constant (pKa) of the drug. The theoretical calculation shows that the maximum activity will be shown by that drug whose pKa value is 6·7. On this basis the maximum activity is almost reached in sulphathiazole (pKa=7·12), sulphadiazine (pKa = 6·48) and sulphamerazine (pKa = 7·06). That no drug has so far been discovered which shows greater activity than the above, indicates that the theoretical reasoning is sound. This is the first time in the history of chemotherapy that a physicochemical property of a compound could be used to predict its antibacterial activity. In the light of this, attempts are also being made to treat the problem on a physicochemical basis. As stated before, this has introduced a new outlook and logic in the field of chemotherapy which has earned for it the status of a science.

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MEDIUM OF STUDIES IN COLLEGES

IN the Dominon Parliament, on the first of March, Maulana Abul Kalam Azad, Education Minister, said in reply to Seth Govind Das that as far as primary and secondary education was concerned, the Provincial Governments had accepted the principle that the medium of instruction should be the mother-tongue. Every effort was being made to put this into practice.

The Central Advisory Board of Education and the Educational Conference both came to the conclusion that the change in University education should be by stages, so that the standard of education did not suffer. It was agreed that the change-over should be spread over five years, and in the sixth year all education should be in the Indian language or languages which should be the medium of instruction. The English language would, however, continue to be a second language and a subject for post-graduate studies.