PHARMACOLOGICAL STUDIES ON SOME SULPHONAMIDE DERIVATIVES
CHAPTER I

GENERAL INTRODUCTION

The introduction of sulphonamides to combat the bacterial infections ushered in a new epoch in the history of modern medicine. The benefit of this revolutionary change in therapy can never be over-emphasised. But bacterial chemotherapy may be said to have originated long before the advent of the sulphonamides. As a matter of fact, this study followed closely attempts to control the protozoal infections, initiated by Erhlich, which led to the introduction of Salvarsan.

The introduction of anti-septics and disinfectants in the treatment of septic wounds may be regarded as attempts to local chemotherapy. Control of systemic infection was a natural urge for extension into wider fields of usefulness.

Initial interest fell on the studies of dyes, like methylene blue, gentian violet etc, which were found to be active bactericidal agents in vitro. By patient research, Hietzsch and Klarer, in collaboration with Domagk, working in the laboratories of I.G.Farbenindustrie of Germany, developed atebrin, an acridine-dye compound, which was found to be a successful remedy against human malaria. Nevertheless, bacterial chemotherapy was still far off.
Morgenroth and Levy (1911) attained partial success in combating bacterial infection of the bloodstream with ethyl-hydrocupreine or OPTOCIN, by protecting mice infected with small inocula of pneumococci. But the clinical trial of the drug did not show any promise; besides, it had an undesirable toxic effect on the optic nerve of the patients. Heidelberger and Jacobs (1919) synthesised a compound by coupling diazotised p-aminobenzenesulphonamide with dihydrocupreine. Unfortunately, the compound was also devoid of any appreciable activity.

The real turning point in the history of chemotherapy came in the year 1935, when Domagk observed the remarkable protective action of the red dye, the hydrochloride of 4-sulphonamido-2,4-diamino-azobenzol against virulent streptococcal infections in mice, and Trefouel, Trefouel, Sitté, and Bovet (1935) demonstrated the real active component of the dye to be the simple chemical compound, p-aminobenzenesulphonamide, synthesised long ago in 1908 by Gelmo, Goissedet, Despois, Gailliot and Mayer (1936) confirmed the work of Trefouel et al. (loc. cit) showing that dyes were not essential for activity.

Remarkable clinical efficacy of sulphenilamide (p-aminobenzenesulphonamide) was reported by Colebrook and Kenny (1936). Buttle, Gray, and Stephenson (1936) working on mice, observed the effectiveness of the compound against streptococcal
and meningococcal infections. The results of these findings were so convincing that attention of scientists throughout the world was drawn towards the object of further contribution in the field.

As a result, a vast number of compounds were synthesized in different laboratories. Northy (1948) has put the figure over 5000. Complete pharmacological data on all of these are not available, but extensive researches on some of them have established a number of compounds useful in clinical medicine.

CHEMICAL CONSIDERATIONS

p-Aminobenzene sulphonamide is a simple chemical compound consisting of three parts, the nuclear benzene ring, the p-amino group, and the sulphonamido part. The nitrogens at these two places are designated as $N^4$ and $N^1$ positions. The $-\text{NH}_2$ group attached to the sulphone part is

![Chemical structure of p-aminobenzene sulphonamide](attachment:chemical_structure.png)

termed as the amido group to distinguish it from the amino group, which is attached to the benzene ring at the para position.
Compounds obtained by substitution at the amido group are known as \(^1\)-substituted compounds. Most of the active sulphonamides fall under this group, which includes sulphapyridine, sulphathiazole, sulphadiazine, sulphamerazine, and sulphamethazine. These latter compounds are all heterocyclic derivatives directly attached to the sulphonamido (\(-\text{SO}_2\text{NH}_2\)) group, and with each a distinct widening in the range of antibacterial activity can be observed. Thus, sulphanilamide is found to be particularly active against \(\beta\)-haemolytic streptococci, meningococci and to some extent gonococci. Its anti-pneumococcal activity is definitely low. In sulphapyridine, a powerful anti-pneumococcal potency is obtained, apart from its action on haemolytic streptococci, gonococci and meningococci. Sulphathiazole possesses in addition to these activities, a strong anti-staphylococcal action. Sulphadiazine, while having a slightly lower potency index than sulphathiazole, has a selective activity against \(E.\)friedlanderi, which is unaffected by any other sulphonamide derivative (Whitby, 1944). The antibacterial action of sulphamerazine and sulphamethazine, which are mono-methyl and dimethyl derivative of sulphadiazine respectively, is similar to that of the parent compound.

The compounds which are prepared by substitution of one or both hydrogen of the p-amino group are called \(^2\)-substituted derivatives. While most of these are less active than the \(^1\)-substituted compounds, there are some very useful ones, which occupy
some distinct place in therapeutics. Further discussion on these compounds will be made in the relevant sections of the Thesis.

CHEMICAL CONSTITUTION AND PHARMACOLOGICAL ACTION

From the study of a vast number of compounds, prepared by various substitutions in the different parts of the sulphanilamide molecule, certain generalisations have been arrived at regarding the correlation of chemical constitution of these compounds with their pharmacological action. These can be summarised below:

(1) The presence of the amino group in the para position induces free sulphonamide-type of activity. Shifting of the amino group to the ortho - or meta - position causes inactivity of the compound.

(2) Substitution in the p-amino group generally lowers activity.

(3) Activity of any sulphanilamide derivative containing NH—R group attached to the para position of the benzene ring depends upon the splitting of the R-radical, and the subsequent reversion of the compound to free sulphanilamide.

(4) Para-amino grouping is not absolutely essential for activity, since p-nitrobenzenesulphonamide is also an effective compound, though not to the same extent as that of sulphanilamide.

(5) Substitutions in the sulphonamido group usually widen the activity, and the compounds derive their activity from the whole molecule. That means SO$_2$NH—R linkage is firm. An exception will be found for example in N$^1$-(aminobenzene sulphonyl)-1:4-oxazine.
(6) Replacement of both hydrogens of the \( \text{SO}_2\text{Hg} \) group results in loss of activity, as the compounds become non-ionisable.

(7) The activity is restricted not only to sulphonamido group as sulphur and groups like sulphoxide and sulphone also induce varying degree of activity, provided they are placed para to the anilino group. Generally however, these derivatives are more toxic than sulphonamido derivatives.

(8) Some findings show that even sulphur containing radicals are unnecessary for activity, since \( p \)-nitrobenzoic acid is chemotherapeutically effective against experimental streptococcal infection.

(9) Nuclear substitutions usually cause a loss of activity.

**BACTERIOLOGICAL CHARACTERISTICS OF SULPHONAMIDE COMPOUNDS**

Sulphonamide derivatives are generally bacteriostatic in action. In presence of serum, defibrinated blood, and under certain conditions, a bacteriolytic effect is also demonstrated (Colebrook et al., 1936; White and Parker, 1936). There is an appreciable lag in the development of sulphonamide bacteriostasis, regardless of the time of addition of the drug to the culture medium. In animals, attainment of a satisfactory concentration before inoculation does not prevent organisms to grow for the first few hours (Lockwood, 1936). This suggests that during the "lag" phase of the growth sulphonamide is unable to exert its action and the phenomenon is explained by the presence of pre-formed metabolites, which get exhausted with the first phase of the growth.
There is divergence of opinion as to whether sulphonamides stimulate the body defence mechanisms. Long and Bliss (1937) observed a stimulation of phagocytes six to fifteen hours after treatment of infected mice. Fleming (1938) demonstrated the importance of leucocytes and antibodies in overcoming infection of pneumococci by sulphapyridine. Kufer and Rantz (1939) observed that samples of blood containing sulphanilamide show a bactericidal effect when natural antibodies are present. Menefee and Poston (1939) found that in some cases of brucellosis which responded well to sulphanilamide therapy, the patients' sera showed a high agglutinating titre. From these observations, it appears reasonable to conclude that for effective eradication of an infection, not only the bacteriostatic concentration of the chemotherapeutic agent, but phagocytosis and the immune response of the host, are factors likely to play an important part. In the present Thesis, some experimental observations have been incorporated on this aspect of the problem (Part III).

However, according to other workers, phagocytosis is neither stimulated nor the speed of production, quality or quantity of immune bodies affected by sulphonamides when exerting the therapeutie action (Bayliss 1940).

MODE OF ACTION OF SULPHONAMIDES

A. BACTERIOLOGICAL ASPECT

The cause of bacteriostatic action of sulphanilamide and its derivatives has been a matter of wide speculation from the
beginning of its introduction. The compounds do not possess characteristics of strong antiseptics' or germicides already known. They do not behave as general protoplasmic poisons or strong adsorbents of bacterial proteins. The effect on the bacterial growth is rather a slow process. Naturally, various theories have been put forward from time to time to explain the cause of sulphonamide bacteriostasis. But most of them have fallen through in course of time. Thus, the earlier ideas of stimulation of body defenses (Long and Bliss, 1937; McIntosh and Whitby, 1939; Reed and Orr, 1944), decapsulation of virulent organisms (Levaditi and Vaisman, 1935a), production of more antibodies (Mitte and Bovet, 1936), detoxication of toxic materials of bacterial metabolism (Levaditi and Vaisman, 1935b, 1936; Rake and Harare, 1944) and many such ideas have failed to solve the mechanism of activity of these compounds. Chemical and biochemical explanations, such as the oxidation of the molecule in the system (Mayer, 1937; Mayer and Oechslin, 1939), or the anti-catalase theory (Main, Shinn, and Mellon, 1939) also have not yielded any satisfactory picture.

**WOOD-FILDES THEORY**

In 1940, it was observed (Green, 1940; Woods, 1940) that yeast extracts contain a powerful sulphonamide counteracting factor, which might be similar to p-aminobenzoic acid (PABA). Shortly after, Woods and Fildes (1940) demonstrated the antagonism of sulphonamide inhibition of bacterial growth of PABA which was confirmed
by many other workers. Based on this finding, they put forward the theory (cf. Fildes, 1940) that sulphonamides function by interfering with an essential metabolite required for carrying out anabolic reactions and this leads to blocking of the chain of enzymic synthesis, so necessary for growth. PABA was considered to be the essential metabolite, and on account of its close structural similarity with sulphanilamide, it was proposed that the antagonism was due to a competition between the sulphonamide and PABA for an enzyme surface.

Woods-Fildes theory blazed a new trial in the complicated field of mode of action of sulphonamides. The explanation was limited to the ultimate depletion from the substrate of the essential metabolites or growth factors for the bacteria. Sevag and his co-workers (1942, 1946), however, offered a different theory based on the competitive inhibition of bacterial respiration, instead of inhibition of food synthesis, which Wood-Fildes theory envisages (cf. Bose, 1949). While there was logic in their arguments, the fact that relatively high concentration of sulphonamides is needed for respiratory inhibitions, suggests that in some cases the effect might be secondary to a primary inhibition elsewhere in the cell. Considering that sulphonamides, like narcotics, stimulate in low and inhibit in higher concentrations (Finklestone-Sayliss et al., 1937; Lamsanna and Shapiro, 1943), Henry (1943) grouped them as a class of "indifferent" cell inhibitors, which inhibit a specific fraction of the total oxidative reactions of the cell upon which cell division depends.
The idea of competition between the drug and an essential metabolite, structurally similar, for the same locus on the enzyme surface, and leading to bacteriostasis, has been verified by synthesis of various structural analogues of vitamins, purines, and amino acids (cf. Ghosh, 1954).

\[
\text{INDOLE} \quad \text{TRYPTOPHANE} \quad \beta\text{-INDOLE ACRYLIC ACID}
\]

\[
\text{BIOTIN} \quad \text{BIOTIN-SULPHINE}
\]

\[
\text{PANTOTHENIC ACID} \quad \text{PANTOYL TAUROPS}
\]

\[
\text{THIAMINE} \quad \text{PYRITHIAMINE}
\]

\[
\beta\text{-AMINOBENZOC ACID} \quad \text{SULPHANILANIDE}
\]
Strong support for this hypothesis was received on the discovery of pteroylglutamic acid (folic acid, PGA) which contains a para-aminobenzoic acid radical. It is now accepted that a sulphonamide inhibits bacterial growth by preventing PABA from being incorporated into the PGA molecule (Lampen and Jones, 1946; Morgan, 1948). Sulphonamide-sensitive bacteria synthesise their own PGA. Bacteria which do not require PGA or can utilise preformed PGA are not affected by sulphonamides. This antagonism is found both in-vitro and in-vivo, and applies not only to bacteria but also to certain viruses and fungi, which are inhibited by sulphonamides. Why animal cells, which require PGA for their own synthesis, are not injured in the same manner as the bacteria may be explained by conceiving animal cells to be similar to sulphonamide-insensitive bacteria.

Besides PABA, methionine, various amino acids and purines have been found to counteract sulphonamide action under certain conditions. The action of methionine has received considerable attention. Harris and Kohn (1941) noted that methionine is effective only against low concentrations of sulphonamides, but unlike PABA there seems to be no constant ratio between the amount of sulphanilamide and the amount of methionine present. Shive and Roberts (1946) have shown that sulphanilamide inhibits sequentially the synthesis of methionine and xanthine by the organism. Guanine and Xanthine in the presence of methionine antagonise sulphanilamide action, but in the absence of methionine, they
increase it (Harris and Kohn 1941; Kohn and Harris, 1943).
These were puzzling facts, which could not be explained by the mode of action of sulphonamide based on PABA competition. But the discovery of PGA has thrown new light on the action of methionine and purines. It is now considered likely that PABA participates in the synthesis of methionine (Winkler and DeHaan, 1948). Winkler (1948) discussed three steps in the inhibition of enzymes involving PABA by sulphonamides in E. coli.

I. The first step most sensitive to the action of sulphonamide is the synthesis of methionine.

II. The next step involves production of xanthine and other purines.

III. The third step relates to the inhibition of synthesis of serine and PGA, at only higher concentrations.

But the theory as discussed above cannot explain all the known facts about the action of sulphonamides on bacteria. The basic postulate for competitive antagonists is that they act by virtue of their structural similarity, but instances are available of structurally dissimilar molecules competing with an essential metabolite (Barlett, 1942; Pfeiffer, 1943). Wooley (1947) has raised certain pertinent questions, citing facts which are difficult to reconcile with the theory of competition between a drug and an essential metabolite. In a competitive inhibition, if the concentration of inhibitor is great enough in comparison
to that of the metabolite, the latter is displaced and the biological system is thereby deprived of it. But there is little experimental proof that the analogues really do displace the metabolites from protein combinations. Dittmar and du Vigneaud (1944) only showed that when the analogue of biotin, biotin sulphone, was added to the complex formed between biotin and the specific protein antibiotin (avidin), the vitamin was liberated. It is a fact that subinhibitory amounts of metabolite analogues e.g., sulphonamides, pyrithiamin, benzimidazole produce stimulation of growth, but why this should be so, is a question still to be answered. If the analogue merely displaces the metabolite, why should small amounts have this stimulating effect? Moreover, according to Henry (1943) the presence of PABA in growth of pathogenic organisms in PABA-free medium has not yet been demonstrated unequivocally. Uptil now, PABA has been demonstrated in various biological systems including yeast, but not in the pathogens. Another very disappointing fact is that inspite of synthesis of so many inhibitors of essential metabolites or growth factors, none as yet has proved effective in clinical medicine.

In a recent note, Alichandani and Sreenivasan (1955) have observed that when at the maximum solubility of the drug in the medium employed the five metabolites (methionine - xanthine - serine - thymine or PGA - valine) failed, amino acid mixtures particularly those containing glycine or threonine and B-vitamins could reverse
considerably the inhibition of growth by the drug. Vitamin B<sub>12</sub> alone among the B-vitamins showed a reversal effect. It was found that a combination of vitamin B<sub>12</sub> and glycine was nearly as active as PABA. Shive (1950, 1951) had reported on the potentiating action of vitamin B<sub>12</sub> on methionine, xanthine and serine. But it is difficult to explain the relationship between the action of PABA and that of vitamin B<sub>12</sub> solely on the basis of an effect of the former on the synthesis of latter.

**BELL-ROBLIN'S THEORY**

Bell and Roblin (1942) accepting Wood-Fildes' mechanism of competition between the drug and the essential metabolite, PABA, put forward a physico-chemical theory based on the ionisation of the compounds as noted by Cowles (1942). They showed a definite correlation of acid-dissociation (pKa) with the in-vitro bacteriostatic activity of the drugs. The compounds whose pKa value is close to the physiological pH (7.2) are likely to be the most effective. Others deviating on either side of this value, are likely to be less effective. The basis of their theory lies on the statement: "The more negative the SO₂ group of a N<sup>1</sup>-substituted sulphonamide the greater the bacteriostatic power". They have argued that to exert effective competition between the drug and the essential metabolite, the physical and electrokinetic properties of their ions
ought to be similar. Considering the steric angle of the valences of S, O, and N atoms to be equivalent, they demonstrated by geometric means the close relationship between the p-aminobenzoate ion and a sulphanilamide derivative.

By measuring the acid-dissociation of over a hundred compounds of the sulphonamide class, Bell and Roblin noted a remarkable correlation of activity with acid dissociation. By plotting these data, a curve was obtained which showed the highest activity with sulphathiazole. One very significant inference of these workers is that the active compounds in the sulphonamide series have already been evolved and further possibilities in this line are limited. This theory however cannot explain the activity of some compounds like sulphaguanidine, sulphanilylbenzamide and sulphanilyl-3:4-dimethyl-benzamide(Irgafen).
SPECIFICITY OF SULPHONAMIDE COMPOUNDS

There is some difference of opinion as to whether the sulpha drugs possess any specificity of action towards the different pathogens. There is considerable evidence to support the argument that such specificity is non-existent. There is individual variation in potency no doubt, but the relation between the potencies of different compounds appears to be the same, irrespective of the organisms tested (Northey, 1949). But many clinicians maintain that in-vitro results are not valid evidence for establishing the point, and that other pharmacological and clinical behaviour are factors which should determine the specificity of action in-vivo.

Marshall et al. (1942) after a study of thirty-three sulphonamide derivatives by the drug-diet method in mice, observed that the activity of all the compounds against β-haemolytic streptococci (C203 strain) was similar to sulphadimidine when judged on the basis of median survival blood concentration. But against pneumococci they observed a much greater activity in the case of N1-heterocyclic derivatives. On this basis they argued that there was definite specificity in the action of sulphonamide compounds against various infections of mice. The findings of Laiiger, Suter and Martin (1944) with N1-(3,4, dimethyl benzoyl) sulphanilamide, N1-(4-methylbenzoyl) sulphanilamide, and N1-Iso-valeryl-sulphanilamide also suggest such specificity of action against different organisms.
B. MODE OF ACTION FROM PHARMACOLOGICAL ASPECT

The difficulty of explaining all the observations in relation to sulphonamide action on the basis of a single unitary theory would therefore be apparent from what has been discussed above. Though a theory based on clear-cut biochemical inter-relationship between the drug, bacteria and the host would be most welcome to a rational scientific mind, the fact remains that all observations cannot be so fitted in as yet (Bose, 1949). It appears that all biological phenomena still possess dark facets, which cannot be illuminated all at a time. Therefore, to study a drug, not to speak of the sulphonamides, a thorough pharmacological study from all aspects is deemed essential (Bose, 1949). As a matter of fact, many of the sulphonamides have been scrutinised from various angles, with respect to their in-vitro and in-vivo potencies against diverse pathogens, their relative toxicology, absorption and excretion, their proportion of plasma binding, distribution in tissues, and finally their clinical evaluation. Though Bell-Hoblin's inference precludes the possibility of new finds in this field, it would be no mean consolation if by patience and perseverance, and still by that well-known method of trial and error, the search in chemotherapy is kept going with the hope that fresh laurels can still be attained in this field of sulphonamides.