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Review Article

Sulfonamides, Structure-Activity Relationship, and Mode of Action

Structural Problems of the Antibacterial Action of 4-Aminobenzoic Acid (PABA) Antagonists

By JOACHIM K. SEYDEL

THIS ARTICLE attempts to review the current knowledge of sulfonamides (SNS),[1] their structure-activity relations, and the mode of action involved. The problem of the mode of action and the relationship between structure and biological and antibacterial action of SNS, which was detected in 1933, are still not completely elucidated. Therefore, it would appear necessary to discuss all important experimental results concerning these problems. However, this is impossible considering that Northey (1) (review, 1948) required 200 pages to discuss the results that were available. Hundreds of publications about the synthesis of new compounds, their chemotherapeutic activity, their biological properties, and their kinetic and enzymatic approaches have been published since then to give an insight into the mode of action. This demonstrates the importance of SNS, which have revolutionized the treatment of bacterial infections. Only some of the important facts can be discussed in this paper, which includes a subjective selection.

There are several statements of many authors concerning the problem of structure-activity rela-

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[1] In this review SNS is used as abbreviation for N^1-substituted sulfanilamides.

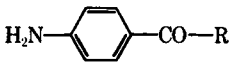
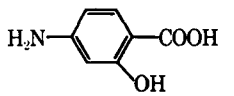
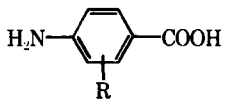
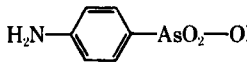
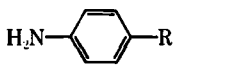
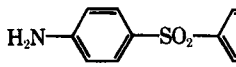
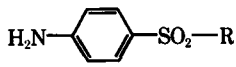
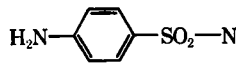
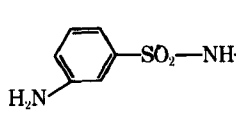
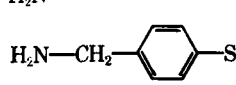
tionships of different chemotherapeutics, but among these statements, none has general validity. Definite results and answers to this question can be expected only if one restricts his attention to a very closely related group of chemical substances. SNS belong to such a group and have been studied in great detail. Setting up an extensive theory of the mode of action of chemotherapeutic agents is not only an academic problem, it should also give quantitative meaning to the chemical, physicochemical, and biological properties of the compound for the therapeutic effects. This can serve not only as a basis for the development of new drugs, but also for the evaluation of different drugs. The increasing difficulties in clinical screening tests (2) necessitate exact experimental standards.

The mode of action of chemotherapeutics, including all chemical, physicochemical, and biochemical reactions involved, can be divided into the pharmacodynamic, toxicological, and the antibacterial mode of action. The antibacterial mode of action consists of (*a*) the transport mechanism to the place of bacterial infection (pharmacokinetic) and through the bacterial membrane and (*b*) the chemical reaction which causes bacterial death or inhibition of growth. The reaction may induce sequential biochemical reactions in bacterial metabolism.

The classification of chemotherapeutics by their mode of action can be based only on the chemistry of the chemical reactions involved in the antibacterial action. As long as the chemistry of the responsible reaction is not clarified, the classification of the chemotherapeutic is doubtful. What are the criteria which decide if an antibacterial compound belongs to the group of SNS? There are substituted SNS, the substituents of which have an antibacterial activity by themselves, for instance sulfanilthiocarbamide (3), some sulfathiazoles, sulfathiadiazoles, and sulfapyridine (4–7), sulfa-3,5-dibromobenzene (8), and some sulfapyrimidine derivatives (9, 10).

If the antibacterial activity of the substituents gets the upper hand, the compound does not belong to the group of SNS with respect to the mode of action. Two criteria which are not identical can be used for classification: the

TABLE I—CLASSIFICATION OF 4-AMINOBENZOIC ACID ANTAGONISTS AND OTHER SULFONAMIDES WITH ANTIBACTERIAL ACTIVITY

4-Aminobenzoic acid antagonists (4-substituted aniline derivatives)		4-Aminobenzoyl[a] derivatives
		4-Aminosalicylic acid[a]
		Other ring substituted[a] 4-aminobenzoic acid derivatives
		4-Aminophenylarsonic acid
		Other 4-substituted aniline derivatives, not sulfone derivatives
		Sulfanilic anilines ("sulfones," DDS derivatives)[b]
		Other sulfanilic acid derivatives (not sulfonamides)[b]
		*p*-Sulfanilamides[b,c]
Other sulfonamides with antibacterial action, which are not 4-aminobenzoic acid antagonists		*m*-Sulfanilamides[c]
		4-(Amino-methyl)[c] benzol sulfonamide (homosulfanilamide)

[a] 4-Aminobenzoic acid derivatives. [b] 4-Sulfanilic acid derivatives. [c] Benzolsulfonamides. [Reproduced with permission from *Jahresber. Borstel*, **5**, 651(1961).]

antagonism of PABA against the antibacterial activity of SNS (11, 12) and the cross-resistance against sulfanilamide (13). By testing the cross-resistance of sulfanilamide and PAS resistant strains, it is possible to obtain a differentiation of the group of PABA antagonists (Table I).

CHEMICAL STRUCTURE AS REASON FOR BIOLOGICAL ACTION

Structure Specificity of the Activity of PABA and PAS—If the antagonistic action of PABA is used as a criterion for the activity of one group of compounds, knowledge is needed of the structure specificity of the action. In the following the rules of Johnson, Green, and Pauli (14), Zetterberg (15), Hossack (16), and others—completed with some additional facts—are given.

1. The antagonistic action against SNS is afforded by the amino group in the 4 position of the carboxyl group (17, 18).

2. Exchange of the amino group for other substituents with the exception of the nitro or acetyl amino group results in ineffective compounds (18).

3. Exchange of the benzene ring for other ring systems causes loss of activity (19).

4. Monosubstitution in the 3 or 2 position of the PABA molecule by halogen, alkyl, and alkoxy substituents results either in bacteriostatic compounds (20–22) or compounds with antagonistic action against SNS. There are completely different statements about the activity of these compounds in the literature; PAS belongs in this group of compounds.

5. Variation in the carboxyl group by exchange or substitution results in compounds which may possess either antagonistic properties (local anesthetics) (23–25), antibacterial, or no activity (26–30).

6. Simultaneous variation at the amino group and substitution at the benzene ring results in inactive compounds.

As previously stated, among the PABA derivatives are compounds which may act as antagonists against SNS, others which have antibacterial properties, and others which are inactive. Some of these compounds are judged differently by different authors, using different concentrations of the drug or different strains. These results are explainable according to Ariens *et al.* (31, 32) by two independent qualities of PABA, PABA derivatives, and SNS, the so-called "affinity" (reciprocal of the dissociation constant of the compound-receptor complex), and the "intrinsic" activity (metabolic activity).

Structure Specificity of the Activity of SNS—Northey (1) developed the following rules about the structure specificity of SNS. These rules are based on a tremendous mass of literature available from several large chemical companies and have been completed by the addition of some new results. The sequence used is the same which is used for PABA and its derivatives.

1. SNS antagonized by PABA possess an amino group in the 4 position of the sulfone group.

2. Exchange of the amino group by H—, HO—, RO—, HOOC—, $H_2N—SO_2—$, alkyl, or halogen substituents causes loss of activity or results in compounds which are not antagonized by PABA (homosulfanilamide). N^4-derivatives are active if the free amino group is obtainable either hydrolytically or enzymatically.

3. Exchange of the benzene ring for other ring systems decreases the activity of SNS.

4. SNS substituted in the benzene ring are mostly inactive; however, there are exceptions (33, 34).

5. Exchange of the SO_2NH group for NH_2—, —CN, $—SO_3H$ (see, however, 35), $—AsO_3H_2$, —NH—CO—CH_3, or $—NO_2$ results in inactive compounds. The activity maintains—in some cases, however, it decreases—if the sulfonamide group is exchanged for $—SO_2H$, $—SO_2C_6H_4—R$, $—SO—C_6H_4—NH_2$, —S—S—$C_6H_4—NH_2$, —SO —SO—$C_6H_4—NH_2$, $—S—(CH_3)_2$, —SO—CH_2 —$C_6H_4—NH_2$, —CO—NH_2, $—CO—C_6H_4—R$, or $—PO(OH)_2$ (28, 36–38).

Monosubstitution in the N^1 position results in essentially more active compounds as compared to the action of sulfanilamide. The most active compounds were obtained with substituents belonging to a heterocyclic system. Acylderivatives at the N^1-nitrogen atom are active in most cases (39). The rule that disubstitution at the N^1-nitrogen decreases the activity (40, 41) corresponds to the hypothesis of Bell and Roblin (41, 42). However there are some exceptions (41, 43–45). One of the most active SNS *in vitro* is sulfanil-(3,4-dimethyl-isoxazole-5-)-acetamide (see Table II).

6. Simultaneous variation at the amino and sulfonamido group performing Assumptions 2 and 5 results in *in vivo* active compounds.

Table I shows some chemotherapeutics and their classification using the given rules. The structure specificity of the antibacterial action of SNS is not easily seen; the same holds true for the structure specificity of the antagonistic action of PABA and its derivatives. The only rule that can be accepted with certainty is that the benzene

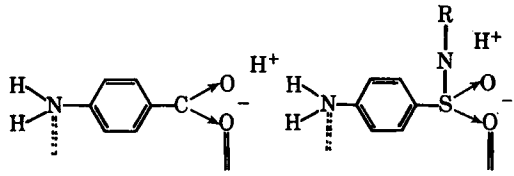
TABLE II—MINIMUM INHIBITION CONCENTRATION (MIC) OF SOME SULFONAMIDES AND THEIR N^1-ACETYL DERIVATIVES

Substance	pKa₂′	MHK *E. coli* μmole/l.
2-Sulfanilamido-pyridine	8.4	8
N^1-Acetyl-2-sulfanilamido-pyridine	—	4
2-Sulfanilamido-5-methyl-pyrimidine	6.8	1
N^1-Acetyl-2-sulfanilamido-5-methyl-pyrimidine	—	1
2-Sulfanilamido-5-ethyl-pyrimidine	6.7	1
N^1-Acetyl-2-sulfanilamido-5-ethyl-pyrimidine	—	1
2-Sulfanilamido-5-isopropyl-pyrimidine	6.7	1
N^1-Acetyl-2-sulfanilamido-5-isopropyl-pyrimidine	—	1
2-Sulfanilamido-5-methoxy-pyrimidine	6.5	2
N^1-Acetyl-2-sulfanilamido-5-methoxy-pyrimidine	—	1
2-Sulfanilamido-3-methoxy-pyrazine	6.1	2
N^1-Acetyl-2-sulfanilamido-3-methoxy-pyrazine	—	0.5
4-Sulfanilamido-2,6-dimethoxy-pyrimidine	5.9	1
N^1-Acetyl-4-sulfanilamido-2,6-dimethoxy-pyrimidine	—	1
4-Sulfanilamido-5,6-dimethoxy-pyrimidine	5.83	1
N^1-Acetyl-4-sulfanilamido-5,6-dimethoxy-pyrimidine	—	1
3-Sulfanilamido-5-methyl-isoxazole	5.7	1
N^1-Acetyl-3-sulfanilamido-5-methyl-isoxazole	—	1
5-Sulfanilamido-3,4-dimethyl-isoxazole	4.9	2
N^1-Acetyl-5-sulfanilamido-3,4-dimethyl-isoxazole	—	0.5
5-Sulfanilamido-1-phenyl-pyrazole	5.8	1
N^1-Acetyl-5-sulfanilamido-1-phenyl-pyrazole	—	0.5

[Reproduced with permission from *Arzneimittel-Forsch.*, 16, 1447(1966).]

ring and the primary aromatic amino group are essential for the biological action. The same is valid for some other PABA antagonists as for instance 4-aminobenzene-arsenic-acid (27, 46), diaminobenzil (36). Obviously the aromatic amino group in the 4 position in SNS is an important or decisive factor for the antibacterial action. Other conclusions are uncertain, for instance, the necessity of an electronegatively charged substituent (but not ionized) in the *para*-position of the amino group, and the distance of the charge from the amino group must be the same as that of the amino group in PABA (41).

There is no doubt that the aromatic amino group of PABA antagonists is involved in a chemical reaction at the site of action (35). Until now the assumption was made that sulfanilamide and PABA have the same partner in reaction. It has been reported that a twofold binding according to Scheme I takes place:



Scheme I

This picture of the geometrical similarity of PABA and sulfonamide molecules obviously was influenced by the ideas of Bell and Roblin (41), see however Alléaume (47, 48).

There is no experimental proof for such a twofold binding. It is better not to call the amino group "haptophore" according to Ehrlich (49) or "toxophile" according to Albert (50), but preferably it should be called the reactive group of the molecule. An explanation will be attempted for the different antibacterial activities of SNS by the different reactivities of their primary aromatic amino groups. A criterion for the reactivity is the "affinity constant" according to Ariens (31). The affinity constant of the amino group in a certain chemical reaction is influenced by intramolecular forces of the molecule. Therefore, it is not necessary to look for a special name for the rest of the molecule as long as the mode of action is discussed. Another question is the meaning of the rest of the molecule for transport mechanism (see protein binding, lipid partition, and ionization).

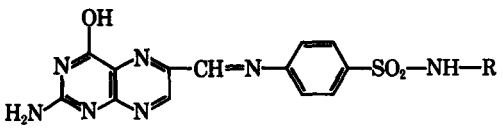
If the structure specificity of SNS is considered from this point of view it is unlikely that structural formulas written in the normal one-dimensional form can contribute to the solution of the question. Even a qualitative grouping of substituents in electropositive and electronegative properties is not sufficient (50). Physical and chemical parameters are needed which describe the condition of the amino group under the influence of certain substituents, for instance, σ-functions according to Hammett (51), rate constants according to Hirsch (52), Kohn and Harris (53), Seydel (43, 54), Brown *et al.* (55), Garrett (56), or optical data for electronic distribution from UV spectra (57–59), IR and NMR spectra (43, 44, 60–62), crystal optical measurements (47), and LCAO calculations (63–67). In considering this question, only a few of Northey's (1) critically discussed mode of action "theories" are of interest. A hypothesis may keep its value if the above-mentioned criteria about the membership of a compound to the group of SNS are considered and correct assumptions are made about the reaction mechanism.

Independent from the metabolic reactions in which SNS may be involved, the question of whether there are any physical or chemical properties of SNS which correlate with the observed antibacterial activity *in vitro* will be discussed. The problem is answered if the minimum inhibitory concentration (MIC) can be calculated by physical or chemical parameters without exception. Bell and Roblin (41) have tried to solve this problem.

CHEMICAL REACTIVITY OF THE PABA ANTAGONISTS

First, some hypotheses and experimental results concerning the reactivity of the primary aromatic amino group will be discussed. In contradiction to the statement of Bell and Roblin (41), there exists a different chemical reactivity of the primary aromatic amino group of different SNS. This can be expressed in reaction rates, pKa values, or electron-density measurements.

Tschesche (68) has developed a hypothesis that SNS are competing with PABA by forming a Schiff base with a pteridinealdehyde. The degree of displacement is a function of concentration; the involved reaction was not considered to be enzymatic but chemical, delivering the following compound:



This compound was considered to be insoluble, thus crystallizing and accumulating within the bacterial cells. In 1950 Tschesche (69) was able to show, that 2-amino-6,9-dioxy-pteridinealdehyde (8) and other pteridines can antagonize the inhibition caused by sulfathiazole. The hydrolysis of the Schiff base is very slow according to Tschesche. From experiments of Wacker, Trebst, and Simon (70) with radioactive SNS, it was not possible to make certain that a Schiff base of that type was formed. This does not mean, however, that the reaction postulated by Tschesche is not possible. It is very likely that under the conditions of the wash process used by Wacker *et al.* (70) a Schiff base could be hydrolyzed. That is even more likely because the action of SNS is considered to be of the bacteriostatic type, which means the bacteria can be liberated from the drug by washing the cells. If the hypothesis is correct, then the sequence of the reactivity of SNS with the pteridinealdehyde should be the same as the sequence of their minimum inhibitory concentration *in vitro*.

A very similar hypothesis was formulated by O'Meara, McNally, and Nelson (71), Bell, Cocker, and O'Meara (72, 73), and Forrest and Walker (74, 75) based on the assumption that SNS was reacting with reductone—an assumed precursor in pteridinealdehyde synthesis—to form a Schiff base. Forrest and Walker (75) were able to support the assumption that the Schiff base from reductone and PABA is a precursor of folic acid by synthesizing pteridine carboxylic acid from 2,4,5-triamino-6-hydroxypyrimidine and reductone. Bell (72) suggested that this reaction was not only blocking folic acid synthesis but also several other reactions where reductone was involved as metabolic compound.

According to O'Meara *et al.* (71) the chemotherapeutic activity of SNS and its susceptibility to the antagonistic action of PABA runs parallel with the stability of the Schiff base formed with glucoreductone. Only qualitative data about the stability are given including a statement that the Schiff base of sulfathiazole is about two times more stable than the Schiff base of PABA.

Bell *et al.* (73), however, obtained the opposite result. They heated a mixture of the Schiff base of sulfathiazole and reductone in sodium acetate with PABA. After 5 min. they obtained crystals which were identified as the Schiff base of PABA and reductone. This result was taken as evidence that SNS can be displaced in Schiff base by PABA in the physiological pH range and therefore would be a chemical explanation of the antagonism. However, there are some objections against this experiment. Reductones were easily oxidized, no exclusion of nitrogen was reported, and the reaction was carried out in heterogeneous mixture. Therefore, the results obtained not only include stability but also solubility constants. The solubility of the Schiff base is very low and differs with different SNS components.

Schiff base formation proceeds in a step reaction, mainly catalyzed by hydroxonium and other oxonium ions (76–78). It is a complex reaction mechanism strongly dependent on pH and changing type of mechanism as a function of pH (79). Therefore, it is more convenient to follow the rate of hydrolysis. Rate constants from such kinetic studies of the hydrolysis of Schiff bases from PABA and SNS with 3-methylreductone were obtained by Seydel *et al.* (44, 54). The sequence of rate constants is similar to the semiquantitative data of O'Meara *et al.* (71); an example is given in Fig. 1. For all studied compounds a minimum of hydrolysis was observed

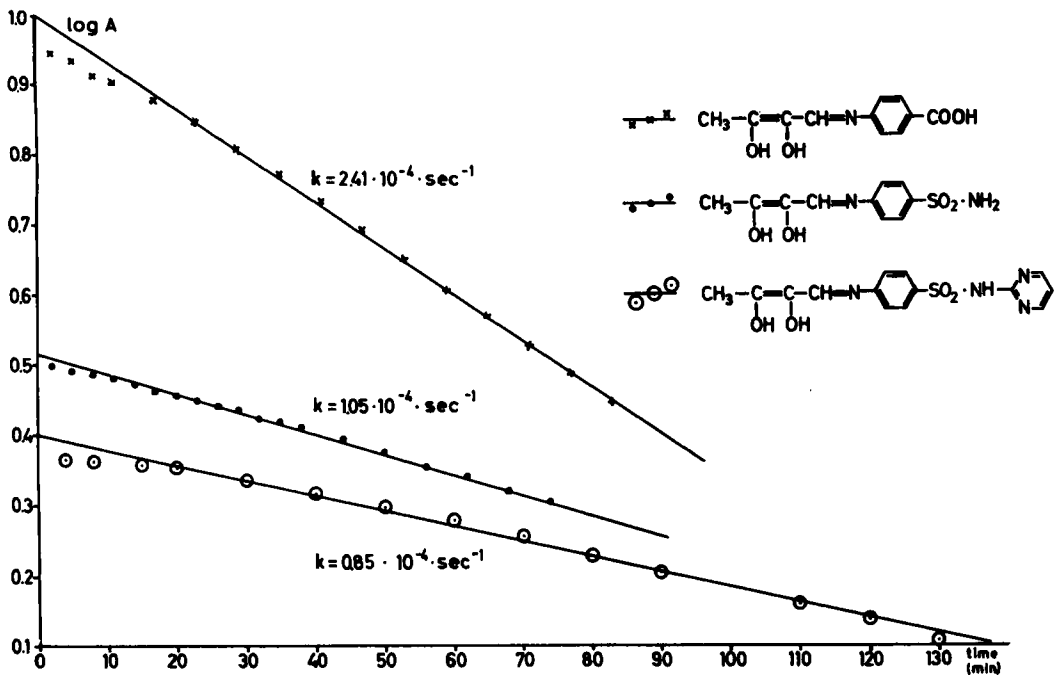


Fig. 1—Apparent first-order plots of Schiff base solvolysis of some sulfonamides, 40°, pH 6.0 phosphate buffer.

between pH 5 and 7. Most of the Schiff bases of SNS studied were more stable than the Schiff base of PABA, but this does not mean that a chemical antagonism of PABA and SNS is not possible. The PABA-reductone compound may be metabolized under *in vivo* conditions thus changing the equilibrium of hydrolysis in favor of the formation of the Schiff base. The antagonism of PABA and SNS may have a metabolic kinetic character in contradiction to the assumption of a more static equilibrium (31, 80, 81). It was not possible, however, to isolate reductone from bacteria (82), and it is known from studies of Brown (83) and Jaenicke and Chan (84) that the biosynthesis of folic acid does not include Schiff base formation, followed by hydration of the double bond. Even *in vivo*, it was not possible to hydrate the double bond of the Schiff base (85). Whatever the reliability of these results may be—with respect to the mode of action—they show that the amino groups of SNS are the reactive groups and that there is a differentiation of the reactivity by the structure of the rest of the molecule. The inductive or conjugative effect of different substituents on the N^1-nitrogen atom of SNS on the primary amino group is shown by some other parameters. Jacobson (86) has studied the effect of substrate structure on the activity of pigeon liver acetyl transferase and compared the effectiveness of several aniline derivatives as acetyl acceptors and donators. The rate of acetyl transfer was seen to decrease as the electronegativity of the *para* substituent decreased; the relative rate of the acceptors was vice versa. Among the compounds studied were sulfanilamide and PABA, with a rate of 6:1 (see Table III).

IONIZATION AND DIPOLE

Ionization of the Amino Group—Although not all of the bacteriostatic acting PABA antagonists possess an ionizing group with a negative charge (—SO_2—NH—), all of them have a primary amino group with a basic character. Tolstoouhov (87) reported that the basic pK_a' values of all active sulfonamides are in the range

Table III—Comparison of Various Derivatives as Acetyl Acceptors[a]

Acetyl Acceptor	Relative Rate
Aniline	0.70
p-Methylaniline	1.00
p-Chloroaniline	1.09
p-Bromoaniline	1.12
p-Nitroaniline	0.34
p-Aminohippuric acid	0.25
Sulfanilamide	0.18
p-Aminosalicylic acid	0.15
p-Aminobenzoic acid	0.03
Sulfanilic acid	0.01

[a] Reaction conditions were as follows: 0.1 *M* Tris pH 8.1; 0.01 *M* EDTA pH 8; 0.03 *M* cysteine HCl(neutralized); 5×10^{-5} *M* acetyl-AABS, A-60 enzyme; and 1×10^{-4} *M* acetyl acceptor amine and temperature 29°. Reaction was followed at 460 mμ. [Reproduced with permission from *J. Biol. Chem.*, **236**, 343 (1961).]

of 1.64–2.34, the most active compounds having a basic pKa_1' in the narrow range of 1.91–2.34. Most emphasis was put on the different pKa_1' values of *p*-, *m*-, and *o*-sulfanilamide (pKa_1' 2.16, 2.89, 1.80), which would explain quite simply the differences in therapeutic activity. The best correlation to activity was obtained by $pH_i = (pKa_1 + pKa_2)/2$.

The earlier results of Bell and Roblin (41), obtained with a larger number of SNS, do not confirm the results of Tolstoouhov (87). This however may be due to insufficient data. In an excellent paper Yoshioka (88) reported the basic pKa_1' values of a homologous series of N^1-arylsulfanilamides. The same compounds were used by Seydel (45, 54, 62) for structure-activity studies. Thus, it was possible to correlate the basic pKa_1' values reported by Yoshioka (88) with the minimum inhibition concentrations reported by Seydel. Figure 2 gives the relationship between those parameters. There is an approximate linear relationship between the MIC and the basic pKa_1' of the N^1-arylsulfonamides. The pKa_1' values of the homologous series of compounds were found to have a linear relationship to the σ-Hammett values of the substituents:

$$\log K_{NH_3}{}^{+} = 0.212\ (\sigma + 0.48\Delta\sigma^{-}R) - 1.815$$

The transmission of the additional resonance effect was considered to be of the cross-conjugation type rather than by a function of —NH—SO_2— like —C=C— (66, 88).

Ionization of the Sulfonamido Group—Bell and Roblin (41, 42) were the first to look for an explanation for the different activity of SNS. The theory of Bell and Roblin published in 1942 is the most important and stimulating one for investigations of structure-activity correlations of SNS. The theory was modified and differentiated by Cowles (89), Fox and Rose (90), Schmelkes *et al.* (91), Brueckner (92), and Klotz (93). The authors stated a functional connection between the acid dissociation constant (pKa_2') and the MIC of SNS. Since then this connection has not been questioned (Fig. 3).

Different hypotheses were developed to explain the observed relation between the acid pKa_2' values and the *in vitro* MIC of SNS. Attention was paid to a possible connection between pKa_2' and the MIC of SNS by the observation of the pH dependency of the inhibitory activity (94) and the antagonizing effect of PABA.

Fox and Rose (90) discovered that the ratio of the MIC of sulfanilamide, sulfathiazole, and sulfapyridine to the antagonistic concentration of PABA varied in the range of 5,000:1–8:1.

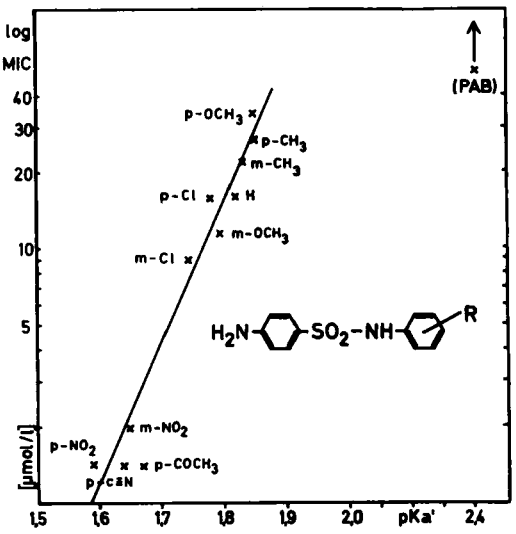


Fig. 2—Correlation between the basic dissociation constant (pKa_1) (88) of some N^1-benzenesulfonamides and their minimum inhibition concentration (MIC) against E. coli.

However, if only the ionized fraction at pH 7 was considered instead of the total concentration, the scattering of the ratio was limited to 1.4:1 up to 6.4:1. The conclusion was derived that only the ionized fraction of the MIC is responsible for the antibacterial action. Schmelkes *et al.* (91) reported that the MIC of sulfanilamide decreased with increasing pH of the culture medium, whereas the activity of sulfadiazine decreased with increasing pH. Cowles (89) considered only the negatively charged form of SNS as active and only the neutral form as able to penetrate the bacterial cell wall. Brueckner (92) differentiated this hypothesis by the assumption of different intra- and extracellular pH values. The results of his experiments only indicate that the molecular fraction of a sulfonamide solution may play an important role in determining the intracellular concentration of the active fraction and that the influence of pH must have a more complex basis than a simple change in the concentration of SNS ions in culture media. To interpret these results, knowledge is needed of the intracellular pH of the bacterial cell and its change as a function of extracellular pH.

Bell and Roblin (41, 42) explained the dependency between pKa_2' and the MIC by the assumption that "the more negative the SO_2 group of N^1-substituted sulfonamide derivatives the more bacteriostatic the compound will be." The acid dissociation constants were shown to furnish an indirect measure of the negative character of the SO_2 group. The hypothesis of Bell and Roblin stated that the unionized molecules

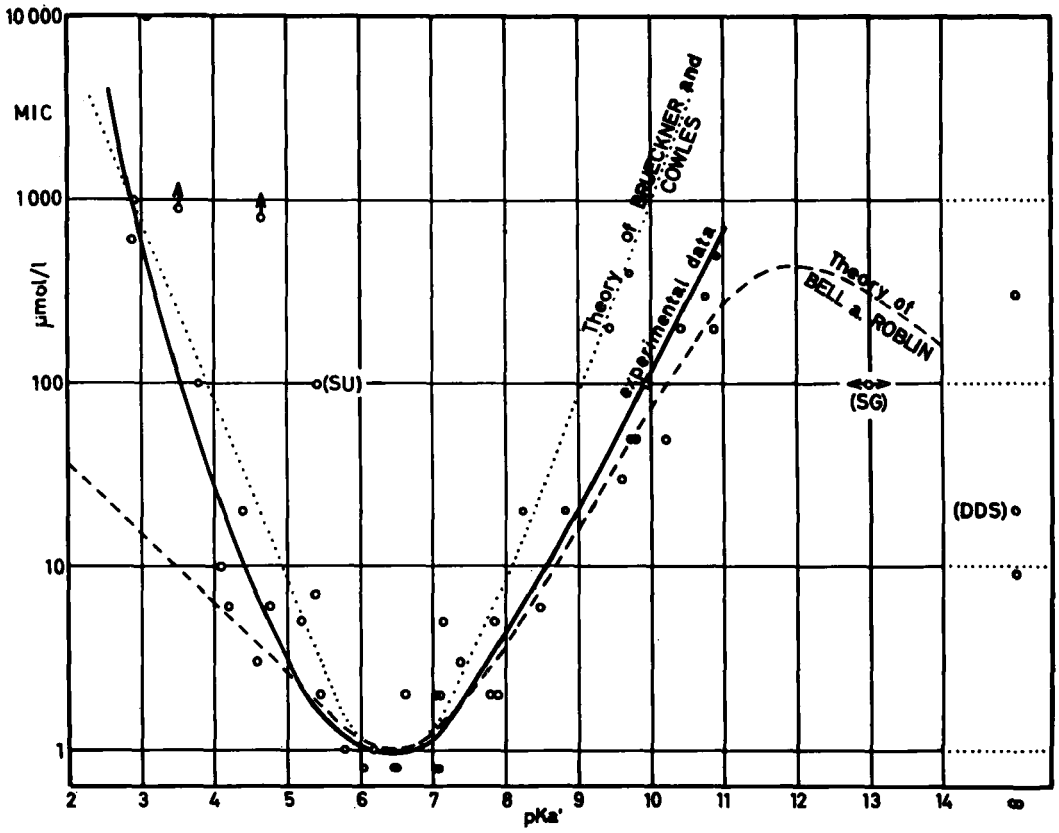


Fig. 3—Plot of the minimum inhibition concentration (MIC, µmole/l.) against the acid dissociation constant (pKa_2) of sulfonamides compared with the theoretical curves of Cowles (89) and Brueckner (92) (···), and of Bell and Roblin (41) (- - -). [Reproduced with permission from Z. Naturforsch., 15b, *628(1960).]*

had a bacteriostatic activity too, however essentially weaker than the ionized form. Further it was supposed that increasing acidity of a compound decreased the negativity of the sulfone group, thus decreasing the bacteriostatic activity of the charged and uncharged molecules. Plotting the MIC against the pKa_2' values of several sulfonamides, Bell and Roblin found a bell-shaped curve with a minimum for the MIC at pKa_2' 6.7. This relationship was used to predict the MIC of a sulfonamide by measuring its pKa_2' value (Fig. 3). There are, however, several exceptions to this hypothesis in addition to the exceptions, sulfaguanidine and sulfanilurea, already stated by Bell and Roblin.

1. There are SNS with pKa_2' values as low as 5.0 or more and still as active as those with pKa values of 6–7. There are SNS with the same pKa_2' but different MIC (44) (see Fig. 4). A striking example is N^1-(3,4-dimethylbenzoyl)-sulfanilamide which has a MIC of 2–4 µmoles/1., the pKa_2' being 4.52 (4.86), and the 2,5- and 2,4-isomers which have a MIC of 22.5 µmoles/1., the pKa_2' being 4.7 [5.1] and 4.6 [5.05], respectively (1, 61). The pKa_2' values in brackets are determined according to Bell and Roblin (41),

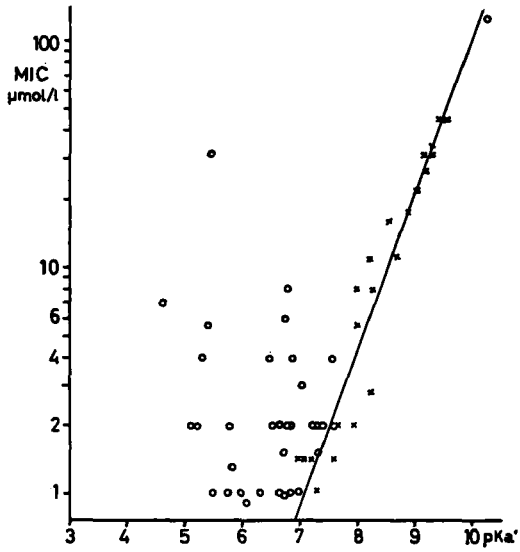


Fig. 4—Relation between the acid dissocation (pKa_2') and the in vitro activity against E. coli *of different sulfonamides* (○) *and of benzenesulfonamides* (×).

for the others a photometric titration method was applied.

It is possible to show in a very homologous series of SNS that there is a *linear* relationship of the pKa_2' and the MIC (45, 54) (see Fig. 4). Using pKa_2' values and MIC data obtained by Bell and Roblin (41), Seydel *et al.* (43), and Seydel and Wempe (61), the bell-shaped curve for the pKa_2'-MIC relation can be separated into linear functions with different slopes (positive and negative) for certain series of substituents (95) (Fig. 5). This provides little evidence in support of a definite pKa_2' which a sulfonamide must possess in order to exhibit maximum activity.

2. N^1-dimethylsulfanilamide is as active as sulfanilamide.

3. N^1-acetylated SNS are as active or even more active than the unacetylated derivatives (Table II) (45). This type of compound has no acidic dissociation constant. The polarization of the SO_2 group should be less in the case of the N^1-acetylated derivatives.

4. There are compounds with a sulfonamide mode of action which do not possess a sulfonamido group, *i.e.*, aminophenylsulfones (96), sulfanilic acid (35), and amino benzil (36), or aminophenyl ketones (37, 28).

5. Furthermore there is no experimental proof that there is a correlation between the degree of polarization of the SO_2 group and the antibacterial activity of SNS (see 32, 34, 35). Therefore it is suggested that the hypothesis of Bell and Roblin fulfills only a minimum condition for the MIC. It is possible to predict from the pKa_2' the MIC of SNS within certain limits of accuracy, but there is no proof for the mode of action theory involved.

Although the relation of ionization to the antibacterial activity and to the mode of action of SNS is very complex, there is no question about the importance of ionization to the physicochemical properties of SNS. The pKa_2' of SNS is directly related to the solubility, the distribution, the partition coefficient and the reabsorption in the kidneys, the permeability of membranes, and the protein binding, to mention only some of the important factors. The solubility is of interest with respect to the toxicologic (crystalluria) (97–100) and the pharmaceutical (absorption) properties of SNS. Therefore the solubilities of SNS have been studied very often (97–104); the functional dependency of pH, pKa_2', the solubility(s), and the base solubility(s_o) is given by the following equation:

$$s = s_o(1 + 10^{pH - pK_a})$$

The distribution has an important influence on the therapeutic effect of SNS; the time needed to reach equilibrium is a function of pH. Sulfathiazole, with pKa_2' of 7, established its equilibrium between blood plasma and erythrocytes *in vitro* in about 4 min., whereas sulfanilacetamide with a pKa_2' of 5.3 needs about 1 hr. (105, 106).

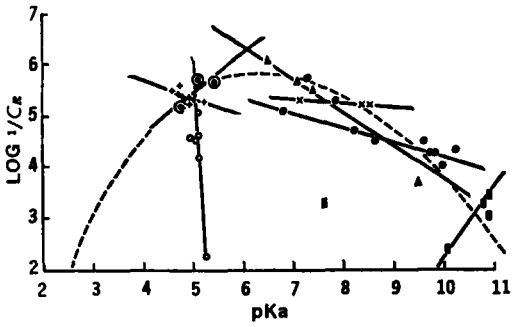


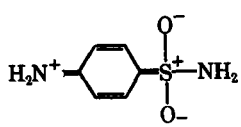
Fig. 5—Correlation between the acid dissociation (pKa_2') and the minimum inhibition concentration (MIC), Key: ⊙, sulfanilamido (substituted) thiazoles, +meta and/or parasubstituted N^1-benzoylsulfanilamides, ○, orthosubstituted N^1-benzoylsulfanilamides; ▲, 2-sulfanilamido (substituted) pyrimidines; ×, 2-sulfanilamido (substituted) pyridines; ●, N^1-(substituted) phenylsulfanilamides; ■ N^1-(substituted) methylsulfanilamides [Reproduced with permission from J. Pharm. Sci., **56**, *640(1967).]*

The partition coefficient plays an important role in the reabsorption of SNS in the kidneys (107). Dependency of the partition coefficient (Q) on the pKa_2' is given by the following equation:

$$Q = \frac{m_o - m_1}{m_1}(1 + 10^{pH - pK_a})$$

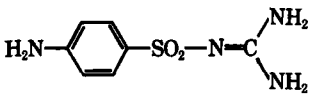
In a detailed study Dettli *et al.* (108) and Portnoff *et al.* (109) were able to demonstrate the change of the excretion rate of SNS by acidifying or alkalizing the urine. The importance of the dissociation constant for the transport of chemotherapeutic agents through membranes is known from several studies (92, 110) and is discussed for SNS by Brueckner (92), and Brown (35), and many other authors.

Dipole Moment of SNS—Kumler and Halverstadt (111) published a paper about the dipole moment of sulfanilamide and related compounds. They stated that there is a contribution of about 3% of the resonance structure in sulfanilamide. Later, Kumler and Daniels (112) and Kumler and Strait (113) proposed a theory that a coplanar amino group resulting from a resonance form

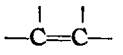


is the main factor effecting activity (see also 114,

115). According to this hypothesis 3-aminobenzene-sulfonamide is inactive because of the inability to build a resonating form; 2-aminobenzene-sulfonamide is inactive in spite of the ability to form a resonance structure, but a hydrogen bond is established between the amino group and the SO_2 group with the consequence of reduction of the active resonance form. Kumler and Daniels (112) explained the low activity of sulfanilurea and the very high activity of sulfaguanidine, which are exceptions of the theory of Bell and Roblin (41) by a resonance form of the ion in which the charge goes mainly to the oxygen rather than to the SO_2 group in case of the sulfanilurea, and by a negative charge appearing on the amide nitrogen in case of the sulfaguanidine. This increases the negativity of the SO_2 group which in turn enhances the coplanar amino resonance form of sulfaguanidine. Since publication of these papers, the resonance hypothesis has been disproved by the papers of Bell, Bone and Roblin (116), Quan *et al.* (33), Quan *et al.* (34), and by NMR measurements, showing that in the case of sulfaguanidine the following form exists (117):



From the paper of Yoshioka *et al.* (88), however, it appears very likely that there is a resonance effect of the R group through the entire molecule and that substituents with a large effect increase the activity of SNS (45). The resonance however is not performed by a SO_2 group acting like



but by cross-conjugation (88).

SPECTRAL DATA AND LCAO PARAMETERS OF SULFONAMIDES AND THEIR PRECURSOR SUBSTANCES IN SYNTHESIS

UV Optical Properties and Structure of SNS—The reactivity of a functional group in a molecule depends on the total structure of the molecule which determines the properties of the compound, for instance, the absorption spectra. UV, IR, and NMR spectra are of special interest because of the relation to the properties of the nucleus and electron density; this again is related to the reactivity of a functional group. Kimmig (57) was the first to study the UV spectra of SNS. He noted that the absorption maxima of active SNS were in the range of 33,000–40,000 cm.$^{-1}$ (300–250 mμ); with increasing activity the maxima shifted toward 33,000 cm.$^{-1}$. The UV absorption spectra of SNS have been studied by several authors (34, 58, 59, 118–123). It was not possible, however, to deduce a general rule for the relation between the position of maximum absorbance and the activity. This is based partly on the fact that the absorbance of the heterocyclic substituents is stronger and shifted toward longer wavelengths (124) in contrast to the absorbance of the important sulfanilamide moiety which is shifted to 38,000 or 40,000 cm.$^{-1}$ and is weak in most cases. The group of interest, the amino group, has no absorbance in the UV range; there is only a certain influence of the primary amino group on the benzene ring absorbance.

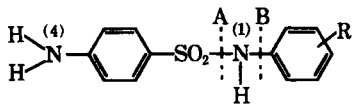
IR Data of the Sulfone Group and the Aromatic Amino Group—Therefore it is much more promising to study the IR spectra of SNS. Vibration frequencies in the infrared characterize the relation between two or three adjacent nuclei in a molecule influenced by other nuclei of the molecule. It is known from several IR-relationship studies that the stretching frequencies arising from vibrations localized in functional groups attached to an aromatic ring can be related to the electronic nature of the ring substituent constant (125).

Many IR measurements of SNS were done for assay methods of drug analysis (126, 127). Other IR measurements were performed to study the imido–amido tautomerism (128) or to see if there is any change of the electronegativity of the SO_2 group (43, 44, 60), which may be correlated to the *in vitro* activity of SNS according to the hypothesis of Bell and Roblin. IR vibrations are suitable for the study of the physical properties of the SO_2 group because of the absence of mass and coupling effects (129). There are two IR absorption frequencies of the SO_2 group, the symmetrical stretching frequency (130) which is little affected by changes in the molecule (1,128–1,665 cm.$^{-1}$ in studied SNS), and the asymmetrical frequency (131), which is in the range of 1,360–1,260 cm.$^{-1}$ in SNS. The exact position of these two frequencies is determined by the electrical character of the substituents adjacent to the sulfur atom. From the position of the IR frequencies it is possible to calculate two parameters with a descriptive physical meaning, the force constant k and the bond angle θ. For both parameters no correlation with the *in vitro* activity of the corresponding sulfonamide was obtained (43, 44, 60, 61). Some of the most active sulfonamides have force constants for the S=O bond of $9.8 \cdot 10^5$ dyne/cm., *i.e.*, the degree of polarization is small.

These data are limited by the fact that these measurements must be performed principally in

solid state. Therefore, the data include association effects. The same is true for the IR data obtained from the vibration frequencies of the primary aromatic amino group, which show some correlation to the activity of SNS (43, 44). However, data obtained from an additional sequence of SNS (61) and data obtained by Brandmüller (60) did not support these findings.

IR and NMR Data of the Aromatic Amino Group of the Precursor Substances in Synthesis (Amines)—To avoid the difficulties arising from the insolubility of SNS in nonpolar solvents, it seems better to study the substituents rather than the complete sulfa drug (45, 62, 132). For this purpose one may split the sulfonamide molecule according to the dotted line B, studying for example substituted chlorobenzene, which may be used for the synthesis of sulfa drugs. Since it is known for sulfathiazole and sulfapyridines (40, 124) and for some 4-sulfapyrimidines and sulfaphenazole (133, 134) that the substituents may be bound to the N^1-nitrogen atom by a double bond, it seems to be more appropriate to split the sulfa drug molecule according to the dotted line A, thus studying the substituted anilines.



To keep the model as simple as possible, anilines were chosen which were substituted in the *ortho*, *meta*, and *para* positions. As shown by Fig. 6 there is an approximately linear relationship between the σ-Hammett values of the substituents in the anilines and the MIC of the corresponding sulfonamides. Similar approaches for other systems have been made by Hansch (135, 136), Zahradnick (137), and Bocek *et al.* (138). It can be seen from Fig. 6 that the more positive the σ-Hammett constant of the amine the more active the corresponding sulfonamide. The IR data obtained from the stretching frequencies of the amino group of the anilines were used to calculate the force constant, the bond angle, and the "s" character of the nitrogen orbitals of the N—H bond expressed as coefficient *b*. The constant *b* is the coefficient of the 2*s* orbital of nitrogen in the hybrid (125, 139).

Plotting the *b* values of the amines against the logarithm of the MIC of the corresponding SNS, an approximately linear relationship was obtained (Fig. 7) (45, 62, 132). The aniline derivatives with *ortho* substituents deviated from the line in most cases. This behavior can be explained by intramolecular hydrogen binding; the hydrogen binding causes a larger bond angle than can be attributed only to the delocalization of the lone electron pair.

More detailed and more sensitive data for all protons of the amines were obtained from NMR measurements (54, 132). The shielding effect of electrons as a function of the substituents were easily followed in these simple molecules. Very small energy differences can be verified; association effects were excluded by dilution technique. Plotting the chemical shift of the amino group protons as a function of substituents against the log MIC of the corresponding SNS, a linear relationship was obtained (Fig. 8) (132). The chemical shift of the amino group was directly related to the pKa of the anilines.

It can be concluded that it is possible to predict the *in vitro* activity of SNS from structural parameters of the basic amines using either NMR or IR data or σ-Hammett constants for calculation;

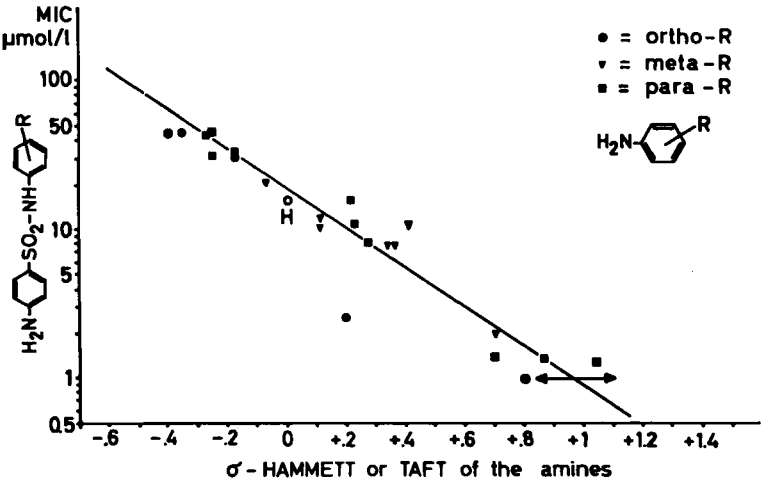


Fig. 6—Relation between the corrected σ-Hammett (210) of the anilines used for the synthesis of sulfonamides and the minimum inhibition concentration of the corresponding sulfonamides against E. coli *(µmole/l.)* [*Reproduced with permission from* Arzneimittel-Forsch., **16**, *1447 (1966).*]

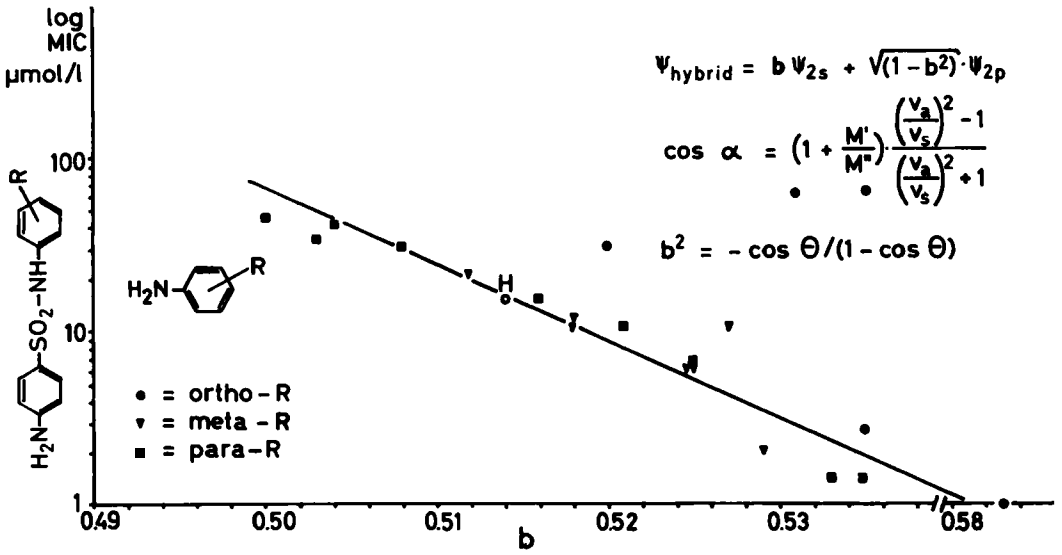


Fig. 7—Correlation between the b *values of the anilines used for the synthesis and the logarithm of the minimum inhibition concentration (MIC) of the corresponding sulfonamides.* [*Reproduced with permission from* Mol. Pharmacol., **2**, *259(1966).*]

this means one can estimate the *in vitro* activity of SNS before synthesis.

Parameters of Crystal Optical Measurements—Alléaume (47) has evaluated from crystal optical measurements the interatomic distances and bond angles of sulfanilamide, 4,4′-diaminodiphenylsulfone, *p*-aminobenzamide, and *p*-aminobenzoic acid. It was concluded by Alléaume that the data support the hypothesis that the different configuration of the primary aromatic amino group may be responsible for the antibacterial activity of SNS. According to Alléaume the configuration of the primary amino group is more of the type sp^3 than sp^2. A hyperconjugation of the substituents at the N^1-nitrogen atom is very likely thus influencing the electron distribution of the primary amino group. More substances have to be measured to come to a final conclusion. It was very clearly shown, however (Fig. 9), that the interatomic distances of the nitrogen atom of the amino group from the benzene carbon are different in PABA on one side and sulfanilamide and diaminodiphenylsulfone on the other side; this is in agreement with the observed different pKa_1' values (87, 88).

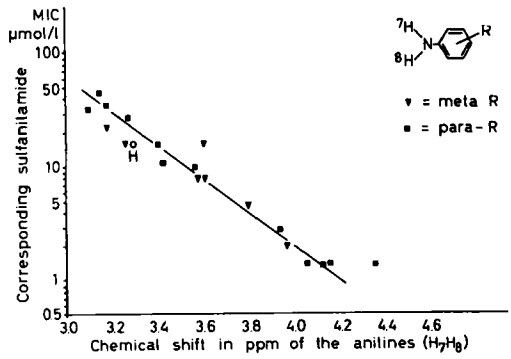


Fig. 8—Relationship between the chemical shift (p.p.m., TMS as internal standard, $CHCl_3$ solution) of the anilines ($^7H^8H$) and the minimum inhibition concentration of the corresponding sulfonamides against E. coli.

Large differences were observed for the bond angle and the interatomic distances of the carboxyl group and the sulfone group. This again makes the proposed similarity (41) of these two functional groups very unlikely.

Parameters from LCAO Calculations—Following the successful application of quantum chemistry to organic chemistry (140–142) and biochemistry (143, 144), greater interest in this method has also been shown in drug research. First calculations have been done for sulfonamide molecules (63–67) and for the sulfone group (66, 145). The data of Schnaare and Martin (63) and Foernzler and Martin (64) show some degree of correlation between the acidic dissociation constant and the electronic charge at the N^1-nitrogen atom. The linear relation which was expected, however, was not obtained. The electronic charge calculated for the oxygen atom of the sulfone group was nearly constant for about 50 different sulfonamides; the oxygen of the sulfone group of the unsubstituted sulfanilamide was more negatively charged than, for instance, the SO_2 of N^1-phenylsulfanilamide. This latter re-

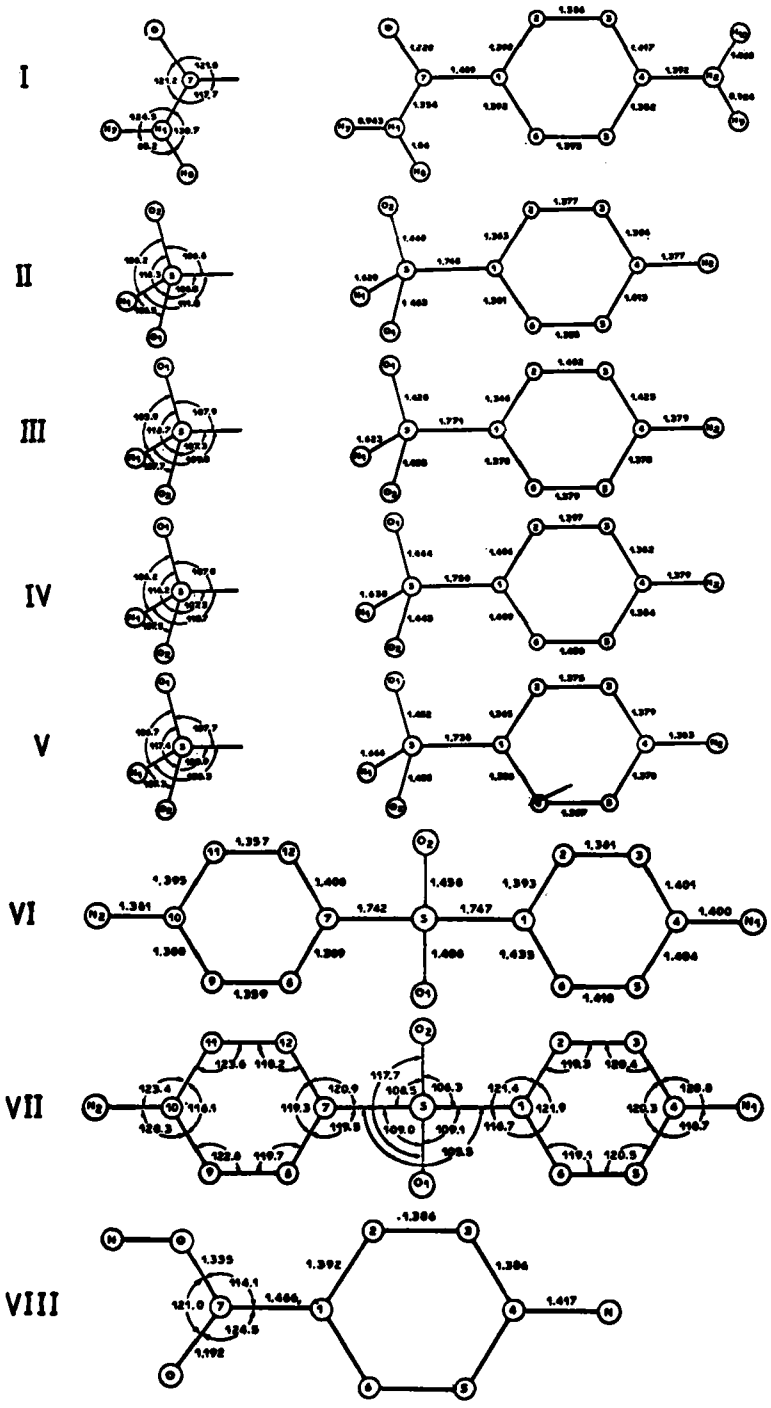


Fig. 9—Atomic distances and valence angles of 4-aminobenzamide, sulfanilamide, diaminodiphenylsulfone, and 4-amino-benzoic acid according to Alléaume (1966, 1967). Key: I, 4-aminobenzamide; II, sulfanilamide monohydrate; III, sulfanilamide α, crystallized from butanol; IV, sulfanilamide β, crystallized from methanol; V, sulfanilamide γ, crystallized from isoamyl alcohol; VI, VII diaminodiphenylsulfone; VIII, 4-aminobenzoic acid [From Alléaume, M., Doctoral dissertation, University of Bordeaux, 1967).]

sult is in contradiction to the hypothesis of Bell and Roblin.

The obtained data for the electronic charge of the primary aromatic amino group, however, show that the parameters used in these calculations are not correct, and the obtained correlation even between pKa_2' and the N^1-nitrogen atom is doubtful. As shown by the pKa_1' values of the aromatic amino group, there are different electronic charges at the N^4-nitrogen atom. If differences of 0.12 pKa units for the acid dissociation (N^1-nitrogen atom) can be described by LCAO method then it should be asked why these differences in electronic charge are not described for the N^4-nitrogen atom. One reason for these difficulties is the treatment of the SO_2 group in LCAO cal-

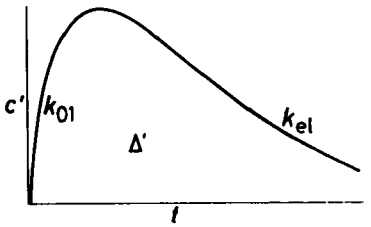
culations. Including the contribution by the sulfur *d*-orbitals in the overall conjugation, Cammarata (65) was able to show in three selected sulfonamide-type compounds that variation in charge at the primary aromatic amino group does occur.

RELATIONSHIP BETWEEN CHEMICAL STRUCTURE OF SNS AND THEIR BIOLOGICAL AND PHARMACOKINETIC PROPERTIES

Besides the *in vitro* activity many other factors are involved in the estimation of the *in vivo* activity of SNS. In spite of the prediction of Bell and Roblin in 1942 (41) that no sulfonamide with a higher activity could be expected (and this is certainly true) and in spite of the evaluation of penicillin which was able to displace SNS from the market for a short time, better SNS were developed in 1950 and 1960 and play an important role in the therapy of infections. This was possible by changing special pharmacokinetic factors, thus reducing the dosage required for therapy (Tables IV and V). These pharmacokinetic factors are mainly absorption, distribution, and elimination coefficients and protein binding, to name some of them. The functional connection of these parameters and their meaning for the dosage regimen of SNS have been studied by Bünger *et al.* (146), Dettli (147), Diller (148), Dost (149, 150), Garrett (151), Krüger-Thiemer (152–154), Nelson (155, 156), Swintosky (157), Wagner (158), and many other authors and is

TABLE IV—DEFINITIONS AND DETERMINATIONS OF THE BIOLOGICAL CONSTANTS NECESSARY FOR THE CALCULATION OF THE DOSAGE SCHEDULES OF SULFA DRUGS

No.	Biological Constants	Symbol	Unit	Experimental Methods of Determination
1	Rate constant of absorption	k'_{01}	h^{-1}	Evaluation of the curve of blood content (po.) in blood plasma of humans
2	Rate constant of elimination; time of half elimination	k'_{el} $t'_{50\%}$	h^{-1} h	
3	Coefficient of distribution relative to the blood plasma	Δ'	ml./g.	
4	Drug concentration in plasma water at half saturation binding	K''	μmole/l.	Evaluation of bound and free drug in human blood plasma by ultrafiltration, dialysis, or ultracentrifugation. Calculation by the law of mass action
5	Maximum specific binding capacity of the plasma proteins	β	μmole/g.	
6	Minimum inhibition concentration	μ	μmole/l.	Evaluation of μ against *E. coli* or other pathogen bacteria in a medium free of antagonists
7	Proportionality constant between μ and $c_{min.}$ for a therapeutical effect of 95%	σ	—	In analogy calculated from the dosage schedules of other clinically effective sulfa drugs



[Reproduced with permission from *Chemotherapia*, **10**, 61(1965).]

TABLE V—CALCULATION OF MAINTENANCE DOSE[a]

Name	Mol. Wt.	μ μmole/ l.	$c_{min.}$ $= \mu\sigma$	K (μmole/ l.)	β (μval/ g.)	A	Δ' (ml./g.)	M 1000· $c_{min.}$	Δ	Φ(τ)	$t'_{50\%}$ (h)	τ (h)	Maint. dose D (mg). calc.	used
Kelfizin	280	1.6	16	366	18.2	4.29	0.21	4.48	0.90	0.32	60	24	90	500
Sinomin, Gantanol	253	0.9	9	536	26.3	4.33	0.22	2.28	0.95	1.24	10	12	190	1000
Sulfadiazine	250	1.0	10	892	16.0	2.19	0.92	2.50	2.01	0.27	17	6	100	1000
										0.61		12	220	
Kynex	280	1.0	10	114	23.2	14.0	0.19	2.80	2.66	0.60	35	24	320	500
Sulfamerazine	264	0.9	9	72	7.4	7.33	0.36	2.38	2.64	0.25	23.5	8	110	1000
										0.92		24	410	
Durenat, Kiron	280	2.0	20	119	18.6	10.3	0.26	5.60	2.68	0.57	37	24	600	500
Sulfuno, Nuprin	267	3.4	34	348	27.0	6.36	0.25	9.08	1.59	1.10	11	12	1110	500
Madribon	310	0.8	8	11	20.5	80.7	0.17	2.48	13.7	0.49	41	24	1170	500
Sulfanilamid	172	128	1280	700	7.0	1.20	0.69	220	0.83	0.76	9	8	9700	1000

[a] $G = 70$ kg.; $w = 0.9337$; $p = 70$ g./l.; $\Delta = \left(w + \frac{\beta \cdot p}{K + \mu \cdot \sigma}\right) \cdot \Delta'$; $D = G \cdot \frac{M}{1000} \cdot c_{min.} \cdot \Delta \cdot \Phi(\tau)$; $\Phi = \left(1 - \frac{k'el}{k'_{01}}\right) \cdot \frac{[1 - \exp(-k'_{01}\tau)] \cdot [1 - \exp(-k'_{el}\tau)]}{\exp(-k'_{el}\tau) - \exp(-k_{01}'\tau)}$. [b] $A = \left(w + \frac{\beta \cdot y}{K + \mu \cdot \sigma}\right)$. [Reproduced with permission from *Chemotherapia*, **10**, 61(1965).]

given by the following equation (152):

$$D = G \times \frac{M}{1000} \times \mu \times \sigma \times \left(w + \frac{\beta \times P}{K' + \mu \times \sigma}\right) \times \Delta' \times \Phi$$

Among these pharmacokinetic factors, the protein binding (binding to albumin) is of great importance. The phenomena of protein binding have been discussed several times in the literature. There is no doubt about the importance of protein binding for the antibacterial action, because of the inactivity of the bound fraction (159–165); in addition there is an influence on the pharmacokinetics (for example, excretion rate and metabolism), demonstrated especially in so-called long-acting SNS (98, 166–169). However, there is no proof for the theory of Klotz (93) that the degree of protein binding is related to the activity and, thus, to the mode of action. As shown by Davis (162), Klotz (170), Goldstein (171), Krüger-Thiemer (98, 152), Nakagaki *et al.* (172), and others, protein binding of drugs may be described with sufficient accuracy by the law of mass action, which is sometimes identified with the adsorbance isotherm of Langmuir (173). The law of mass action for protein binding may be written as follows:

$$c_1' = c_1 \left(w \cdot \frac{\beta \cdot p}{K_1 \cdot c_1}\right)$$

where c_1, drug concentration in plasma water; c_1', drug concn. in water; K_1, apparent dissociation constant of drug–protein complex · β_1, specific binding capacity of plasma proteins (μval./g.); p, protein content of blood plasma (g./l.); w, water content of blood plasma (ml./ml.).

For the achievement of the two parameters of protein binding β and K_1, several methods were developed, for example, ultrafiltration, dialysis, gel filtration (174, 175), and ultracentrifugation techniques (176). The importance of the type of binding involved, electrostatic (ionic type of binding) or hydrophobic, dipole, or van der Waal type of binding is still open to question. Scholtan (177) discussed an important influence of hydrophobic binding in highly bound SNS which possess alkyl or alkoxy groups. According to Scholtan, the reaction energy of the binding reaction is proportional to the molecular refraction of the substance. In a homologous series the protein binding increases from alkyl to alkoxy to halogen substituents (Table VI). On the other hand, papers of Klotz (170), Nakagaki *et al.* (172), Dettli and Spring (178), Krüger-Thiemer *et al.* (152, 179), and Bünger *et al.* (146) show that a stoichiometric type of binding occurs. The constant β, the specific binding capacity of plasma proteins, seems to be the same for all studied sulfonamides; the value corresponds to the ratio of two molecules of drug to one molecule of protein. Moreover, the binding affinity of SNS was estimated to be about 6 Kcal./mole (172). From this number it may be concluded that electrostatic forces are the most important factors involved, which means that the dissociation constant of SNS strongly affects the protein binding.

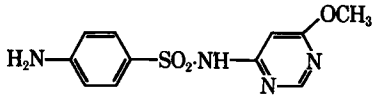
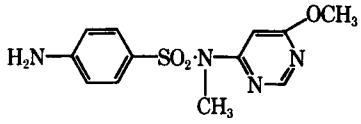
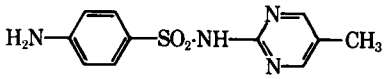
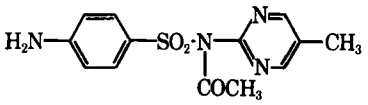
TABLE VI—BINDING CAPACITY OF HUMAN BLOOD SERUM FOR ALKOXY, ALKYL, AND HALOGEN DERIVATIVES OF 2-SULFAPYRIMIDINES AT 37°

No.	Substituent	Position	K	$K\ 10^{-3}$ l./mole	$\Delta F°$ cal./mole
1	H		0.75	1.3	4,430
2	CH_3	5	4.1	7.1	5,480
3	C_2H_5	5	9.5	16.4	6,000
4	C_3H_7 iso	5	28.8	50.0	6,700
5	C_5H_{11}	5	237	445	8,010
6	C_6H_5	5	280	483	8,070
7	OCH_3	5	5.0	8.65	5,640
8	OC_2H_5	5	19.0	33.0	6,400
9	OC_3H_7	5	60.0	104.0	7,100
10	OC_3H_7 iso	5	14.5	25.1	6,240
11	OC_4H_9	5	120	208.0	7,550
12	Cl	5	17	29.4	6,370
13	Br	5	34	59.0	6,770
14	J	5	118	205.0	7,550

[Reproduced with permission from *Arzneimittel-Forsch.*, 14, 348(1964).]

Many studies on structure-protein affinity relations have been conducted (168, 177, 180). The substances used, however, did not belong to a homologous series of compounds or if they did, only alkyl or alkoxy groups with different lengths of the hydrocarbon chain were studied and compared. The influence of the ionization was studied by Seydel (45, 132, 181) using N^1-methylated or N^1-acetylated SNS. In both types of compounds the protein binding was strongly decreased (Table VII). The accuracy of the protein-binding determination in the case of the N^1-acetyl derivative was limited because of the instability of the compound. In a very closely related series of compounds the influence of the type of substituent and of the position of the substituent was studied (45, 132, 181). The influence of different types of substituents was clearly demonstrated (Table VIII). In addition there was a strong influence of the position of the substituent. Obviously the *ortho* substitution resulted in the lowest protein binding among the isomers. This may be explained, at least in part, by a steric hinderance of the N^1-nitrogen atom, which is involved in protein binding by electrostatic forces. The strong overlapping of a methoxy group can be demonstrated by a stereo model. However, this phenomenon is not limited to the benzene sulfonamides. The influence of the position of the substituent on protein binding can be demonstrated also with two pairs of isomeric heterocyclic SNS which are on the market (Table IX). It is difficult to explain these results ac-

TABLE VII—PROTEIN BINDING OF SULFONAMIDES AND THEIR N^1-METHYLATED OR N^1-ACETYLATED DERIVATIVES

Substance	M.p., °C.	MIC [μmole/l.] *E. coli*	Protein Binding Free SNS	
			%	C_o [μmole/l.]
	210–212	0.5	10.5	72
	120–124	≥128	73	106
	274–276	1	11	180
	206	1	~42	56

cording to Scholtan (177) by hydrophobic binding. In addition the data do not support the studies of specific molecular interactions of SNS and protein, shown by NMR relaxation measurements published by Jardetzky (182). Jardetzky was using the broadening of the signals originating from the *p*-aminophenyl moiety in his studies, whereas the lines originating from the substituent ring hydrogens are not involved. The influence of this moiety on protein binding, however, is clearly shown by the data given above. Moreover the N^1-hydrogen is very quickly exchanged and is not observable in NMR and the stated binding energy of 6 Kcal./mole (172) is not coincident with a van der Waal binding type.

Another parameter which may be important for pharmacokinetic properties of the molecule is the partition coefficient, the lipid solubility. The importance of the lipid partition was shown by Koizumi *et al.* (107) and used by Krüger-Thiemer (183) in a new clearance model. Higher lipid solubility means a higher rate of reabsorption in the kidneys, thus increasing the half-life of the drug and decreasing the dosage required. A large number of experimental results obtained for different SNS were published by Rieder (184). Because of the very different substituents used in this study, it is difficult to evaluate structure relationships. In a homologous series the influence of the length of the hydrocarbon chain can be demonstrated (181) (Table X) and again there seems to be a certain influence of the position of the substituent. In a comparison of the partition coefficients of isomers, the *ortho*-substituted compound was found to have an increased lipid solubility. This might be due to steric hinderance of ionization thus increasing lipid solubility. Further studies are necessary to come to a final conclusion.

MODE OF ACTION OF SNS

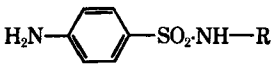
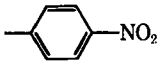
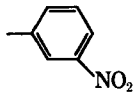
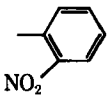
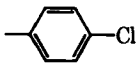
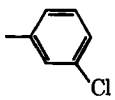
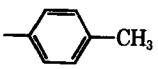
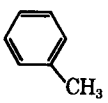
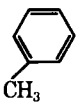
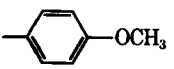
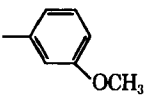
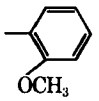
The data discussed above do not give an answer to the mode of action. The developed structure-activity correlations do not include, for instance, that the N^1-nitrogen atom is the reactive center of SNS. From the data it may be derived that there are correlations between the physicochemical properties of the N^1-nitrogen atom and the reactive center, which is very likely the primary aromatic amino group.

In the following the discussion will be restricted to the action of SNS on bacterial strains which are inhibited by SNS and the inhibition of which can be antagonized by PABA or by *p*-aminobenzoyl glutamic acid (82).

What is known about the antibacterial action of SNS besides the structural properties necessary for action?

1. The inhibition by SNS is bacteriostatic, which is not the case for several other drugs like penicillin or isoniazid. This was shown by experiments of Muir *et al.* (185) and Hirsch (52) using a Warburg equipment. Using concentrations in the range of the minimum inhibitory concentration, it was shown in a kinetic approach that there is no death of bacterial cells. It was also shown that the antagonistic effect of PABA is reached in the range of the same molarity as the concentration of sulfathiazole applied for inhibition. The bacteriostatic effect of sulfa drugs can only be obtained in growing cultures. Other studies of a kinetic nature to obtain growth

TABLE VIII—CORRELATION BETWEEN TYPE AND POSITION OF THE SUBSTITUENT AND THE pKa_2', R_f VALUE, AND PROTEIN BINDING OF SOME SUBSTITUTED BENZENE SULFANILAMIDES

	pKa'	R_f Value $CHCl_3/CH_3OH$ 80:15 Silica Gel GF 254	% Unbound[a] $(c.100)/c'$
	6.97	0.56	14.5
	7.67	0.52	20.4
	7.20	0.67	18.3
	8.56	0.58	8.1
	8.28	0.55	12.5
	8.29	0.71	18.6
	9.25	0.54	15.6
	9.05	0.52	20.4
	9.34	0.62	25.2
	9.34	0.61	22.9
	8.72	0.58	25.7
	9.43	0.65	32.3

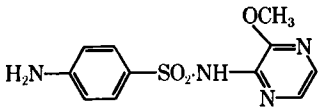
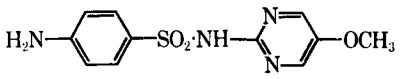
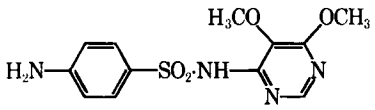
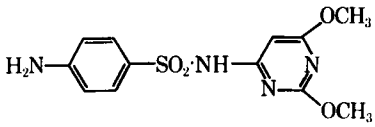
[a] Range of concentration used was 100–400 μmoles/l. Data given are averaged values from 4 runs.

rates and to establish the influence of sulfa drugs on the growth rate were done by Kohn and Harris (53, 186). A more sophisticated approach is that of Garrett *et al.* (56), using the Coulter-counting technique for studying the change of growth rates by changing sulfa drug concentration and reversibility of drug action by dilution technique or addition of PABA.

2. Inhibition of growth by sulfa drugs does not occur immediately, contrary to the activity of many other drugs. Evidence was obtained by respiration curves (52, 53, 186, 187), and a lag phase of about 1–2 hr. was observed. More accurate data were obtained by Garrett (56), who was able to show that this lag phase is 3.7 ± 0.3 generation times. The same lag time was obtained for the different SNS and was interpreted as being the result of the depletion of stored PABA metabolites (56). After a sequential addition of SNS, however, there is again a lag phase until the new steady state is established.

3. The antagonism between sulfa drugs and PABA has been shown by several authors. The antagonistic action of PABA took place imme-

TABLE IX—CORRELATION BETWEEN POSITION OF THE SUBSTITUENT AND THE pKa′ AND PROTEIN BINDING OF SOME SULFONAMIDES USED IN THERAPY

Substance		pKa	% Unbound[a] $(c.100)/c'$
	(Kelfizin)	6.1	54.5
	(Durenat)	6.54	12.7
	(Fanasil)	5.75	14.5
	(Madribon)	5.95	6.2

[a] Range of concentration used was 200–400 μmoles/l. Data given are averaged values from several runs.

diately, whereas the action of sulfa drug needed a lag phase of about 4 mitosic divisions.

In accordance with the well-established bacteriostatic nature of the mode of action of sulfa drugs, Wacker *et al.* (70) could not detect any metabolic transformation or fixation of sulfanilamide using the isotope tracer technique. Since the discovery of the competitive antagonism of PABA (11) against the antibacterial activity of the sulfa drugs, this antagonism has been interpreted by all authors in such a way that SNS displaces the PABA from its site of action at an unknown enzyme system (188) which would be necessary for the biosynthesis of the tetrahydrofolic acid (THFA). As already pointed out, Bell and Roblin (41) have taken a theoretical approach to relate the chemical structure and the strength of the antibacterial activity of SNS. The theory is based on the assumption that the sulfonamide molecule with a more polarized SO_2 group has more structural similarity to the bacterial growth factor PABA, thus fitting in a receptor on an enzyme surface. There is no proof, however, for a structure similarity of the SO_2 group and the carboxy group (see 32, 34, 35). The physicochemical part of the hypothesis of Bell and Roblin (41) was based on analogies rather than on actual measurements of properties of the SO_2 group.

Other review articles concerning the mode of action problems of SNS have been published by

TABLE X—PARTITION COEFFICIENT $[Q = (c_0 + c_1)/c_1]\ (1 + 10^{pH - pKa})$ OF SOME HOMOLOGOUS AND ISOMERIC SULFONAMIDES IN THE SYSTEM OCTANOL/WATER

Substance	C_0 μmole/l.	C_1 μmole/l.	pH − pKa	$1 + 10^{pH-pKa}$	$\frac{K}{\frac{c_0 - c_1}{c_1}}$	$\frac{Q}{K(1 + 10^{pH-pKa})}$
2-Sulfapyrimidine	190	85.5	5.1–6.4	1.05	1.22	1.28
	190	100	6.56–6.4	2.44	0.9	2.20
	357.6	170.8	5.1–6.4	1.05	1.09	1.14
	357.6	220.5	6.56–6.4	2.44	0.62	1.52
2-Sulfa-5-methyl-pyrimidine	50.7	19.2	5.1–6.8	1.02	1.64	1.67
	50.7	27.6	6.56–6.8	1.57	0.84	1.32
2-Sulfa-5-ethyl-pyrimidine	364.8	43.0	5.1–6.7	1.02	7.49	7.68
	364.8	78.0	6.56–6.7	1.72	3.68	6.34
	404.8	58.79	5.1–6.7	1.02	5.90	6.05
	404.8	87.97	6.56–6.7	1.72	3.60	6.20
	372.9	62.2	6.50–6.7	1.63	4.99	8.14
2-Sulfa-5-isopropyl-pyrimidine	484	29.4	5.1–6.6	1.03	15.46	15.9
	484	47.4	6.56–6.6	1.91	9.21	17.8
2-Sulfa-5-methoxy-pyrimidine	217.0	65.6	5.1–6.9	1.02	2.3	2.34
	217.0	100.4	6.56–6.9	1.46	1.16	1.64
2-Sulfa-3-methoxy-pyrazine	6,615	4,110	7.3–6.1	16.85	0.366	6.16
4-Sulfa-2,6-dimethoxy-pyrimidine	3,630	1,725	7.4–6.1	20.95	0.52	10.89
4-Sulfa-5,6-dimethoxy-pyrimidine	16,600	12,800	7.4–5.75	45.67	0.296	13.5

Work and Work (189), Tschesche (82), Mietzsch and Behnisch (115), Kallinich (81), Sexton (190), Wacker (191), and Albert (192). All studies in this field have been done without the knowledge of the reaction chain of the biosynthesis of THFA. Jaenicke and Chan (84) and Brown *et al.* (55, 83, 193) published experimental data which gave proof for the biosynthesis in *E. coli* along the following reaction sequence (Fig. 10). Starting from a pyrimidine derivative, probable guanosine (I) (194, 195), 2-amino-4-hydroxy-6-hydroxymethyl-7,8-dihydropteridine (II) was synthesized. In a cell-free enzyme extract of *E. coli*, to which adenosintriphosphoric acid (ATP) and Mg^{2+} ions were added, the alcohol reacted in a two-step reaction with ATP and ADP to form the corresponding monophosphoric ester (IV) and pyrophosphoric ester (V) according to Jaenicke and Chan (84) or the pyrophosphoric ester was formed enzymatically by the transfer of a pyrophosphate group from ATP to hydroxymethyldihydropteridine according to Weisman and Brown (196). The pyrophosphoric ester then combined with *p*-aminobenzoyl glutamic acid(PAG)or PABA to form dihydrofolic acid (DHFA) or dihydropteroic acid, respectively.

The same reaction took place if formylpteridine, formyltetrahydropteridine (III), or hydroxymethylpteridine was used as the substrate (55, 84); formylpteridine was many times more active

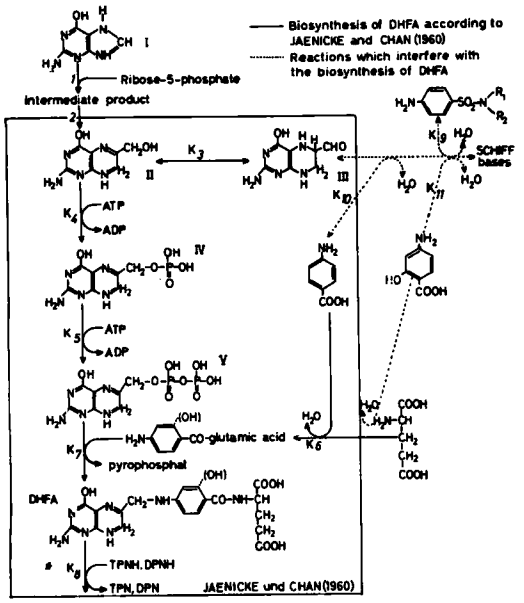


Fig. 10—Pathway for the biosynthesis of DHFA according to Jaenicke and Chan (1960) (——) and reactions which might interfere with the biosynthesis of DHFA (···). [From Jahresber. Borstel, **5,** *651 (1961).]*

than hydroxymethylpteridine (55). It may be correct to assume that a tautomeric equilibrium or a disproportion of the aldehyde and the alcohol exists in watery solutions (74, 75, 84, 193, 197). Dihydro- or tetrahydrofolic acid may act in a similar way as a biological redox system (198).

Brown (35) has used a cell-free enzyme extract in a detailed study of the inhibition of "folate synthesis" by different SNS and of the antagonistic action of PABA. The main results were:

1. The enzymatic inhibition indexes (SNS/PABA) were calculated to be 18 and 0.71 for sulfanilamide and sulfathiazole, respectively, whereas the index published in other papers from studies with bacterial cultures stated 1:1000.

2. In general the sequence of effective inhibitors of enzymatic synthesis of "folate" was the same as in growth inhibition. The exception was sulfanilic acid, which was quite effective in inhibiting folate synthesis but had no effect on growth.

3. A procedure was developed to prove whether or not the inhibition was of the competitive type. From the results it can be seen that the degree of inhibition was different depending on the addition of PABA at the same time as sulfonamide or after a preliminary incubation time. PABA (0.067 mmole) did not reverse the inhibition of folate synthesis by sulfathiazole (0.017 mmole) in the reaction mixture from which PABA was omitted in the first incubation time (2 hr.). On the other hand when PABA (0.067 mmole) and sulfathiazole (0.017 mmole) were added to the reaction mixture at the same time, little inhibition occurred. According to Brown, "the experiment described above indicated that the sulfonamide inhibition could not be described solely as a competitive type" (Table XI). The results were interpreted by Brown in such a way that the irreversible effect might be due merely to depletion of pteridine substrate.

4. Almost complete enzymatic activity was recovered by dialyzing the enzyme system which had been exposed to sulfathiazole during the first incubation period and after new addition of hydroxymethyltetrahydropteridine in the second incubation period. "The fact indicates that the enzyme was not inactivated irreversibly by reaction with sulfathiazole." (Table XI.)

These results indicate that there is not only competition on the receptor site, as suggested by Wood (12) and Bell and Roblin (41). In this case there should be no dependency on the sequence of incubation of sulfonamide and PABA.

5. In another experiment the relation of incubation time to the irreversible inhibition of

TABLE XI—IRREVERSIBLE INHIBITION BY SULFATHIAZOLE OF "FOLATE" SYNTHESIS[a]

Reaction Mixture No.	Component Omitted in First Incubation Period	Component Added Between First and Second Incubation Periods	Folate Equivalents Produced, mcg.
1	Sulfathiazole	None	5.8
2	None	None	5.2
3	Sulfathiazole, *p*-aminobenzoate	*p*-Aminobenzoate	6.4
4	*p*-Aminobenzoate	*p*-Aminobenzoate	1.1
5[a]	Sulfathiazole, *p*-aminobenzoate	All components, except enzyme and sulfathiazole	6.5
6[b]	*p*-Aminobenzoate	All components, except enzyme and sulfathiazole	5.5

[a] The complete reaction mixture contained *p*-aminobenzoate, 0.067 m*M*; hydroxymethyltetrahydropteridine, 0.033 m*M*; ATP, 3.3 m*M*, TPN, 0.067 m*M*; $MgCl_2$, 6.7 m*M*; sulfathiazole, 0.017 m*M*; phosphate buffer, 0.07 *M*; mercaptoethanol, 67 m*M*; and enzyme preparation equivalent to 6 mg. of protein; the total volume of each reaction mixture was 1.5 ml. The first incubation was for 2 hr. anaerobically at 37° in special tubes. Missing components were then added to the reaction mixtures (except for Reaction Mixtures 5 and 6; see footnote *b*) by means of a syringe equipped with a hypodermic needle in order not to disturb anaerobic conditions, and the reaction mixtures were reincubated for 2 hr. at 37°. [b] After the first incubation period, these reaction mixtures were dialyzed for 16 hr. against 2000 vol. of 0.1 *M* phosphate buffer. New substrates and cofactors were then added to the dialyzed solutions, and the reaction mixtures were incubated a second time. [Reproduced with permission from *J. Biol. Chem.*, **237**, 536(1962).]

folate synthesis by sulfathiazole was studied. It was shown that sulfathiazole needs about 2 hr. for full irreversible inhibition, which means the reaction between the sulfonamide and the pteridine derivative is relatively slow. (The concentration used is 0.017 m*M* sulfathiazole and 0.033 m*M* hydroxymethyltetrahydropteridine.) Marked inhibition occurred after approximately 30–60 min. It is interesting to note that about the same time was observed in kinetic studies of growth inhibition by SNS (52, 53, 56), whereas the antagonistic action of PABA occurred immediately.

6. The irreversible inhibition can be overcome, at least to a certain degree, by increasing the concentration of the hydroxymethyltetrahydropteridine. In the author's opinion this cannot be interpreted as a competition on the enzyme surface between PABA and sulfonamide. The antagonistic effect of pteridine substrates was also observed in growing cultures (69, 197).

7. In an isotope tracer experiment in a cell-free enzyme extract where hydroxymethyltetrahydropteridine (0.17 m*M* and sulfanilic-^{14}C acid (0.67 m*M*) were incubated, one or two radioactive compounds were obtained with different R_f values. The nature of these compounds is unknown, but suggested to be a reaction product between sulfanilic acid and the pteridine alcohol. The reaction requires Mg^{2+} ions (Table XII).

TABLE XII—PTERIDINE AND Mg^{++} REQUIREMENTS FOR IRREVERSIBLE INHIBITION BY SULFATHIAZOLE OF "FOLATE" SYNTHESIS

(Reaction mixtures were prepared and incubation procedures were followed as described in Table XI.)

Component Omitted from First and Added Before Second Incubation	Folate Equivalents Produced, mcg.
None	5.6
p-Aminobenzoate	1.2
p-Aminobenzoate, hydroxymethyltetrahydropteridine	6.0
Hydroxymethyltetrahydropteridine	5.0
p-Aminobenzoate, $MgCl_2$	5.1
$MgCl_2$	5.1

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It should be mentioned that Wacker *et al.* (70) did not succeed in isolating such a product from bacterial cultures after incubation of sulfanilamide-C^{14}, whereas they were able to isolate a folic acid analog after incubation of *p*-aminosalicylic acid (199).

Under the assumption that the pathway for the biosynthesis of tetrahydrofolic acid and coenzyme F (84) is correct and that an equilibrium between the alcohol and the aldehyde of the pteridine component exists, a hypothesis about the mode of action was developed (44), which may explain the observed phenomena of the antibacterial action of SNS and even 4-aminosalicylic acid without the assumption that PABA and SNS compete in an antagonistic reaction at the same receptor (Fig. 10). Both of the models are in agreement with the kinetically derived equations for the apparent first-order steady-state growth rate (56).

1. Sulfanilamide and other aromatic amines inhibit the biosynthesis of coenzyme F by forming Schiff bases (Fig. 10, reaction sequence *9, 10, 11*) with formyltetrahydropteridine, which is part of a direct reaction sequence (*2, 4, 5, 7*) or in equilibrium with the corresponding alcohol. The relative strength of the inhibitor compounds depends on their affinity for the aldehyde component, according to the different reactivity of the primary amino group and their possible ability to permeate into the bacterial membrane (35, 56, 89, 92). Different heats of activation obtained by Garrett (56), in a kinetic study of growth inhibition by sulfathiazole (ΔH_a = 4.3 Kcal./mole) and by sulfisoxazole (ΔH_a = 15.9 Kcal./mole) were interpreted in such a way "that the receptor site–drug interaction or active transport process have greater significance in the sulfisoxazole than in the sulfathiazole." The ΔH_a value for sulfathiazole justifies the assumption that the passive diffusion process into the bacterial cell is

rate determining. This is not confirmed, however, by the pKa_2' values being 4.9 for sulfisoxazole and 6.8 for sulfathiazole. Later there was no difference in lag phase of 3.7 generation times observed.

The rate of formation of Schiff base depends on the law of mass action. If there is sufficient concentration of sulfonamide, the equilibrium is changed in the direction of the Schiff base. Under these conditions the concentration of aldehyde component decreases and the formation of folic acid also, but it is not completely blocked. This would explain why there is no fixed minimum inhibitory concentration *in vitro*. It would also explain the fact that inhibition does not occur immediately (52, 53, 56, 187). The antagonistic action of PABA, however, does occur immediately.

2. 4-Amino benzoic acid exerts its physiological function and the antagonistic function against SNS in the reaction step *6* or *7* for 4-aminobenzoyl glutamic acid, respectively.

3. All substituted *p*-aminobenzoic acids, which do not inhibit the reaction *6* and *7* and which are incorporated in an active coenzyme F analog may act like PABA as antagonists of SNS [see *p*-aminosalicylic acid (PAS) in enterococci (199)] and the antagonistic action of PAS (200).

4. If a substituted PABA derivative is incorporated in a precursor of coenzyme F, like PABA, however, the further synthesis of the enzyme is blocked or the activity of the coenzyme F is decreased or completely absent, then this PABA derivative acts like an inhibitor of bacterial growth. The relative strength of the inhibitory effects depends on two parameters, the affinity of the compound to the other compound involved in the rate-determining step and on the metabolic activity of the coenzyme F analog (32). These compounds may act like SNS and form Schiff bases (see Statement 1).

5. The relation between PABA and the effect of compounds which act as in Statement 4 depend on the concentration used. In the range of low concentrations PABA acts as an antagonist if the affinity and the metabolic activity is stronger than that of the inhibitors. At high concentrations PABA acts like an inhibitor (see Statement 1).

6. A certain limit of concentration of coenzyme F is necessary for a normal metabolism. Concentrations which are too high or too low disturb bacterial growth.

The new assumption of this hypothesis is that SNS and PABA do not compete in the same reaction. The competitive antagonism between PABA and SNS occurs along the reaction chain *9*, *3*, *4*, *5*, *7*, and *6*, and is not a direct one. Some experimental results shall be discussed concerning this hypothesis.

The PABA antagonists can be divided into two groups, those which react like sulfanilamide, *etc.* (see Statement 1) and compounds which react like compounds mentioned under Statement 4 [*e.g.*, 4-aminosalicylic acid which can be incorporated in a coenzyme F analog (199) and salicylic acid which can be aminated in the 4 position by some bacterial strains (199)]. The division of the compounds into these two groups is supported by some bacteriological experiments (43, 44). According to these results SNS is much more active against a strain of *Mycobacterium smegmatis* than against some strains of *Mycobacterium tuberculosis* and *Mycobacterium bovis*, and the activity of 4-aminosalicylic acid is the reverse. This is in contradiction to the relatively close relation of the MIC of different sulfonamides on different bacterial strains. The relatively strong inhibitory effect of PABA against *Mycobacterium smegmatis* is striking, whereas this compound has nearly no inhibitory effect against *Mycobacterium tuberculosis*. Therefore the inhibitory effect of PABA, which was already noted by Woods (11), belongs to the sulfonamide type (see Statement 1 and Reaction *10* in Fig. 10). The inhibitory effect of PABA occurs only at much higher concentrations than those which afford antagonistic action against SNS. Therefore it could be assumed that this inhibition is caused by Schiff base formation. The experimental results support the assumption that the folic acid metabolism is different in *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*.

The 4-aminosalicylic acid (PAS) holds a special position (see Statement 4). The experimental results may be explained by the assumption that the metabolic activity of the PAS analog of coenzyme F is nearly the same as that of the natural coenzyme F, therefore, PAS may act in these bacterial strains (*E. coli*, enterococci) according to Statement 3 of the hypothesis. This explains that the antibacterial activity of SNS against *Staphylococcus* can be antagonized either by PABA or by PAS (200).

With Statement 6 of the hypothesis it may be possible to explain the results of Emmerson and Cushing (201) and Emmerson (202, 203) and Zalokar (204) about mutants of *Neurospora crassa* which either need SNS or SNS and PABA for growth. The results were interpreted as showing that the mutant was producing too much PABA, and the excess PABA was producing a

toxic compound. This process should be more easily inhibited by sulfonamide than the normal function of PABA. According to the new hypothesis it is very likely that the toxic compound is an excess of coenzyme F. It is well known that sulfonamide-resistant strains produce much more PABA (205, 206) and that other sulfonamide-resistant strains need folic acid, which is not the case in normal strains (207, 208). The antagonistic action of PABA against PAS is essentially weaker (209) and can be taken as support of the hypothesis.

The given examples, however, are not sufficient to prove the hypothesis which was developed before the data of Brown (35) were available. Nevertheless most of these data can be interpreted by this hypothesis. It should be noted that the experiments done by Brown do not correspond to the steady-state condition in growing cultures. Only if the reactions are operating in the proximity of their equilibrium is there possible validity to the assumption of an indirect competitive antagonism along the given reaction chain (see Fig. 10).

$$\frac{SB}{DHF} = \frac{SNS \cdot K_3 \cdot K_4 \cdot K_5 \cdot K_6 \cdot K_7 \cdot DHF}{PAG \cdot K_9}$$

where SB = Schiff-base; DHF = dihydrofolic acid; SNS = sulfonamide; PAG = 4-aminobenzoylglutamic acid. The final decision of whether PABA and SNS do compete in an enzymatic reaction for the same compound or whether the competitive antagonism between PABA and SNS is not direct but occurs along a reaction sequence is still open. The final conclusion will be the identification of the compound which was found by Brown (35) in his isotope tracer experiment but could not be detected by Wacker (70). This contradiction may be interpreted in favor of the second assumption. This means that the difference of the experimental findings of Wacker (70), who used growing cultures, and Brown (35), who used cell-free enzyme extracts, might be explained in such a way that the product which is formed in both experiments, was hydrolyzed by the necessary washing process used by Wacker (70). This is possible only if the metabolic compound is a Schiff base type, but not if a covalent bond exists. Under the experimental conditions used by Brown (35), a washing procedure was not necessary and the Schiff base could be detected. On the other hand it should be mentioned that the need for Mg^{2+} ions is in favor of an enzymatic reaction. The hypothesis of the mere receptor-blocking action of SNS and the importance of the polarity of the SO_2 group according to Bell and Roblin (41) is not supported by the experimental findings of the last few years. The results support the assumption that the primary aromatic amino group is a decisive factor.

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Keyphrases

Sulfonamides—structure, activity relationship
Biological action, sulfonamides—chemical structure
PAB antagonists—chemical reactivity
Dipole moment—sulfonamides
Spectral properties—sulfonamides, precursors
Mechanism of action—sulfonamides

Research Articles

Solvolysis of 5-Halouridines and Related Nucleosides

By EDWARD R. GARRETT and GERALD J. YAKATAN*

The neutral and alkaline solvolyses of uridine (UD) derivatives substituted in the 5 position with I (IUD), Br (BUD), Cl (CUD), and CH_3 (MUD) were followed spectrophotometrically. The order of reactivity was IUD > BUD >CUD > UD >>> MUD (essentially stable). Ribosylbarbituric acid (RBA) was identified as a product of solvolysis of IUD and BUD in strong alkali and is itself unstable. The halouridines also degrade to nonchromophoric compounds. The rate-pH profiles for all the halouridines are similar and can be explained by hydroxyl ion attack on both the undissociated and dissociated halouridine. Hydroxyl ion attack on the undissociated species leads to the formation of nonchromophoric products. Hydroxyl ion attack on the dissociated species forms RBA. 5-Hydroxyuridine (OHUD) and dihydropyrimidines are postulated as intermediates. The lability of the halogen substituent enhances the ease of solvolysis. The rate of alkaline solvolysis of BUD is the same as the rate of bromide ion production and shows that the reaction intermediates are highly unstable. The arabino and lyxo derivatives of 5-fluorouracil are solvolyzed faster than all of the halouridines. The Arrhenius parameters for all compounds were determined.

SEVERAL NUCLEOSIDES have been shown to be active chemotherapeutic agents in the treatment of viral diseases (1, 2). Quantitative studies on the stability of nucleosides and the effects of structure and substituents should provide insight into their possible metabolic transformations, and they are of pharmaceutical importance for the estimation of maximum stability and inhibition of the formation of toxic side products (3,

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