A Discussion of Sulfanilamide and Related Compounds

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Sulfanilamide, a drug having only two substitutions in its benzene ring, but an organic compound having potentialities many times the number of letters occurring in the word itself. Sulfanilamide, a term appearing in all medical journals of the world, a drug used by all medical and professional men. Sulfanilamide, a term found upon the lips of all doctors, a term found in the hearts of all the faithful, and a drug found as an answer for the prayers of all. Sulfanilamide, the greatest drug of the century, but yet, an unbelievable drug; a drug still feared by many and revered by others.

1. Story of Sulfanilamide

The story starts in 1904, when Ehrlich and Shiga first showed that a fatal experimental infection of mice caused by a trypanosome could be cured by a single injection of a relatively harmless acid dye. In 1911 Morgenroth and Levy found that a chemical related to quinine - ethylhydrocupreine - would cure pneumococcic septicemia in mice. However, when this remedy was tried in pneumonia in man it was found too toxic to be given in sufficient dosage for effective therapy. In 1908 Sulfanilamide was prepared by a chemist, Gelmo, working at the Royal Technical Hochschule of Vienna. A year later chemists at Elberfeld, Germany prepared azo dyes with sulfonamide groups and found that they were distinguished by greater fastness to washing and milling than the corresponding sulfonamide-free products. No attempt was made at this time to apply these compounds to the control of bacterial infections. Domagk announced in 1935 that an azo dye containing the sulfonamide group prepared by Mietzsch and Klarer would cure an otherwise fatal streptococcic infection in mice. This compound, azosulfanilamide, was originally called prontosil. Clinical reports attesting the efficacy of azosulfanilamide in patients with streptococcic infections appeared shortly before and at the same time as Domagk's announcement of his discovery. Workers at the Pasteur Institute late in 1935 suggested that azosulfanilamide was broken down in the body to form sulfanilamide, which was found to be as effective as azosulfanilamide. This important observation demonstrated that a relatively simple organic compound was effective as a chemotherapeutic agent in streptococcic infections. English investigators in 1936 confirmed, as Long and Bliss did later in America, the fact that both azosulfanilamide and sulfanilamide would cure an otherwise fatal streptococcic infection in mice. With the publication of a paper in 1936 by Colebrook and Kenny of Queen Charlotte Square Hospital in London on the results in child-bed fever, much interest was awakened in these new drugs. Sulfanilamide has been proved to be a specific in certain infectious diseases in human beings. It produces however at the same time in some cases toxic effects, some of which may endanger the patient's life. The question of the mechanism of action of these new drugs is important. If it could be solved, the search for still better drugs for treating infections would be put on a firm scientific basis. Research is needed to complete this picture.

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2. Chemistry of Sulfanilamide and its derivatives

It is most important that one understands the numbering of the radicals and carbon atoms of the parent compound sulfanilamide since this compound and the name "sulfanilamide" has been adopted officially by the A.M.A. It seems reasonable, therefore, that all new derivatives of this compound should have names, where possible, related to the parent sulfanilamide. The following is the structural formulas with the numbering system generally accepted.

\[
\text{(Sulfonamide group)}(N')
\]

\[
\text{(Amino Group)}(N^4)
\]

The following illustrate the method of naming as suggested by Crossley, et al is being used in most scientific journals.

\[
\text{N-Methylsulfanilamide} \quad 3-\text{Methylsulfanilamide}
\]

If, however, the derivatives have complex substituents a more elaborate method of naming is necessary. In such cases radical names are useful. The following radical names are suggested.

Sulfanilyl- Orthoanilyl- Methanilyl- Naphthionyl-

To illustrate a simple case using one of the above radicals let us name the structural formula.* This is an important intermediate which is called \(N^4\)-acetylsulfanilyl chloride, and is made as follows:

\[
\text{Acetanilid}
\]

Other useful radicals are

Sulfanilamido- Orthanilamido- Metanilamido-
In September of 1938 there was on record over 300 references concerning sulfanilamide and its derivatives but only about five of these summarized the work on the derivatives alone. The published work at that date, had indicated the following important generalities on the relationship if chemical structure of the derivatives of sulfanilamide and antistreptoccal activity.

1. Little or no activity was found in mononuclear compounds in which either the amino or sulfonamide groups of sulfanilamide were replaced.

2. Shifting the amino group to the meta or ortho position results in marked loss of activity.

3. A third group on the ring lowered the activity.

4. Substitution of the amino group with alkyl (CH₃CH₂) substituted allyl(CH₂=CH-CH₂) or aryl, etc., groups had less effect but in general lowered the activity.

5. Substitution of the amide nitrogen had a variable effect.

a. Aminoarylsulfonamidoarylsulfonic acids and aminoarylsulfonamidoarylcroxylic acids.

Due to the low solubility of sulfanilamide and its toxicity at effective dosage levels, new derivatives were sought of increased solubility which might also have a higher therapeutic index and more advantageous physical properties. First attempts were to combine acetylsulfanilyl chloride (see above) with various aminobenzene-sulfonic acids -

\[
\text{NH}_2 \quad \text{SO}_3 \text{H}
\]

- in aqueous solutions at pH 8-10. This reaction takes place readily and the acetyl group can then be hydrolyzed with H⁺ or OH⁻ to give a sulfanilyl derivative.

Orthanilyl and metanilyl derivatives are made by treating the corresponding nitrobenzenesulfonyl chloride - \(\text{SO}_2\text{Cl}\) - with the desired amino and, followed by reduction of \(\text{NO}_2\) the nitroamide with ammonium sulfide or in some cases iron in neutral solution. In the form of the neutral salts of sulfonic or carboxylic acids, these derivatives have high water solubility, while the free acids were, in general, only slightly soluble. Those compounds which are superior to sulfanilamide when tested on mice infected with b-hemolytic streptococci are N-sulfanylorthanilic acid (C₁₃H₁₉O₅N₂S₂O); Sodium 2,4,-bis sulfanilamidobenzene-sulfonate (C₁₈H₁₇O₇N₄S₃Na); 2,5 bis Sulfanilamido-benzenesulfonic acid (C₁₈H₁₇O₇N₄S₃H₂O) and 2-Sulfanilamido-benzoic (C₁₃H₁₂O₄N₂S) which may be illustrated structurally.
In summing up this group of derivatives it may be said that derivatives based on the above series of radicals to give highly soluble salts of sulfanilamidobenzene sulfonic acids and sulfanilamido-benzene carboxylic acids show greater therapeutic effect provided the second ring containing the carboxyl (COOH) or sulfonic group (SO₃⁻) is ortho to the amido group (SO₂NH₂). On the other hand in the parent aminobenzensulfamide the para position gives the greatest therapeutic effect.

p-Acylamidobenzensulfonalkanolamines and p-Aminobenzensulfonalkanolamines

As pointed out above, in the parent aminobenzene-sulfamide the para position gives the greatest effect. Likewise, mononuclear derivatives of sulfanilamide show the most effectiveness when the added groups are also in the para position. The mononuclear and other derivatives of this group are prepared by the condensation of the proper p-aminobenzene-sulfonyl chloride with an alkanolamine (NH₂CH₂CH₂OH), ammonia (NH₃), morpholine (NH₂CH₂-CH₂-O) or p-aminobenzensulfonalkanolamide. The general reaction may be illustrated as follows:

\[ \text{RHNCC₆H₄SO₂Cl + NH₂CH₂CHOHR} \rightarrow \text{RNHC₆H₄SO₂NH₂CH₂CHOHR (alkyl = R)} \]

The multitued of derivatives which were produced by Adams et al using the above reaction are of interest in that they all show less antistreptococcal activity than sulfanilamide but practically all were much less toxic. The antineuritic activity of many was found equivalent to sulfanilamide.

In general it was found that the acyl groups in the acylamidobenzensulfonalkanolamides were responsible for the reduction in the toxicity of the molecule. As a result a number of sulfanilamide derivatives were prepared by Adams et al in which the amino group was substituted by various complex acyl groups and the amido group by ethanol and isopropanolamine. None, however, showed activity comparable with sulfanilamide. A typical compound of this type may be illustrated as

\[ R' \text{NH} \underbrace{\text{SO₂NHR}}_R \]

\[ R = -\text{CH}_2\text{CHOHCH}_3 \]

\[ R' = \text{HOOC(CH}_2\text{CO)}_2 \text{CO}_\text{complex acyl} \]

\[ \text{C}_2\text{H}_5\text{OOC(CH}_2\text{CO)}_2 \text{CO}_\text{groups} \]

N₁-Acylsulfanilamides and N₁,N₄-Diacyl-Sulfanilamide

These derivatives have the general formula respectively of \( \text{NH₂} \underbrace{\text{SO₂NXCOR}}_R \) where \( R = \text{alkyl (1-17 carbons), alkenyl, aryl, etc.} \), and \( x = \text{hydrogen, alkyl or a cation, and R¹CONH} \underbrace{\text{SO₂NXCOR}}_R \) in which \( R¹ \) was usually a methyl group, but sometimes \( R¹ \) and \( R \) were the same. The compounds of these series of derivatives have not been investigated in detail pharmacologically. However, result on N₁-Dodecanoylsulfanilamide (dodecan = C₁₂) on rats infected with b-hemolytic strep, were encouraging and also arrested the spread of tuberculosis in cavities.
2a. Preparation of Sulfanilamide

\[
\begin{align*}
\text{HNO}_3 & \rightarrow \text{NO}_2^- \rightarrow \text{NH}_4^+ \\
\text{CH}_3\text{COOH} & \rightarrow \text{NH}_4\text{COC}_2H_5 \\
\text{H}_2\text{SO}_4 & \rightarrow \text{H}_2\text{SO}_4\text{C}_2H_5 \\
\end{align*}
\]

*acetanilide chlorosulfonic acid

analine para sulfonamide acetonilide para acetonilide para (Sulfanilamide) sulfonamide sulfonyl chloride

The nitro group is introduced by the use of concentrated nitric acid; this in turn is reduced by nascent hydrogen to form the amino group. The analine is then acetylated with acetic acid to form acetonilide. The acetonilide, in its turn, is treated with chlorosulfonic acid to form acetonilide para sulfonyl chloride. The latter product is treated with ammonia to form acetonilide para sulfonamide which upon hydrolysis forms the analine para sulfonamide.

*I followed the preparation of Adams and Johnson which starts with acetonilide. The following steps are as those above.

2b. Preparation of Sulfapyridine (2-Sulfanilamido-pyridine)*

2-(N^4-Acetyl-sulfanilamido)-pyridine. 18.8 g. (0.2mole) of 2-aminopyridine (purified by double vacuum fractionation and three recrystallizations from 1 part benzene and 2 parts hexane; melting range 57.0-58.0°); setting point 57.900° was dissolved in 70 cc. of anhydrous dioxane; 3.5 g. (0.1 mole) of N-acetylsulfanilamide chloride (recrystallized twice from toluene and once from chloroform; m.p. 148.5-149.5°) was added. The mixture was warmed to 95° and held fifteen minutes. On cooling and stirring, the oily layer crystallized. The crystals were filtered and washed with 25 cc. of dioxane, then 25 cc. of alcohol, and finally with 150 cc. of water. The dry weight was 27 g. or 92.8% based on N-acetylsulfanilamide chloride.

The crude 2-(N^4-Acetyl-sulfanilamido)-pyridine was recrystallized from 200 cc. of dioxane and 105 cc. of water with use of activated charcoal and the crystals were washed with dilute dioxane. On drying, the material partially melted and changed crystalline form indicating loss of dioxane of crystallization. After two recrystallizations from 70% alcohol, the melting range was 226.20° (first softening), 226.60° (first meniscus), 228.10° (final melting). This value was unchanged by recrystallization from 30% acetic acid, diacetone alcohol, and 70% alcohol. On the Dennis block, the material melted at 230.5° when placed on the hot bar or at 229.0° when warmed from room temperature. When a sample was heated at 230° for five minutes, then remelted, the range was 190°-227.5°, indicating thermal decomposition.

The condensation was also run in pyridine in which case equimolecular amounts of 2-aminopyridine and N-acetylsulfanilamide chloride were used. The crude product so obtained contained a yellow impurity which was difficult to remove.

(continued)
2-Sulfanilamidopyridine. The acetyl derivative was hydrolyzed by boiling 29.1 g. (0.1 mole) with 10 g. (0.25 mole) of sodium hydroxide and 150 cc. of water for two hours. The excess sodium hydroxide was neutralized to pH 11, activated charcoal was added, and the hot mixture clarified, giving a colorless solution. On acidifying to pH 6, 23.1 g. of colorless crystals was obtained equal to 92.5%.

After two recrystallizations from 70% alcohol, the compound had the properties noted above, which remained unchanged on further recrystallizations from alcohol and chlorobenzene.

(* According to Crossley, Northey and Hultquist)

Preparation of Sulfapyridine*

Acetylsulfanilyl chloride (10 g.) and 2-aminopyridine (4 g.) were dissolved in 34 cc. of acetone containing 5 cc. of pyridine. Upon standing overnight 5 g. of almost pure product separated as a white deposit. The filtrate upon dilution with water gave an additional 4 g. For analysis it was recrystallized from acetone as small white needles. Removal of the acetyl group was effected by treating 1 g. of the crude acetyl compound with 10 cc. of ethanol and 2 cc. of concd. hydrochloric acid. After refluxing for twenty minutes, the reaction mixture was diluted with water and made basic with ammonium hydroxide. It was recrystallized from ethanol; yield, 0.67 g. (75%).

(* According to Winterbottom)

2c. Preparation of 5-\(\text{N}^2\)-Acetylsulfanilamido-2-acetylaminopyridine*

Using the same procedure as above, 3.0 g. of 5-amino-2-acetylamino pyridine and 4.0 g. of acetyl sulfanilyl chloride gave 6.0 g. (94%) of crude 5-\(\text{N}^2\)-acetyl sulfanilamido-2-acetylamino pyridine. This was converted to 5-sulfanilamido-2-aminopyridine by the same procedure used below for 3-sulfanilamidoquinoline.

2d. Preparation of 3-Sulfanilamidoquinoline.*

3-Aminoquinoline (2.5 g.) and acetylsulfanilyl chloride (4.0 g.) were dissolved in 30 cc. of dry pyridine. After heating for two hours on the steam bath, the reaction mixture was poured into 400 cc. of cold water. The product separated as a white crystalline mass weighing 5.4 g. when dry. For analysis a small amount was washed successively with water and ethanol and dried at 100°. One gram of the crude acetyl compound was boiled for a half hour with 25 cc. of 12% HCl. Upon cooling and neutralizing with ammonium hydroxide, the sulfonamide was obtained; yield 0.8 g. (91%). It was recrystallized from ethanol.

(* According to Winterbottom)

2e. Disulfanilamides and Related Compounds

The disulfanilamides which according to previous statements on nomenclature should be called \(\text{N}^2\)-sulfanilylsulfanilamide may be represented as

\[
\begin{align*}
\text{NH}_2 & \quad \text{S}^\text{O}_{2}\text{NH} & \quad \text{SO}_2\text{NH}_2 & \quad \text{or} & \quad \text{(NH}_2\text{S}^\text{O}_2\text{NH})_2
\end{align*}
\]

\(\text{N}^2\)-Sulfanilyl Sulfanilamide

The disulfanilamide - \(\text{SO}_2\text{NH}\text{SO}_2\) - reacts with many bases because of their highly acidic character - form a series of neutral salts, most of which are highly water soluble and very stable towards heat.
However, if the hydrogen of the disulfonamide is replaced by alkyl groups the compounds become water and alkali insoluble and do not form salts. These compounds, however, appear to have high antistreptococcal activities and in the case of N^1-alkyl derivatives, virucidal activity as well. Thus N^1-methyldisulfanilamide (N^1-Hg << >> SO_2)N^-CH_3 and N-ethyldisulfanilamide are considered more therapeutically effective than sulfanilamide while soluble N-sodium disulfanilamide is also considered more effective. Some of the trade-marked derivatives belonging to this group are: Albucid, Uleron or Leptal-A, Disepcal-B, Diacul.

2f. Strepto-N-Polysulfanilylsulfanilamides and Related Compounds

These are nothing more than compounds which contain more than one sulfanilyl group NH_2 << >> SO_2 and arranged in a chain-like structure (Strepto) rather than being separately attached. Furthermore, they may be derivatives of aminobenzenesulfonic acids and carboxylic acids, hydroxyalkylamines, sulfonamides, and disulfonamides. Three compounds of this group which appear more effective than sulfanilamide are N^1-(2-Hydroxyethyl) - N^-Sulfanilylsulfanilamide (C_{14}H_{17}O_{5}F_{3}S_{9}); N^-Sulfanilylsulfanilamide and Strepto- N^4-Disulfanilylsulfanilamide (C_{18}H_{18}O_{6}N_{4}S_{3}) which may be illustrated:

\[
\text{NH}_2 \quad \text{SO}_2 \quad \text{NH} \quad \text{SO}_2 \quad \text{NH} \quad \text{SO}_2 \quad \text{NH}_2
\]

It should be pointed out that at the present time no general conclusions can be drawn concerning the effect of increasing the number of sulfanilyl groups. Certain of these compounds appear effective in virus diseases, but caution is expressed in assuming that the results of preliminary studies in mice are translatable to human therapy.

Derivatives of Sulfanilamide as reported by Hugo Bauer of the U.S. Public Health Service. A series of derivatives of sulfanilamide were prepared for the purpose of chemotherapeutic studies. Of the compounds synthesized of which there were nineteen recorded only two exhibited greater effective therapeutic activity in mice infected with B-hemolytic strep. These were sulfanilyl-d-nitroaniline which is two to three times as active but twice as toxic as sulfanilamide and sulfanilyl-4-aminoaniline which is two times as active and of the same toxicity as sulfanilamide. The structural formulas of these two derivatives are:

\[
\text{SO}_2 \quad \text{NH} \quad \text{NH}_2 \quad \text{NO}_2 \quad \text{SO}_2 \quad \text{NH} \quad \text{NH}_2
\]

In addition to the nineteen derivatives of sulfanilamide mentioned above, Bauer also prepared the formaldehyde sulfonate derivative of sulfanilamide. In this case the chemotherapeutic index was inferior to sulfanilamide per se.

To this group of compounds belongs, "Aldainil", a trade-marked derivative of Sulfanilamide; N^4-Acyl Derivatives (Sharpe & Dohms). These derivatives have already been discussed to a degree elsewhere when it was pointed out that the antistreptococcal activity of sulfanilamide was reduced by the introduction of an acyl group such as the formyl or acetyl (CH_3CO) group on the 4-amino nitrogen. However, according to Ellis Miller et al (Sharpe & Dohms) it was found that the N^4-n-capreyl derivative of
sulfanilamide is as active as sulfanilamide itself and much less toxic, in the protection of mice against \( \beta \)-hemolytic streptococci. The general formula of this compound is: \( N^2 \)-capreylsulfanilamide

\[
C_{11}H_{13}CONH \overset{\text{ac}}{\longrightarrow} SO_{2}NH_{2} \quad \text{ac = CH}_{3}CO (\text{acetyl or formyl}) \\
C_{6}H_{5}CO (\text{propionyl}) \\
C_{3}H_{7}CO (\text{n-Butyl}) etc.
\]

2g. Thiazole Derivatives

These compounds are sulfanilamide variants of the heterocyclic amines. They were originally developed by Fosbinder and Walter along with other compounds of a similar nature.

Sulfathiazole is chemically 2-(p-aminobenzene-sulfonamide) thiazole or better still it is \( \beta \)-sulfanilylaminothiazole. It is as effective as sulfapyridine in pneumococcal pneumonia and superior to the latter substance in staphlococcal infections. Toxicity is equal to sulfapyridine and sulfanilamide.

\[
\begin{align*}
N\text{H}_2 & \text{SO}_2 - \text{NH} \\
& \text{Sulfathiazole}
\end{align*}
\]

The other compound of interest in this group is Sulfamethylthiazole.

\[
\begin{align*}
N\text{H}_2 & \text{SO}_2 - \text{NH} \\
& \text{Sulfamethylthiazole}
\end{align*}
\]

There are other compounds in this group such as sulfaphenylthiazole but less is known about them.

2h. Azo Derivatives

Azosulfamide which is a non-proprietary name for prontosil and neoprontosil is chemically 4-sulfamide benzene-2-azo-1-hydroxy-7-acetylamine naphthalene-3; 6 disodium sulfonate. This may be illustrated as:

\[
\begin{align*}
\text{N} & = \text{N} \\
\text{SO}_2 \text{NH}_2 & \text{OH} \\
& \text{Prontosil}
\end{align*}
\]

The chemical structure of prontosil may be illustrated as:

\[
\begin{align*}
\text{NH}_2 \text{SO}_2 \overset{\text{CN = NC}}{\longrightarrow} \text{NH}_2 \\
& \text{Neoprontosil}
\end{align*}
\]

chemically, neoprontosil is Disodium sulfaminophenylazo-hydroxy-acetylamino phenyl disulfonic acid. Some of the trade-marked drugs belonging to this group of azo derivatives of Sulfanilamide are: "Azosulfamide", "Prontosil Flavum", "Streptocid Rubrum", "Streptozen".

It must be apparent to the reader of the tremendous possibilities of having varied sulfanilamide derivatives. Without a doubt the next few years will see many more derivatives of this drug on the market. Today there are only about four well-known derivatives: Sulfapyridine, Sulfathiazole, Prontosil and Neoprontosil. Sulfanil-Guanadine, recently patented, may or may not
join in the popular group. It was hoped that the simple structure of sulfanilamide per se and its derivatives would help to clarify the knowledge of the as yet unsolved problem of the correlation of chemical constitution with chemotherapeutic activity. While there can be no doubt that the sulfamide group is of importance for chemotherapeutic action, it has been found that the effect is not restricted to this radical yet on the basis of the infinite number of compounds which must be studied and have been studied up to the present time, it is not possible to generalize upon the essential nature of certain chemical groups. "All that can be said is, that given a fundamental atom or radical, substituents of a different kind produce certain changes in the chemical and physical properties which enable the resulting molecule in its entirety to exert characteristic and specific chemotherapeutic effects."

2I. Pyridine Derivatives

Sulfapyridine is probably the only compound of this group at the present time of any interest. Chemically it is 2-sulfanilylaminopyridine or 2-(paraminobenzenesulfonamido)pyridine.

\[
\text{N}^\text{H}_2 \text{C} \text{H} = \text{C} \text{SO}_2 \text{N}^\text{H} \rightarrow \text{N}^\text{H}_2 \text{C} \text{H} = \text{C} \text{Na} 
\]

(Dagenan)

replacing the "H" with the "Na" renders it 1800 times more soluble. This is called Sodium sulfapyridine monohydrate or Dagenan soluble.

To the Pyridine derivatives belong the following trademarked drugs:

- "Coccoclaste"
- "Eubasin"
- "M & B 693"
- "Pyriamid"

2J. According to Northey*

The frenzied research of the past five years has resulted in the synthesis and disclosure of about thirteen hundred new compounds derived from the parent sulfanilamide. When allied compounds and undisclosed sulfanilamide derivatives are added to these, it is probable that more than three thousand new compounds are available for chemotherapeutic study. Almost every class of sulfanilamide derivative has now been explored. Inevitably, there has been an enormous duplication in synthesis, so that often four or more groups have synthesized the same compound, independently, and within a few days or weeks of each other.

While sulfanilamide derivatives have been well explored from the chemical side, the bacteriological and pharmacological studies have been superficial and wholly inadequate. Obvious reasons for this are that pharmacologists have had a great amount of work in widening the field of usefulness of sulfanilamide and its commercial derivatives, in investigating the numerous toxic reactions, and in laying a foundation of test methods. Each new derivative calls for several weeks' work at a cost of many experimental animals before even a preliminary estimation of its therapeutic value against a single disease can be given. When this is multiplied by the number of diseases now known to be susceptible to treatment by this group of drugs, it will be appreciated that each pharmaceutical chemist should be backed by a staff of at least ten bacteriologists and pharmacologists if they are to keep pace with synthesis in this field. Unhappily the ratio is apt to be the reverse!
Much of the work done above on the derivatives of sulfanilamide is duplicated in the journal completed by E. H. Northey of the American Cyanamid Company, Bound Brook, New Jersey, in the past month of August, 1940. For completion here is the classification by Northey of the sulfanilamide derivatives:

I. Nuclear-substituted sulfanilamides.
II. N₁-Substituted sulfanilamides.
III. N₂-Substituted sulfanilamides.
IV. Nuclear,N₁-substituted sulfanilamides.
V. Nuclear,N₄-substituted sulfanilamides.
VI. N₁,N₄-Substituted sulfanilamides.
VII. Nuclear,N₁,N₄-substituted sulfanilamides.
VIII. Salts of sulfanilamide.
IX. Unclassified sulfanilamide derivatives.

Each of the above main divisions is further subdivided into the following:

a. Inorganic substituents.
b. Acyclic substituents.
c. Isocyclic substituents.
d. Heterocyclic substituents.
e. Acyl substituents.
f. Sulfonoyl substituents.
g. Anils (Schiff bases).
h. Azo derivatives.

(*Structure and Chemotherapeutic Activities of Sulfanilamide Derivatives by E. H. Northey of the Calco Chemical Division, American Cyanamid Company, Bound Brook, New Jersey)

3. Experimental Chemotherapy with Sulfanilamide and related compounds*

The acute toxicity of sulfanilamide for several species of higher animals has been adequately established. Given in single doses by mouth, it is tolerated in amounts of from 1.5 to 3 gm. per kilogram, depending on the species. Contrary to prevalent opinion, water increases the toxicity of sulfanilamide. There is a definite need for some standardization of the experimental assay of chemotherapeutic activity of these new drugs. Apart from such factors as toxicity and the rate of absorption and excretion, therapeutic effectiveness is influenced by the dosage, the route of administration, the onset and duration of therapy, the period of observation following therapy, the strain of organism, the infecting dose and virulence of the organisms, the species and number of the animals and the condition of the animals employed. Sulfanilamide has given encouraging results when used to treat the infections, streptococci and meningococci. On staphylococci infections in mice sulfanilamide has only a slight activity, but more favorable effects have been obtained with sulfanilamide, and recently with sulfapyridine.

Now to consider the relation of chemical structure to chemotherapeutic action. Since the discovery of prontosil, an impressive number of compounds have been investigated for antibacterial action. Of the numerous derivatives of sulfanilamide few possess greater activity than the parent compound against streptococci, but sulfapyridine is one illustration that derivatives can be obtained with enhanced activity against specific infections. The discovery that 4,4'-diamino diphenylsulfone and certain derivatives were many times as active against streptococci as sulfanilamide marked an important advance, since these compounds contain no sulfonamide group. The active radical common
to this entire class of compounds may be characterized N. M. Activity has been reported for dihydroxy diphenylsulfone, demonstrating that nitrogen is not in all cases necessary for activity.

That sulfur is not essential for activity has been shown in that asymmetrical arsenic derivatives analogous to the sulfones

\[
\begin{align*}
\text{NHCOCH}_3 & \quad \text{NHCOCH}_3 \\
\text{SO}_2 & \quad \text{NO}_2
\end{align*}
\]

possess antistreptococcic action. p-Nitrobenzoic acid NO\textsubscript{2}COOH has a slight action against pneumococcic and streptococcic infections in mice. These brief examples serve to illustrate the wide chemical front along which the conquest of bacterial infections may be approached. A survey of the numerous derivatives brings out the importance of an amino or nitro group in the para position in the benzene ring. Substitutions in the amino group of sulfanilamide are much more prone to diminish antibacterial activity than substitution in the sulfonamide group.

Relative large doses of sulfanilamide and repeated therapy are required to bring about a high percentage of cures in streptococcic infections in mice, with pneumococcic infections, marked variation in response to therapy occurs with different species of animals. Combination of sulfanilamide therapy with specific antiserum in pneumococcic, meningococcic and streptococcic infections in mice brings about more favorable results than those obtained with either type of therapy used alone. It has been shown that sulfur is not essential to therapeutic activity. Antibacterial properties have been demonstrated for some aromatic arsenic compounds, as well as for p-nitro benzoic acid. Following the oral administration of sulfanilamide to rabbits, a small percentage of an oxidation product of the amino group(hydroxylation) has been detected in the urine. Other investigations have suggested the possible significance of this derivative in the mechanism of action of sulfanilamide.

(*J.A.M.A. Vol 113: p 1710-14 (1919))


The method of analysis in blood and urine (as well as other body fluids) is based on deozotization of the aminobenzenesulphonamide with nitrous acid and coupling the resulting diazo compound in acid solution with dimethyl a-naphthylamine to produce a purplish red azo dye which can easily be estimated by colorimetric comparison. This reaction depends on the presence of an amino group substituted in the benzene ring and will estimate any compound to which the sulfonamide is changed in the organism and in which the amino group is intact. This color reaction is exceedingly delicate, being detectable in a solution of the sulfonamide of one part in twenty million of water.

For the determination the following reagents are required:
1. N/10 HCl, 2. NaN\textsubscript{2}O\textsubscript{2} 1% freshly prepared, 3. Ethyl Alcohol 95%, 4. Dimethyl-a-naphthylamine, 1cc-100 cc. of alcohol, 5. A standard solution of para-aminobenzenesulphonamide, 200 mg. per liter. From this solution, standard solutions containing 1.0, 0.5, and 0.2 mg. per 100 cc. can be prepared.
The following procedure is used: One volume of blood is run slowly with shaking into 9 volumes of alcohol, and the flask is stoppered and allowed to stand ten minutes or longer. The mixture is now filtered and 10 cc. of the filtrate measured into a small flask. 5 cc. of water, 2 cc. of HCl, 1 cc. of NaNOp and 1 cc. of dimethyl a-naphthylamine are successively added. The colored solution is slightly turbid but after standing five minutes can be filtered and the clear filtrate used for colorimetric comparison. An appropriate standard is prepared at the same time by adding 1 cc. of a standard solution of the sulfonamide to 9 cc. of alcohol and treating the solution as described for the blood filtrate. Color comparison is best made about 15 or 20 minutes after the reagents have been added. Since only 92% of the sulfonamide is recovered from blood by this procedure, the final result is divided by 0.92 to obtain the correct concentration of blood. Subsequently it was found that if the dimethyl-a-naphthylamine is added about 3 minutes after the NaNOp, a more intense color is obtained and the recovery is practically 100%.

The method originally used for the determination of sulfanilamide in blood and urine both in experimental work and in controlling the dosage of the drug for patients has recently encountered certain disadvantages. The use of N,N-dimethyl-1-naphthylamine (dimethyl-a-naphthylamine) as the coupling component for the diazotized sulfanilamide is not entirely satisfactory on account of the necessity of a catalyst for rapid development of color in dilute solutions, the need of a large excess of the reagent, and the necessity of a certain amount of alcohol to keep the resultant azo dye in solution. A coupling component which can be obtained in the form of a crystalline salt of reproducible composition and which gives a soluble azo dye in acid solution appeared desirable. Other components which can be used, along with their advantages, are shown in the following table:
4a. Suitability of some Coupling Components for Sulfanilamide Determination

<table>
<thead>
<tr>
<th>Compound</th>
<th>Speed</th>
<th>Color of dye</th>
<th>Influence on coupling speed</th>
<th>Sensitivity, 1 mg.</th>
<th>Ppt., 10 mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>for 0.1% sol.</td>
<td>at pH 1.3</td>
<td>0.1 mg. % sol.</td>
<td>pH 1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>1. N,N-Dimethyl-1-naphthylamine</td>
<td>*</td>
<td>Purple-red</td>
<td>*</td>
<td>**</td>
<td>***</td>
</tr>
<tr>
<td>2. N-(1-Naphthyl) glucamine</td>
<td>***</td>
<td>Purple-red</td>
<td>****</td>
<td>****</td>
<td>**** ****</td>
</tr>
<tr>
<td>3. N,N-Di-(hydroxyethyl)-1-naphthylamine</td>
<td>**</td>
<td>Violet</td>
<td>*</td>
<td>****</td>
<td>**** ****</td>
</tr>
<tr>
<td>4. Sulfonated N-ethyl-1-naphthylamine</td>
<td>*</td>
<td>Violet-red</td>
<td>*</td>
<td>*</td>
<td>***</td>
</tr>
<tr>
<td>5. N-(1-Naphthyl) ethylenedi-amino dihydrochloride</td>
<td>***</td>
<td>Purple-red</td>
<td>****</td>
<td>**** ****</td>
<td>**** ****</td>
</tr>
<tr>
<td>6. 2-Naphthylamine</td>
<td>O</td>
<td>Orange</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7. N-Ethyl-1-naphthylamine</td>
<td>***</td>
<td>Purple</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>8. N-Methyl-1-naphthylamine</td>
<td>**</td>
<td>Violet</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>9. 1-Naphthylamine</td>
<td>*</td>
<td>Violet-red</td>
<td>-</td>
<td>-</td>
<td>*</td>
</tr>
<tr>
<td>10. 1-Hydroxyethylamin-8-naphthol</td>
<td>*</td>
<td>Purple-red</td>
<td>-</td>
<td>-</td>
<td>o</td>
</tr>
<tr>
<td>11. 1-Amino-5-naphthol</td>
<td>*</td>
<td>Violet</td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>12. Phenyl J acid(N-phenyl-2-amino-5-naphthol-7-sulfonic acid)</td>
<td></td>
<td>Orange-red</td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>13. N,N-Diethyl-1-naphthylamine</td>
<td>*</td>
<td>Red</td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>14. H acid(1-amino-8-naphthol-3,6-disulfonic acid)</td>
<td>*</td>
<td>Red</td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>Compound</td>
<td>Color</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>°</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
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</tr>
<tr>
<td>15. Phenyl peri acid (phenyl- ** Violet</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>1-naphthalamine-8-sulfonic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16. Tolyl peri acid (p-tolyl- * Purple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>o</td>
</tr>
<tr>
<td>1-naphthalamine-8-sulfonic acid</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17. N-Phenyl-1-naphthyl- Purple</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>amine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

° = color no greater than blank; - = ppt. formation; * = degree of color, speed, etc.
(J. of Biological Chemistry Vol 128: p 537 (1939))
5. Determination of Sulfapyridine in the Blood and Urine

The following reagents are required: 1. A solution of trichloroacetic acid containing 15 gm. dissolved in water and diluted to 100 cc., 2. A 0.1% solution of sodium nitrite, 3. An aqueous solution of N-(1-naphthyl)ethylenediamine dihydrochloride containing 100 mg. per 100 cc., 4. A solution of saponin containing 0.5 gm. per liter, 5. 4N HCl, 6. A solution of ammonium sulfamate, containing 0.5 gm. per 100 cc., 7. A stock solution of sulfapyridine in water containing 200 mg. per liter. This solution is diluted and used to establish a calibration curve.

Procedure for Blood: 2 cc. of oxalated blood are measured into a flask and diluted with 30 cc. of saponin solution, and after 1 or 2 minutes precipitated with 8 cc. of the solution of trichloroacetic acid. The free sulfapyridine is determined in the filtrate as follows: 1 cc. of the sodium nitrite solution is added to 10 cc. of the filtrate. After 3 minutes' standing, 1 cc. of the sulfamate solution is added, and after 2 minutes' standing, 1 cc. of the solution of N-(1-naphthyl)ethylenediamine dihydrochloride is added. The unknown is compared with an appropriate standard which has been treated as above. This comparison may be made immediately and no change in color is observed for 1 hour or more. To determine the total sulfapyridine, 10 cc. of the filtrate are treated with 0.5 cc. of 4N hydrochloric acid, heated in a boiling water bath for 1 hour, cooled, and the volume adjusted to 10 cc. The subsequent procedure is as stated above for determining free sulfapyridine.

Procedure for Urine: Protein-free urine is diluted to contain about 1 to 2 mg. of sulfapyridine per 100 cc., and 50 cc. of the diluted urine plus 5 cc. of the 4N hydrochloric acid are diluted to 100 cc. 10 cc. of the product of this second dilution are treated as a blood filtrate for free sulfapyridine, and 10 cc. are heated without further addition of acid, for total sulfapyridine. If the urine contains protein, it is diluted and treated by the procedure for blood.

In the determination of sulfapyridine, the following may be mentioned. To a sample of mixed human blood, sulfapyridine was added to make about 10 mg. per 100 cc. Precipitation in a 1:4 dilution gave 80.8% recovery; in a 1:20 dilution, 93.7%; and in a 1:50 dilution, 99.4%. A number of other experiments indicate incomplete recovery (average 91%) in 1:20 dilution with 5 to 10 mg. per 100 cc. in blood, but essentially complete with values below 5 mg. per 100 cc. With a 1:50 or greater dilution, recovery is quantitative.

5a. A Few Details of Sulfapyridine

Introduction of Sulfapyridine: In the May 28, 1838, issue of the Lancet, Whitby reported that 2-sulfanilyl aminopyridine, one of the many substances synthesized by Dr. A.J. Twins and Mr. M.A. Phillips in the chemical research laboratories of May & Baker, Ltd., at Dagenham, England, was effective in experimental pneumococcic infections of mice. Whitby also published the constitutional formula of 2-sulfanilyl aminopyridine, as established by Twins and Phillips.

Chemical Description: Sulfapyridine Merck ("Dagenan") is a white crystalline powder, melting at 190.5° to 191.5°. The sulfapyridine is almost 30 times less soluble in water than sulfanilamide, but the solubilities of both substances increase rapidly with rising temperature. At 27.5°C, a saturated aqueous solution of sulfanilamide
contains 800 milligrams of sulfanilamide per 100 cc., whereas a saturated solution of sulfapyridine contains only 28 milligrams of sulfapyridine per 100 cc.

Clinical Pharmacology: Absorption - When sulfapyridine is administered orally, it is absorbed fairly rapidly from the gastro-intestinal tract, although not in such constant amounts as sulfanilamide. Within a short time after administration, sulfapyridine may be detected in the blood by means of a test in which it is transformed into a colored dye substance. This is then compared with known standards prepared from pure sulfapyridine. Fate - Aromatic compounds with a free para-amino group when absorbed undergo certain changes effected chiefly in the liver. Acetylation is one mechanism which blocks the free amino group and may render the compound more or less inert. Thus, sulfapyridine after absorption is in part acetylated.

\[
\text{CH}_3\text{CO} \text{NH} \quad \text{SO}_2 \text{NH} \quad \text{N}
\]

The acetyl derivative of sulfapyridine

Both the free compound and conjugated derivative are found in blood, body fluids, and urine. Blood Concentration - The concentration of free sulfapyridine in the blood following the oral administration of a given dose cannot be predicted with the same accuracy as is possible with sulfanilamide. With average effective therapeutic doses, the blood levels of free sulfapyridine usually range from 3 to 6 mg. per 100 cc. In general, blood levels of free sulfapyridine are lower than those obtained with similar doses of sulfanilamide. Excretion - Both the free and acetylated forms of sulfapyridine are apparently excreted almost entirely through the kidney, but excretion does not proceed so rapidly as with sulfanilamide and may be retarded in the presence of renal impairment. If confirmed, this finding, that sulfapyridine with the para-amino group free is conjugated in the body with glucuronic acid to form a very soluble compound, may prove important with relation to the formation of renal calculi. Toxicology - Discussed later with the toxic reactions of Sulfanilamide and its derivatives. Administration - The method and timing of administration, as well as the dose given, are important factors. Nausea and vomiting occurring during the first day or two of administration may disappear even though the treatment is continued. However, nausea and vomiting are major and frequent difficulties in the administration of the drug. Some patients take the 0.5 gm. tablets without difficulty. In some cases, the drug is better tolerated if the tablets are pulverized and suspended in alkalis, milk, or fruit juices. The administration of gelatin, olive oil, or some comparable demulcent substance is occasionally of help. Some tolerate the drug better when it is given before meals; others when it is given after meals. Small doses of barbital or phenobarbital may eliminate nausea.

Dosage: The most effective means of determining the dose is to make a daily estimation of the sulfapyridine in the blood. Blood concentrations ranging between 3 and 6 mg. of free drug per 100 cc. are usually considered adequate.

6. Determination of Sulfathiazole in Blood and Urine

The following reagents are required: 1. A solution of trichloroacetic acid containing 15 gm. dissolved in water and diluted
to 100 cc., 2. A 0.1% solution of sodium nitrite, 3. An aqueous solution of N-(1-naphthyl) ethylenediamine dihydrochloride containing 100 mg. per 100 cc. This solution should be kept in a dark-colored bottle. 4. A solution of saponin containing 0.5 gm. per liter, 5. 4N HCl, 6. A solution of ammonium sulfamate, containing 0.5 gm. per 100 cc., 7. A stock solution of sulfathiazole in water containing 200 mg. per liter. This solution is diluted and used to establish a calibration curve.

Procedure for Blood: 2 cc. of oxalated blood are measured into a flask and diluted with 30 cc. of saponin solution. The procedure is then carried out the same as described above for sulfapyridine. Procedure for Urine: The same as for sulfapyridine.

6a. A Few Details of Sulfathiazole

Chemical Description: Sulfathiazole is a white crystalline powder which melts at 200°C-202°C. At 26°C, sulfathiazole is soluble in water to the extent of 60 mg. in 100 cc. and in alcohol, 525 mg. per 100 cc. The saturated water solution has a pH of 6.0.

Clinical Pharmacology: After oral administration sulfathiazole is readily absorbed from the gastro-intestinal tract, but disappears from the blood very rapidly. In some cases this may be an advantage (e.g. ease of removal in the presence of toxic reactions) and in others may be a disadvantage (e.g. difficulty in maintaining an adequate blood concentration). Following oral administration of sulfathiazole, the percentage of acetyl sulfathiazole in the blood is greater than the percentage of acetyl sulfapyridine under similar circumstances. Long reports that sulfathiazole is distributed in tissues, exudates, and transudates in the same manner as sulfanilamide and sulfapyridine. However, the amount present in specific body fluids and tissues is inconstant. Penetration of the spinal fluid is poor and sulfathiazole is not recommended in meningeal infections. Sulfathiazole is excreted almost entirely through the kidney and at a rapid rate. Under similar conditions the average percentage of acetyl sulfathiazole in the urine is lower than the average percentage of sulfapyridine. Because of the rapid excretion of the drug, it is difficult to maintain an adequate blood level of sulfathiazole even with adequate administration.

Dosage: Sulfathiazole is poorly soluble and hence must be administered by the oral route. In the treatment of pneumococcic pneumonia in adults (patients over 14 years of age) the initial dose of sulfathiazole should be 4.0 gm., to be followed by 1.0 gm. every 4 hours day and night until the patient's temperature has been normal for 72 hours. Then discontinue the drug. Sulfathiazole should not be used in the therapy of any type of meningitis because the drug does not pass over readily into the spinal fluid. The drug should not be used in the treatment of minor staphylococcic infections such as localized boils and small carbuncles or in mild furunculosis.

7. A Few Details of Neoprontosil

As stated before (see page 8), Neoprontosil is disodium 4-sulfamido-phenyl-2-azo-7-acetylamino-1-hydroxynaphthalene 3, 6-disulfonate. It occurs in the form of a dark red, tasteless, odorless powder, soluble in water, but virtually insoluble in organic solvents. For peroral use, it is now available in tablets of 5 grains.
Action of Neoprontosil: The theory that the activity of Neoprontosil is due solely to the sulfanilamide liberated through reduction in the body is not universally accepted. An increasing number of investigators incline to the view that the different components of the complex Neoprontosil molecule contribute to its specific effect. As these authors state, the conclusion seems inevitable that unless one wished to attribute such satisfactory results to the small amount of sulfanilamide, it must be assumed that Neoprontosil is capable of producing other added chemotherapeutic actions in the body.

8. Toxicology of Sulfanilamide and Its Derivatives

No patient should be treated with sulfanilamide or sulfapyridine unless arrangements are made for daily attention by a physician. This is necessary because of the serious toxic effects of these drugs, which, while not frequent, are generally unpredictable in their occurrence and presumably have as their basis a peculiar idiosyncrasy.

8a. Central Nervous System Disturbances

Nausea with Vomiting - sometimes very severe, is the most constant and frequent side effect, occurs whether the drug is given orally or by parenteral injection, are much more frequent in the course of sulfapyridine therapy than with sulfanilamide; discontinuance of drugs is not necessary because the symptoms usually occur early in the treatment and may cease later.

Other disturbances in this system, including vertigo, headache, malaise, mental depression, and toxic psychosis, are often recorded. In such cases discontinuance of drugs is recommended.

Cyanosis - In sulfapyridine Cyanosis is rarely so marked or so frequent as with sulfanilamide, and the withdrawal of the drugs is not necessary.

Drug Rashes - In some cases, observation has been made that photosensitivity increases the severity of this reaction. It is suggested that patients who are receiving these drugs be removed from strong sunlight, or else receive ultraviolet-irradiation. The rashes may be accompanied by fever.

Drug Fevers - A rise in temperature without a change in the white blood cell count is an indication of drug fever, but, in some cases drug fever may also be accompanied by a rise in the white blood count. The reaction generally occurs between the fifth and ninth days of therapy and is less frequent in the course of sulfapyridine therapy.

Acidosis - This may be produced in certain individuals in sulfanilamide therapy and has not been noted in sulfapyridine. The routine, concurrent use of sodium bicarbonate generally prevents this complication.

Hepatitis - Hepatitis, accompanied by jaundice and, in a few instances, ending fatally, is one of the rarer complications of sulfanilamide therapy. In sulfapyridine, serious instances of
hepatitis have been reported. Instances of gross hematuria with and without signs of renal failure have been noted in patients receiving this drug. Calculus formation had been observed in sulfapyridine therapy, the calculi being composed chiefly of acetylsulfapyridine. It is suggested that large quantities of fluid be taken with the drug and that the urine be analyzed by the use of sodium bicarbonate.

Severe Toxic Effects - Serious reactions of granulocytosis or neutropenia and acute hemolytic anemia, could be detected only by daily blood counts and hemoglobin estimation.

A granulocytosis may progress very rapidly and is generally evidenced by a drop below the normal level in the white blood cell count, and particularly the neutrophils. It is advisable to withdraw the drugs immediately.

The acute hemolytic anemia, is not infrequent and may be fairly progressive. This could be controlled by repeated transfusions, and the withdrawal of the drugs should be resorted to only if the intravenous injection failed to control the anemia.

In patients who have a decrease in renal function the normal excretion of the drugs is impaired, and an accumulation of sulfanilamide in the blood and tissues of the patients may occur if care is not taken in regulating the dosage of the drug.

When severe oliguria or anuria occurs, Brown et al. advised the administration of hypertonic dextrose solutions by continuous intravenous drip method. From 300 to 400 cc. of 20 to 25 per cent dextrose solution should be given over a period of two to four hours on three occasions.

It has been observed that patients who have had severe toxic reaction in the course of the administration of sulfanilamide may, if the drugs are given for a second time, develop an even more severe reaction.

The saline laxatives, which caused the development of sulfanemoglobinemia has been attributed by some observers to concurrent magnesium sulfate therapy.

Sulfapyridine is essentially more toxic than sulfanilamide.

9. Mode of Action

Though much has been written about sulfanilamide and its derivatives since it was first introduced to this country a few years ago, very little information exists as to the exact means by which sulfanilamide combats infectious bacteria. In discussing this topic therefore, it is impossible to draw any definite conclusions.

All of the investigators seem of the opinion that sulfanilamide is, in itself, not a bacteriacide, and that while the harmful organisms may be killed after the administration of the sulfanilamides, the destruction is brought about as a result of some other action of the drug.
For means of comparison, the following theories are presented.

(1) Three French workers (Levaditi, Vaisman, and Krasnoff) claim that the sulfanilamides interfere with capsular formation of the streptococci and other organisms, thus rendering them more susceptible to phagocytosis.

(2) Long and Bliss believe that the sulfanilamides act as bacteriostatic agents, delaying the growth of the infectious organisms until the body is capable of overcoming them. They also believe that sulfanilamide may bring about an increase in the number of phagocytes.

(3) Osgood states "the major action of the sulfanilamides is to render inert or destroy the bacterial toxins and to cause a decrease in the rate of division enabling the bactericidal action of the human serum and the phagocytic action of the cells of the marrow and blood to overcome the infection."

(4) Mellon, and this is the most recent, claims that most of the bacteria such as hemolytic streptococci, pneumococci, gonococci, meningococci, and bacillus coli, produce minute quantities of hydrogen peroxide (\( \text{H}_2\text{O}_2 \)) which soon breaks down into oxygen and water. Since the concentration of the hydrogen peroxide is very small, there is no effect on the bacteria. Mellon believes that the sulfanilamides prevent the breakdown of the peroxide with the result that the concentration of the peroxide becomes very large, and the bacteria eventually die in their own product.

(5) The editors of the N.N.R. state the problem as follows: "The mode of action of sulfanilamide on susceptible bacteria is still uncertain. There is evidence that one action (and this is possibly the only one of importance) is to render the blood, spinal fluid, urine and other tissue fluids unfavorable as mediums for supporting the active multiplication of susceptible bacteria. In consequence, tissue invasion by these organisms may be prevented, production of toxic substances reduced, and the antibacterial mechanisms of the host permitted to complete recovery from the infection.

10. Uses of Sulfanilamide and Derivatives

10a. Comparative Clinical Value of Sulfanilamide and Derivatives

Estimates of the comparative clinical value of sulfanilamide, neoprontosil, sulfapyridine or the sulfathiazoles when administered orally in the treatment of infections.

<table>
<thead>
<tr>
<th>DISEASE</th>
<th>Sulfanilamide</th>
<th>Neoprontosil</th>
<th>Sulfapyridine</th>
<th>Sulfathiazole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemolytic Streptococcal Infections</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tonsillitis</td>
<td>4</td>
<td>4</td>
<td>2</td>
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<tr>
<td>Pharyngitis</td>
<td>4</td>
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<td>2</td>
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</tr>
<tr>
<td>Peritonsillar Abscess</td>
<td>3</td>
<td>2</td>
<td>2</td>
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</tr>
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<td>Ludwig's Angina</td>
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<td>Mastoiditis</td>
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<td>Disease</td>
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<td>Neoprontosil</td>
<td>Sulfa-pyridine</td>
<td>Sulfa-thiazole</td>
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**KEY**

4 - This is the preferred drug
2 - The drug is active
1 - The drug is slightly active
x - The drug should not be used

Where no key symbol is used, there are insufficient data for evaluation.

From Modern Medicine, May, 1940, p. 39.

**Bibliography:**
1. Lancet 2:281, July 30, 1938
2. Lancet 2:14, July 2, 1938
4. Lancet 1:1391, June 18, 1938
10b. Excerpts

(1) Bacteriostatic and Antitoxic actions of Sulfanilamide
J.A.M.A. Vol. 113, p. 1704-9 (1939)

It has been known that the blood and serum of human beings and laboratory animals possessed increased bacteriostatic and bactericidal power after the administration of sulfanilamide and the prontosils. The experimenters regarded it as probable that this factor might be important in human infections but expressed surprise that the very low bactericidal power of the blood of mice should be associated with such marked curative effect. A further factor of importance has been emphasized by Lockwood, who finds that peptones interfere with the antibacterial power of sulfanilamide in vitro. Thus the composition of the culture medium must be taken into account. Sulfanilamide does not neutralize toxin. We do know that sulfanilamide exerts a bacteriostatic effect on the three strains of beta hemolytic streptococci studied; that the effect varies directly with the concentration of the drug and inversely with the number of bacteria present; that a decrease in hemolysis is observed when bacteriostasis is observed; that the extent of the decrease of hemolysis does not suggest either toxin neutralization or "prevention of formation" of toxin beyond what might reasonably be expected as a result of the observed bacteriostasis, and that the drug inhibits the development of diffuse peripheries around colonies of beta streptococci. Finally, it has been found that all strains of beta streptococci studied were inhibited. Reduction in hemolysis with the observed bacteriostasis leads to the conclusion that the antihemolytic effect is secondary to bacteriostasis. The drug causes the development of abnormal, long chains of streptococci.

(2) Treatment of Pneumococcic Meningitis
J.A.M.A. Vol. 113, p. 1614-19 (1939)

Beginning with the use of sulfapyridine upon mice infected with pneumococcic infections, many clinical reports now show that the drug is of definite value in the treatment of pneumonia in man. The sodium salt of sulfapyridine also is used; the salt, unlike sulfapyridine itself, is freely soluble. It is most conveniently used in distilled water in 25% solution, which is approximately isotonic. It should be emphasized that such a solution is extremely alkaline (pH almost 11) and cannot be given intrathecally or subcutaneously but only intravenously. During the course of treatment with the large doses of sulfapyridine and its sodium salt which...
were employed, a number of toxic effects of the drug were noted; among these were nausea, vomiting, cyanosis, leukopenia and granulocytopenia. By way of a summary, "sulfapyridine given by mouth is absorbed irregularly and only in limited amounts. It is recommended that the use of the drug be supplemented by the intravenous administration of sodium sulfapyridine at regular intervals. The concentration of free sulfapyridine in the spinal fluid should be maintained at a level of from 10 to 15 mg. per hundred cubic centimeters. Certain toxic symptoms caused by sulfapyridine have been encountered. These include granulocytopenia and hematuria, neither of which was fatal in any of the cases. Hematuria occurring during treatment with sulfapyridine has been shown to be due to the formation of small calculi made up chiefly of the acetyl derivative of sulfapyridine. These calculi apparently produce hematuria by injury to the pelvis, ureters and bladder. It appears that the prognosis in pneumococcal meningitis may be greatly improved by the use of sulfapyridine and its sodium salt."

(3) Treatment of the eye

J.A.M.A. Vol. 112, p. 2025 (1938)

A peculiar experiment was carried out by Bellows and Chinn in which they determined the percentage of sulfanilamide in the eye following oral administration. "Sulfanilamide can be detected in all ocular tissues and fluids within 15 minutes after its oral administration. The concentration of sulfanilamide in the eye has been determined one, two, three, four, six, twenty-four, and forty-eight hours after a single massive dose by mouth. The maximum is reached around the sixth hour. Heat, atropine, and physostigmine applied locally have no effect on the sulfanilamide concentration of the aqueous humor. Methylcholine, similarly applied, increases this value. The second aqueous contains somewhat more sulfanilamide than does the original aqueous. Sulfanilamide has been analyzed in tears.

(4) Use and Abuse of Sulfonamide Derivatives

J.A.M.A. Vol. 114, p. 1298 (1940)

Whitby states that beta hemolytic streptococci, pneumococci, Bacillus coli, meningococci and gonococci are the organisms that used to be considered in relation to treatment with sulfonamide derivatives. It is essential to maintain an effective concentration of the drug in the blood. Because the drugs are quickly excreted, night doses must not be omitted; otherwise the blood concentration falls below the effective level. If the drugs are going to be effective they quickly show some evidence of this. Therefore if no clinical effect is observed in from five to seven days there is no justification for prolonging an adequate course of a particular sulfonamide derivative beyond this period. But if a clinical effect has been observed its administration should be continued for from ten to fourteen days. If not completely effective at the end of this time, a rest period of two days should be given and a fresh course then instituted. When used at all the drugs should be given in full doses; otherwise no deductions can be made as to effectiveness. Rather than administer small doses on chance, it is preferable to withhold the drug until there is some justification for giving full clinically effective doses.
(5) Treatment of Abscesses
J.A.M.A. Vol. 115, p. 322 (1940)
F.R. Adams reported that good results were obtained by the direct injection of a hot sulfanilamide solution into abscesses. There is, however, information available which shows that the local application of crystalline sulfanilamide to infected wounds and open abscesses is a rational therapeutic procedure. Good results have been obtained by the repeated local application of sulfanilamide powder without concurrent oral administration of the drug in infected wounds, superficial ulcers and draining abscesses and sinuses.

(6) Treatment of Peritonitis of appendical origin
J.A.M.A. Vol. 115, p. 162 (1940)
Ravdin and his associates showed that the mortality rate is decreased upon the use of sulfanilamide for peritonitis of appendical origin. Although the reduction in mortality does not appear to be great, the authors are convinced that a number of lives of desperately sick patients have been saved by the administration of sulfanilamide. Drainage is necessary in many instances. In nearly every instance the sulfanilamide has been given by hypodermoclysis. The crystalline sulfanilamide was used. It was used preoperatively in cases of appendical abscess so as to minimize spread of the infection if transperitoneal drainage became necessary. No accessory method of treatment can take the place of early operation and skillful surgery. At a time of late operation, it is in such cases of desperate illness that accessory therapeutic agents can help reduce the mortality.

(7) Use in Surgical Infections - Its possibilities and limitations
J.A.M.A. Vol. 115, p. 1190 (1940)
Only four years has elapsed (1936-40) since the publication by Colebrook and Kenny of the first statistically significant evaluation of sulfonamide therapy in a single disease. Since the publication of Colebrook and Kenny, the application of sulfonamide therapy has been widely extended and now includes almost the whole field of surgical infections.

An adequate explanation of the mode of action of the sulfonamides in biochemical terms has not yet been supplied. It is reasonable to assume, however, on the basis of a considerable amount of laboratory evidence, that these drugs act in infectious lesions by producing an environment in the body tissues and fluids which is unfavorable for bacterial multiplication. As the capacity of the bacteria to multiply is depressed, their invasiveness is diminished and their vitality reduced to a point at which the natural defenses of the body can dispose of the surviving organisms. However, there are factors which modify this so-called bacteriostatic effect of the drug. Among the most important of these are: 1. The ratio between the number of bacteria and the concentration of the drug in the infected zone. 2. The activity of the antibacterial defense in the area of infection. 3. The amount of proteolysis of tissue accompanying the inflammatory process; its content of substances likely to antagonize sulfonamide bacteriostasis.

The prophylactic use of the sulfonamide compounds in prevention of wound infection is a supplementary means of holding down the multiplication of bacteria which gain access to an operative wound or cannot be removed by debridement from a traumatic wound. Prophylactic sulfanilamide is now being used also in industrial practice in an effort to reduce the amount and duration of disability from infection following injuries in shops and factories. There may be
some dangers in this treatment. Besides some toxic effects, there is the added danger that the drug will produce motor incoordination and other effects in the central nervous system. No person should be allowed to operate a machine of any sort while taking sulfanilamide. Therefore it would seem advisable to restrict the prophylactic use of sulfanilamide to patients whose injuries are sufficiently disabling to demand their complete inactivity. Clinical studies now in progress may justify the use of crystalline drug implanted locally in the prevention of infection in other types of surgical wounds, including large hernias, lobectomies and pneumonectomies, and contaminated intestinal resections. But for the present it appears that local implantation of the drug should be used only to supplement oral administration and not in place of it.

However, in evaluating chemotherapeutic effects in animal infections and in man it is advisable to keep in mind that from a practical standpoint the pathologic characteristics of the lesion under treatment are also of great importance. Diffuse infections characterized by the rapid multiplications and dissemination of invasive bacteria in tissues of relatively normal architecture or in fluids closely resembling normal serum or lymph are likely to respond favorably to chemotherapy. This group includes, specifically, the early stages of cellulitis, lymphangitis, erysipelas and lobar pneumonia and probably also acute pyelonephritis and infections of the cerebrospinal fluid. Whether the causative organism is a hemolytic streptococcus, a staphylococcus or a pneumococcus makes some difference in the choice of a drug, perhaps, but these lesions are generally susceptible regardless of the bacterial species. Sulfapyridine, which is apparently a more polyvalent drug than sulfanilamide, will be effective in the great majority of infections of this pathologic type, and the bacteriologic aspects are of secondary importance.

Infections of serous and synovial membranes such as peritonitis, pleuritis and suppurative arthritis (at least in their early stages) constitute another broad group of lesions which are generally susceptible to sulfonamide therapy. Infections of serous and synovial cavities have certain definite pathologic features in common, the most important probably being the early mobilization of an abundant protective mononuclear cell reaction, which Gay has shown is a highly effective mechanism in killing bacteria. This cellular response is given added time to become mobilized when a substance like sulfanilamide is present to hold down the multiplication and toxin production of the infecting organisms.

There are, on the other hand, certain definite types of lesions which are quite resistant to chemical treatment. The most conspicuous in this group are the localized abscesses of soft tissue or bone. Bacteriologic specificity again has only a limited significance in the chemotherapy of localized necrotic lesions in soft tissue, bone, pleura or peritonum. They all tend to be resistant, regardless of the type of organism present. Use of the appropriate drug may have some effect in preventing spread of the infection to adjacent tissues and may actually mask the clinical signs and symptoms of the abscess. Prolonged treatment for a month or two may sometimes allow sterilization of the abscess, but as a general rule such a course will be unpractical and inadvisable. Premature withdrawal of the drug is likely to be followed by reactivation of the infection. Suppurative thrombochlebitis is another type of pathologic lesion which is comparatively resistant to chemotherapy.
The limitations of sulfonamide therapy in terms of pathology are well illustrated in the staphylococcic infections. One may as readily account for the relative resistance of staphylococcic infections on the basis of their pathologic characteristics as on the basis of the comparative resistance to sulfonamide bacteriostasis which the staphylococcus possesses. The conspicuous features of staphylococcic infections are tissue necrosis, supplicative phlebitis and the early tendency to form supplicative metastatic lesions.

In summary, it might be said that a lesion which is characterized by minimal loss of tissue architecture, and an exudate which is "serous", composed chiefly of lymph, bacteria and inflammatory cells, is likely to respond very rapidly to sulfonamide therapy. On the other hand, when the exudate is thick and purulent, having a high content of products of tissue proteolysis and many dead bacteria and phagocytes, the response to chemotherapy will be relatively slight except at the periphery, where bacteria are lodged in tissues which have not yet become broken down. Sulfanilamide will attack the invasive component of bacterial infections, but the localized abscess and the necrotic distributing focus are problems for surgical drainage or excision. In these areas the bacteria remain potentially invasive; if the inhibitory effect of the drug is removed, and host resistance is still inadequate, reinvasion occurs.

(8) Treatment of Gas Gangrene
J.A.M.A. Vol. 115, p. 1192 (1940)
Experimental results with sulfanilamide treatment of Clostridium welchii infections in small animals have not been encouraging. For the present it would seem that our chief reliance should be on prevention of gas gangrene by debridement of susceptible wounds and administrations of prophylactic antitoxin. It is possible that sulfanilamide treatment might have some value in limiting the activity of pyogenic cocci, which are known frequently to prepare the ground for gas gangrene. One would therefore be justified in using one of the sulfonamide preparations in conjunction with other measures, while recognizing the fact that the drug perhaps has little effect on Clostridium itself.

(9) Treatment of Asthma
J.A.M.A. Vol. 115, p. 411 (1940)
The authors do not suggest that sulfanilamide and its derivatives will relieve all cases of status asthmaticus or that they will actually cure asthma. There may be cases of status asthmaticus which result from an overwhelming contact with some extrinsic substance of antigenic quality in which they do not know what effect sulfanilamide may have. When the indications for the use of these drugs, the proper dosage and the duration of treatment have been well established, the outlook for the asthmatic patient may be improved considerably and the late complications of asthma - chronic bronchitis, bronchiectasis, emphysema, pulmonary, fibrosis, cardiac hypertrophy and myocardial damage - prevented.

(10) Treatment of Chancreas
J.A.M.A. Vol 115, p. 1750 (1940)
The authors conclude that there is no advantage in giving sulfanilamide to patients with small and uncomplicated chancreas. Local therapy seems to be sufficient. If prompt control is not
obtained, sulfanilamide is to be given. For large chancroids, sulfanilamide associated with local therapy should be used. In phagedenic chancroid it should be tried in conjunction with local therapy. Sulfanilamide does not decrease the formation of pus. It is most efficacious for ruptured buboes. Sulfanilamide should not by used in a routine manner but with knowledge of its value and indications.

(11) Treatment of Tertian Malaria
J. of Pharmacology and Exp. Therapeutics Vol. 63, p. 353 (1933)
Chills, with characteristic malarial blood cycles continuing in patients on Prontosil and Prontylin over periods of 50, 70, 73, and 120 hours and over, indicated that these new therapeutic agents have no effect on tertian malaria within these intervals, nor does further administration to the point of possible effect on the parasite appear advisable. Considering that prompt response is the primary governing factor in the choice of therapeutic agents, quinine supplemented by atabrine or plasmochin in indicated resistant cases, remains as a quickly acting and satisfactory drug in malaria treatment. Prontosil and Prontylin do not have a place in tertian malaria, according to the experiments conducted in these cases, except as conjectured agents in persistent resistant forms of malaria.

(12) Inhibition of the bacteriostatic action of the Sulfonamides by substances of animal and bacterial origin
J. of Biological Chemistry Vol. 72, p. 217.
The widespread distribution of a substance or substances which annul the bacteriostatic action of the sulfonamide drug is of importance not only in relation to the use of these chemotherapeutic agents in the treatment of infections but also in relation to the mode of action of the drugs upon bacterial cells in vitro. In an important contribution to this subject Woods has demonstrated that p-aminobenzoic acid annuls the bacteriostatic effect of sulfanilamide in vitro, and that the inhibition is not due to a molecular reaction between p-aminobenzoic acid and sulfanilamide. Woods has postulated that p-aminobenzoic acid is essential for the growth of bacteria, and that sulfanilamide causes bacteriostasis by inhibiting the enzyme reaction involved in the utilization of p-aminobenzoic acid. As a corollary to this theory, Woods has suggested that differences in the sensitivity of bacteria to sulfanilamide may be due to quantitative differences in their ability to synthesize p-aminobenzoic acid. Selbie has shown that p-aminobenzoic acid, administered by mouth, inhibits the curative action of sulfanilamide in experimental infections of mice with group A hemolytic streptococci.
Certain of the properties of the sulfonamide inhibitors occurring in enzymatic hydrolysates of casein and in culture supernatants of pneumococcus are similar to those of p-aminobenzoic acid. However, an important difference exists in their solubilities in acid ether. Woods has pointed out that novacaine, which is the hydrochloride of the p-aminobenzoic ester of N-diethyl-amino-ethyl alcohol (HgNOCH2COOCH2CH2N(C2H5)2) is also a very potent sulfonamide inhibitor.
Active inhibitors occur in certain normal animal tissues. It is shown that in rabbit liver and kidney tissue and in human urines, most of the inhibitor is present in a conjugated form so that its activity becomes evident only after acid hydrolysis. The presence
of an inhibitor in many of the tissues and fluids of the body as well as in association with the invading bacteria affords an explanation for the occurrence of localized lesions, resistant to the action of the sulfonamide drugs, which may develop during the course of therapy even though necrosis or cell autolysis is not demonstrable.

Sulfonamide inhibitors have been demonstrated in extracts of fresh normal muscle, pancreas, and spleen of certain animals. When autolysis of tissues takes place the amount of inhibitor is greatly increased. Fresh liver from beef, rabbit, and guinea pig is free of active inhibitor, although inhibitor is demonstrable in autolysates of the tissue. Fresh rabbit kidney is likewise free of active inhibitor. Following acid hydrolysis extracts of fresh rabbit liver and kidney cause sulfonamide inhibition. Normal human urine contains little or no active inhibitor. However, upon acid hydrolysis, inhibitor is uniformly present. Sulfonamide inhibitor is present in some, but not all, sterile serous effusions occurring during certain diseases. Inhibitor was found uniformly in pus. None was found in blood serum. In certain species of bacteria the inhibitor is found in cells only and is not demonstrable in the culture medium, whereas in other species, the inhibitor is found in the culture supernatant, and the cells themselves are relatively free.

(13) Relationship of Sulfapyridine, Nicotinic Acid, and coenzymes to the growth of Staphylococcus Aureus

It is evident that substances other than coenzymes inhibit the bacteriostatic effect of sulfapyridine. Observations show that coenzymes interfere with the bacteriostatic action of sulfapyridine, whereas nicotinic acid fails to have this effect. A possible explanation is that sulfapyridine and nicotinic acid compete for the same position in the coenzyme molecule. Given nicotinic acid, the organism on the basal medium is presumably able to form coenzymes (nicotinic acid amide adenine dinucleotide). However, given nicotinic acid and sulfapyridine, the organism may be unable to form coenzymes or the activity of certain dehydrogenases (coenzymes combined with protein) may be inhibited. When preformed coenzymes are present in the medium, the normal metabolism of the organism is not modified by sulfapyridine and the customary growth curve is seen. As all cultures were aerobic, it seems improbable that coenzymes functioned merely as reducing agents.

(14) Treatment of Conjunctivitis
J.A.M.A. Vol. 115, p. 445 (1940)

The various organisms that have been accused as possible causative agents include the tubercle bacillus (human and bovine), Bacterium tularense, Spirochaeta pallida, the glanders bacillus, Sporothrix, Streptococcus and Leptothrix. In this case, we are interested in that of the virus of venereal lymphogranuloma. Syphilis, tularemia and tuberculosis as etiologic factors of the ocular disease were ruled out. Leptothrix was not seen at biopsy. Sulfanilamide, however, terminated the activity of the process of the eye. Its efficacy in this case points to the infectious nature of the lesion. Besides, sulfanilamide, although to a lesser degree than sodium sulfanilate, seems to be valuable especially in the later stages of venereal lymphogranuloma. It is interesting to note that by intradermal tests on patients the virus was demonstrated in an extract of the tissue but not in washings from the eye.
(15) Cure of a case of acute ulcerative Endocarditis

J.A.M.A. Vol. 115, p. 1712 (1940)

This is a singular case but with much importance. The diagnosis of acute ulcerative endocarditis is established by the changing heart murmurs, the positive blood cultures of hemolytic streptococci and the patient's septic course in the absence of other foci of infection. The absence of embolic phenomena and splenomegaly is not unusual but rather the expected finding in acute endocarditis as contrasted with the manifestations in subacute bacterial endocarditis. While it cannot be proved, it seems probable that the upper respiratory tract was the portal of entry for the infection. The success of therapy with sulfanilamide in this case may be related to two factors: Therapy was instituted very early in the disease, perhaps before deep bacterial invasion had occurred; secondly, the treatment was persisted in for a period of six weeks. There seems little doubt that moderate dosage, long instituted, is more effective for deep seated infections than massive doses for a shorter period of time.

(16) Treatment of Erysipelas

J.A.M.A. Vol. 115, p. 1053 (1940)

Top and his associates treated cases of erysipelas admitted to their hospital with sulfanilamide. They compare these with cases treated with antitoxin during a period of about the same length. The number of patients treated with antitoxin was 76 and the number treated with sulfanilamide was 135. They observed that the proportion of cases that showed no spread was twice as great among those treated with sulfanilamide as among those given antitoxin. The proportion of cases with slight spread was about the same for the two groups, but moderate spread was noted twice as frequently among the antitoxin treated as among the sulfanilamide treated. Marked spread was noted among 10.5% of antitoxin treated, whereas none of the patients receiving sulfanilamide showed this degree of extension.

(17) Treatment of Compound Fractures

J.A.M.A. Vol. 115, p. 164 (1940)

Local implantation of sulfanilamide crystals in compound fractures not only tends to lessen the danger of infection but does not perceptibly interfere with the union of the soft tissues or of the bone. However, such implantation does not permit the closing of grossly contaminated or infected wounds. The wound must be debribed in the usual way and all foreign material and devitalized tissue removed before the infection has gained a foothold and invaded the tissues, preferably within 12 hours after the injury. Had the casualty wounds during the war of 1914-18 been rapidly debribed, sprinkled with sulfanilamide crystals, sutured and the reduced fractures immobilized in casts or splints, the large majority of them would have stayed closed without infection and the soldiers could have been sent directly back to America from the casualty clearing station or field hospital.

(18) Treatment of Gonorrhea in the male

J.A.M.A. Vol. 115, p. 163 (1940)

It was found that the use of sulfanilamide materially enhanced the satisfactory results early in the course of the treatment period. Sulfanilamide plus local therapy was over six times as effective in producing remissions as was local therapy alone in those cases followed during the 15 to 19 day interval. At the
same time, sulfanilamide alone was twice as good as was local treatment alone. By the 49 day, sulfanilamide plus local therapy had obtained nearly twice as many good results as did either local therapy alone or sulfanilamide alone.

(19) Staphylococccic Infections of Kidney
J.A.M.A. Vol. 115, p. 160 (1940)
Staphylococcus aureus is the most frequent invading organism, although any of the other strains of staphylococcus may be responsible. The port of entry is the skin or mucous membranes. Furuncles and carbuncles are the most common cutaneous foci, while infections of the upper respiratory tract are the most frequent mucosal precursors.

Austen administered sulfanilamide directly to ten patients with infections of the upper part of the urinary tract. These patients either did not tolerate the drug well or it was unwise to administer it by the usual routes because of anemia, granulocytopenia, diminished renal function, or the presence of an anatomic or pathologic abnormality preventing high local concentration or because there was interference with renal drainage. The drug administered by direct pelvic instillation was well tolerated by all except one patient. The author feels that although the method has definite limitations the direct pelvic instillation of sulfanilamide is a worthwhile procedure in the treatment of selected cases of renal infection.

(20) Treatment of Lung Infections (Pleuritis and Emphyema)
J.A.M.A. Vol. 115, p. 1194 (1940)
Chemotherapy might be of value if used before and after pneumonectomy or lobectomy. Chemotherapy of pneumonia tends to prevent empyema. Chemotherapy and drainage by aspiration may be used if the exudate is serous. Resolution without the necessity for open drainage is likely under these conditions. Rib resection and drainage will be required as soon as the exudate is frankly purulent. Sulfonamide therapy in small dosages after operation may shorten the period of drainage.

(21) Treatment of Venereal Lymphogranuloma
J.A.M.A. Vol. 115, p. 1484 (1940)
Stein used sulfanilamide in the treatment of 35 cases of the bubonic type of venereal lymphogranuloma. Of the 35 patients treated, the results of three are unknown. The remaining patients were cured, with complete subsidence of the local lesions and relief of all constitutional symptoms. Two patients with elephantiasis improved within two weeks. Few toxic effects were encountered. In no case was it considered necessary to stop medication.

(22) Treatment of Recurrent Lymphocytic Choriomeningitis
J.A.M.A. Vol. 115, p. 436 (1940)
The usual case of lymphocytic choriomeningitis is characterized by a single attack of meningeal irritation followed by prompt recovery. The patient in question had four distinct recurrences in addition to the initial attack in a period of four months. The prompt response and apparent control of this malady by the use of sulfanilamide is of unusual interest. One gets the impression from the literature that sulfanilamide and its derivatives are of little value in the therapy of the human virus diseases. Rosenthal, Wooley and Bauer found that although sulfanilamide and
azosulfamide were ineffective, the original prontosil of Domagk (4-sulfonamido-2,4'-di amino-azobenzene) was effective in the early therapy of lymphocytis choriomeningitis in mice if large doses of the drug and small infective doses of the virus are employed. On the other hand, McKinley, Mack and Acree inoculated mice with large infective doses of the virus and found that azosulfamide was ineffective. They also found that sulfanilamide and sodium sulfanilylsulfanilate were inactive against this virus infection.

(23) Treatment of Acute Purulent otitis media
J.A.M.A. Vol. 115, p. 178 (1940)
If chemotherapy is given early, before bone destruction occurs, the duration of discharge is diminished by about 50% and the number of mastoidectomies is diminished by about 50%. When the clinical picture strongly suggests mastoidectomy, it is safer to operate. After uncomplicated mastoidectomy, it is better not to give the drug. Complicated mastoiditis requires intensive chemotherapy. At times it is necessary to stop the drug in order to obtain the true picture, as the drug cures the middle ear while progressive bone destruction is taking place in the mastoid. Chemotherapy has unquestionably added greatly to the confidence of physicians in their ability to conquer acute otitis media and mastoiditis. They have always been apprehensive of meningitis as long as the ear continued to discharge. Mortality has dropped from 97 to 35% in the last 5 years. This is a great comfort and a signal achievement.

(24) Treatment of Chronic Sinusitis
J.A.M.A. Vol. 115, p. 1569 (1940)
Sulfanilamide and its derivatives have been used with moderate degrees of success in the treatment of acute sinusitis due to beta hemolytic streptococci, pneumococci and staphylococci. In almost all instances, however, some type of drainage has to be used concomitantly with therapy with sulfanilamide or its derivatives. In chronic sinusitis, reports on the use of sulfanilamide or its derivatives have been discouraging. The reason for the failure of sulfanilamide to benefit patients ill with chronic sinusitis probably lies in the fact that reinfection from the nasopharynx and nose is constantly taking place. Hence, while temporary chemotherapeutic effects may be obtained, the cessation of drug therapy is almost always followed by prompt recurrence of the infection in the affected sinus or sinuses.

(25) Treatment of Sunburn
J.A.M.A. Vol. 115, p. 1569 (1940)
Sunburn in itself is not a contraindication to the administration of sulfanilamide or its derivatives, provided there is a good reason for using these drugs. It has been definitely established that it is unwise for a patient who is receiving one or the other of these drugs to expose himself to the direct rays of the sun during a course of treatment, because in a certain number of instances photosensitization will take place and a dermatitis will result. For this reason patients who are receiving these drugs should keep out of the sun until from two to four days after the administration of the drug has ceased. This latter precaution is recommended in order to permit the drug to be completely excreted and hence to avoid possible photosensitization.
(26) Treatment of Trachoma
J.A.M.A. Vol. 115, p. 111 (1940)
Sulfanilamide and its derivatives were found to be definitely effective against trachoma in stages I, II, and III, being most useful in eliminating subjective complaints and in checking and healing pannus and corneal ulcer. Pathologic conditions in the palpebral conjunctiva were improved markedly but always remained in the canthal regions. The upper and lower cul-de-sac were not much influenced by prolonged oral or intramuscular administration of the drugs. Intramuscular administration of sulfanilamide suspended in oil and especially of sulfapyridine has, besides the therapeutic effectiveness of the oral administration, the advantage of prolonged action, decreased dosage, lack of toxic manifestations and increased ease in controlling and managing patients. The disadvantage lies in the pain and transitory fever the injection causes, although they are never severe enough to incapacitate the patient. Blood estimations show that sulfanilamide administered intramuscularly is present in the blood for approximately four days, while sulfapyridine remains in the blood from ten to fourteen days, whether they are suspended in physiologic solution of sodium chloride, olive oil or 0.5% sodium hydroxide. The minimum therapeutic level appears to be between 2 and 2.5 mg. per hundred cubic centimeters.

(27) Treatment of Urinary tract infections
J.A.M.A. Vol. 115, p. 1350 (1940)
The sulfonamide drugs are excreted by the kidneys in a manner exactly similar to phenolsulfonphthalein. The action of sulfonamide drugs in infections of the urinary tract depends more on the tissue reaction than on direct bactericidal action in the urine. Mandelic acid is an excellent drug for infections with colon bacillus and Streptococcus faecalis. A comparison of the colon bacillus infections treated with sulfanilamide and sulfapyridine shows practically the same, or 81%, cured. A comparison of the same drugs in staphylococci infections shows that with sulfapyridine 73% and with sulfanilamide 62.5% were cured. Infections complicated by other pathologic changes do not respond as favorably as the simple infections. A comparison of sulfanilamide and mandelic acid therapy in various types of cases shows that sulfanilamide is usually preferable.

(28) Treatment of Wounds
J.A.M.A. Vol. 115, p. 2194 (1940)
In treatment a few doses of sulfanilamide would on most occasions deal with an acute streptococcic infection but not with a chronic infection. When the infection was in the blood the drug was not of the same value as when it was in the body tissues. The same held for gas gangrene. Colonel Colebrooke said that definite results were to be expected from careful use of sulfonamide derivatives. The first was the prevention of hemolytic streptococcus infection. A sulfonamide derivative would be valuable in a case of secondary operation in which there was a suspicion of hemolytic streptococcus infection. Prophylactic treatment should be administered in four hourly doses. It might also be possible to arrest infection by prompt and vigorous action. In long-standing or superficial cases, such as old burns and road accidents which had led to infection, the application of sulfanilamide powder gave astonishingly good results.
Do not think this is the last you will hear of sulfanilamide. Actually, it is the beginning of a new era. Sulfanilamide is new comparatively speaking; sulfathiazole is even more recent. Strange as it seems, just today as I finish my report (4/19/41), I received word of a new derivative, sulfadiazine (Sulfanilyl-amino-pyrimidine), which is being used in Halifax, N.S. by Doctor John H. Dingle, of the Harvard expedition, for the treatment of meningitis, diphtheria and scarlet fever. Also, Sulfanilyl-guanidine, another new drug, is of value because it is poorly absorbed in the intestinal tract and, therefore, stays there long enough to exert its action against, for example, dysentery. Much research has yet to be conducted; trials and hardships, time and labor, are necessary for the attainment of this goal. As Pasteur once said, "There is no peril in expressing ideas a priori when they are taken as such and can be gradually modified, perhaps even completely transformed according to the result of the observation of facts." It was only in 1937 that 73 deaths resulted from the use of sulfanilamide. A fatal error caused by the Massengill pharmaceutical house when they claimed they had discovered a solvent for sulfanilamide in the use of 70% diethylene-glycol. Experiments warranted the belief that diethylene glycol is the toxic agent in the Elixir of Sulfanilamide-Massengill examined. Ironically, upon the label were these directions, "1 or 2 teaspoonfuls and continue at this dose until recovery." Incidentally, the deaths due to the Elixir of Sulfanilamide were the chief cause in the passage of legislation which is now known as the "Pure Food, Drug and Cosmetic Act."

Unfortunately, a solvent for sulfanilamide is yet to be found without producing toxic effects. Probably today, perhaps tomorrow, or a year's time is necessary for such a discovery. At any rate, sulfanilamide still reigns supreme as the ideal drug discovered in this century.