SULPHANILAMIDE AND DERIVATIVES IN BACTERIAL INFECTIONS

BY

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1. PRONTOSIL AND RELATED DYES

DOMAGK'S sensational discovery\(^1\) of the specific curative effect of 'prontosil' (I) in experimental β-haemolytic streptococcal infections in mice, which is hailed as the "greatest discovery in modern therapeutics", appears to have been made in 1932 as a culmination of his researches dating from 1923-24 in the Elberfeld laboratories of the I. G. Farbenindustrie.\(^2\) Regarding the hosts of compounds that must have been tested systematically in the course of this investigation, we are given no details. The discovery was announced on the 15th February 1935\(^1\) only after it was confirmed by three years of clinical trials at the hands of the Rhineland practitioners, for "by untimely publication he did not want to give false hopes to doctors and patients".\(^2\) This dye (prontosil) being of low solubility in water (about 0.25 per cent), a more soluble form, "prontosil soluble" ("prontosil S", "neoprontosil", II) was introduced (as 2.5 per cent aqueous solution) for parenteral use, while in France, a carboxyl derivative of prontosil, "rubiazol" ("rubiazol C", III), synthesised by Gley and Girard\(^3\) came into use.
2. REDUCTION OF PRONTOSIL IN VIVO: EVOLUTION OF SULPHANILAMIDE

The next great advance in the subject was made by Tréfouël (J. and Mme.), Nitti and Bovet in November 1935. They studied systematically the antistreptococcal properties of forty-five dyes of the azobenzene group with various substituents and found that the replacement of the sulphonamide (SO₃NH₂) group in prontosil (I) by -AsO₃H₂, -SO₂H, -CN, -CONH₂, -CH₂, CO.CN and -O.Ph groups destroyed the activity, while the amino group could be replaced by other groupings without much loss of activity. This led them to formulate the important hypothesis that the therapeutic activity of prontosil is due to para amino benzenesulphonamide (sulphanilamide) liberated in vivo by reduction. In support of this they showed, for the first time, that the simple colourless compound, sulphanilamide (already synthesised by Gmel in 1908 who never dreamt of its therapeutic properties), itself was as active as prontosil in experimental streptococcal infections. The above results were confirmed from various points of view by many workers. As an apparent proof of the hypothesis, Fuller actually isolated sulphanilamide from the urine of a patient treated with prontosil. Ganapathi and Rao have shown that following the feeding of six typical dyes of this group to groups of mice in therapeutic doses (10 mg.), only the therapeutically active ones produce considerable blood concentrations (1-6 to 2-0 mg. per cent.) of sulphanilamide, whereas the very little active or inactive ones produce only traces.

Though till now about seventy dyes of the above group constituting various types have been reported, only a dozen of these have been found to be comparable in antistreptococcal activity to prontosil or sulphanilamide; it is yet to be shown definitely that these active dyes possess any advantage over the parent amines. As regards both the intensity as well as the poly-valency of therapeutic effect, the dyes are inferior to the free amines. For example, sulphanilamide shows a striking therapeutic effect in (β-haemolytic) streptococcal meningococcal, gonococcal, bacillus welchi and B. coli infections; its effect in B. typhosus infection is considerable; in pneumococcal infections, the protection is less and in staphylococcal infections far less. The dyes show considerable therapeutic effect in streptococcal infections and in the rest their efficacy compared to sulphanilamide is negligible. However, both the dyes and sulphanilamide possess considerable protective effect in the virus infection, lymphogranuloma inguinale.

3. SEAT OF THERAPEUTIC ACTIVITY IN SULPHANILAMIDE

The next obvious step of elucidating the seat of chemotherapeutic activity in sulphanilamide was immediately taken up by Fournou, Trefouel, Nitti and Bovet. They studied 130 derivatives of related structures and showed that for the antistreptococcal activity, (i) the amino and sulphonamido groups in the para positions of the benzene ring are necessary, (ii) the presence of an additional grouping in the benzene ring destroys the activity and (iii) the substitutions in the amino and sulphonamido radicals have variable activity depending on the nature of the substituents. These important findings led to such intense activity in the synthesis of new compounds of this group that, till now, about ninety papers have been published and forty patents taken, reporting in all about 600-700 compounds (besides a lot unpublished).

4. COMPOUNDS WITH SUBSTITUENTS IN THE AMINO RADICAL OF SULPHANILAMIDE

About 130 derivatives with various types of substituents in the amino radical of sulphanilamide have been reported, of which only about twenty possess anti-streptococcal activity comparable to that of sulphanilamide. Of these twenty derivatives, fourteen are Schiff’s bases obtained by condensing sulphanilamide with variously substituted benzaldehydes. Of the forty-five acyl derivatives reported, the valeryl, caproyl, and pyrrolidone carboxy derivatives are as active as sulphanilamide. It is conceivable that all the above compounds can yield free sulphanilamide in vivo by hydrolysis. The guanidine and formaldehyde sulphoxide derivatives of sulphanilamide are stated to be quite active.

4-Benzylaminobenzene sulphanilamide (IV) first reported by Goisset et al., and introduced for clinical trials (under the trade names “proseptamine”, “septazine”), has been shown to be inferior to sulphanilamide in experimented infections in mice. It has been suggested by Lockwood and Robinson, though it cannot be considered to be
definitely proved, that the activity of pro-
septazine is due to the sulphanilamidé libe-
rated in vivo. Another colourless compound,
disodium p-(γ-phenylpropyl) aminobenzene-
sulphonamide-α-γ-disulphonate (V), has
been introduced for clinical trials under the
trade name "soluseptazine" for parenteral
use. The only animal experiments reported
about it by Whitby,17 do not indicate it to
be superior to sulphanilamide. Though these

\[
\text{Ph} \cdot \text{CH}_2 \cdot \text{NH} \begin{array}{c}
\text{SO}_2 \text{NH}_2 \\
(\text{IV})
\end{array}
\]

\[
\text{Ph} \cdot \text{CH} \cdot \text{CH}_2 \cdot \text{CH} \cdot \text{NH} \\
\text{SO}_3 \text{Na} \quad \text{SO}_3 \text{Na} \\
(V)
\]
two drugs possess considerable antistrepto-
coccal action, their therapeutic effects in
other bacterial infections compared to
sulphanilamide is negligible.16,17 Schultz19
has reported "soluseptazine" to protect rats
and not mice in experimental P. pestis in-
fecions.

The para nitro, nitroso, hydroxyaminio and
hydrazo derivatives of benzenesulphonamide
have been studied by Mayer.20 The first
two compounds are more active than
sulphanilamide and are more toxic, while the
hydrazo derivative is inactive. The hydroxy-
aminio derivative is about 100 times more
bactericidal than sulphanilamide in vitro,
but not in vivo (this being due to its reduc-
tion in the body to sulphanilamide). Some
significance is attached to this compound in
explaining the mechanism of action and
also some of the toxic manifestations of
sulphanilamide.20,21

5. COMPOUNDS WITH SUBSTITUENTS IN
THE SULPHONAMIDE RADICAL OF SULPHANIL-
AMIDE

Fortiye derivatives with alkyl and aralkyl
groupings substituted in the sulphonamide
radical of sulphanilamide have been synthe-
sised and tested but these have proved to
be of no advantage. Of interest is the
report of Adams et al.13 that five derivatives
with hydroxypropyl substituents in the
sulphonamide radical show striking anti-
meningococcal but no antistreptococcal
activity. Eighty-five derivatives of 4-amino-
benzenesulphonilide with various substit-
uenis in the second benzene ring have been
synthesised and tested. Those with the nitro,
amino, sulphonamido, substituted sulphan-
amido, N1-sulphanilamido, sulphanilic
amino carboxylic acid groupings in the second
benzene rigid are quite active and many
reported to be even superior to sulphanil-
amide in antistreptococcal activity. Domagkal
16, 17 p-aminobenzensulphonamido
benzene-p'-dimethylsulphonamide (known by
the trade names, "Deserital A", "Ularon"
"Ultron", VI) to be superior to sulphanil-
amide in streptococcal and staphylococcal
infections but this has not been confirm-
ed.16,22 It was given a fairly extended clini-
cal trial in gonococcal infections but has
now been withdrawn due to the toxic re-
action of peripheral neuritis. A series of
N1-acyl derivatives of sulphanilamide has
been reported.21 The acetyl derivative
("albusciden", VIL, though far less active that
sulphanilamide in streptococcal infections
has been introduced for clinical trials in
gonococcal infections with the claim that it
is of low toxicity and very little of it is
acetylated in vivo. Crossley et al.24 have
reported five compounds of the diethyl-
amide group (VIII) to be superior to sulpha-
nilamide in antistreptococcal action.

\[
\begin{array}{c}
\text{H}_2 \text{N} \\
\text{SO}_2 \text{NH} \\
(\text{VI})
\end{array}
\]

\[
\begin{array}{c}
\text{H}_2 \text{N} \\
\text{SO}_2 \text{NH} \cdot \text{O} \cdot \text{CH}_3 \\
(\text{VII})
\end{array}
\]

\[
\begin{array}{c}
\text{H}_2 \text{N} \\
\text{SO}_2 \text{N} \cdot \text{SO}_2 \text{NH} \\
R
(\text{VIII})
\end{array}
\]

One of these, the sodium salt of disulphani-
amide (VIII, R = Na), is claimed to protec-
t mice infected with moderate doses of the
influenza virus.26 Adams, Long and John-
son13 have reported thirty compounds with
acyl substituents in the amino, and hydroxy-
alyl substituents in the sulphonamido rad-
cals of sulphanilamide. Of these, only eight
are of interest for they are quite active in
meningococcal but almost inactive in strepto-
coccal infections.

6. DIPHENYLSULPHONE AND RELATED
DERIVATIVES

Almost simultaneously, Buttke et al. in
May 1937,27 and Fournier, et al.28 In June
July of the same year reported the remark-
ably high antistreptococcal action of 4:4'
diaminodiphenylsulphone. This diamine
according to Burtle et al. is about 100 times as active as sulphanilamide and 10 times more toxic in mice. In rabbits and monkeys, and possibly in man also, the toxicity as well as the activity, do not appear to be so high. The diacetetyl derivative (marketed under the name "Rhodilone" in France for use particularly in gonorrhcea) is relatively very little toxic and yet ten times as active as sulphanilamide. The 4:4'-dinitrodiphenyl sulphon is as active as sulphanilamide. The last two sulphone derivatives appear to be converted in vivo into the free amines. The significance about these compounds is: (i) they do not contain the sulphanamide grouping and (ii) they are the best of the till then known compounds giving a definite percentage of survivors in experimental pneumococcal (type I) infections. In other types of pneumococcal infections, the action of the diacetethylamino derivative is not so good. In experimental typhoid infections, it is inferior to sulphanilamide. About fifty related derivatives of this diphenylsulphone group have been tested but only the benzylidene, glucoside and the formaldehyde-sulphonyl derivative of 4:4'-diaminodiphenylsulphone have been suggested to be of some advantage. However these are not in use in practical therapy today.

About seventy related derivatives of the diphenylsulphoxide, diphenylsulphide, diphenyldisulphide and related compounds have been studied but they are generally of far less activity. 4-Nitro-4'-amino diphenylsulphoxide has been claimed by Levačiti et al. to possess such specific effects in pneumococcal infections as the arsenicals in the epidermatidal infections.

7. Heterocyclic Derivatives of Sulphanilamide

The discovery by Whitty, announced in May 1938, of the remarkable therapeutic effect of 2-N1-sulphanilamidopyridine ("Sulphapyridine", "Dagenan", "M. & B. 693", IX), one of the forty-six compounds synthesised by Ewins and Phillips of the firm, "May and Baker", in experimental pneumococcal infections in mice, is indeed a distinct advance in the chemotherapy of bacterial infections. The "dramatic cures" obtained with it in clinical trials in cases of pneumonia, have convinced us about its remarkable therapeutic properties. This compound is more polyvalent in action than sulphanilamide. The only experimental infection in which it is shown to be inferior to sulphanilamide is that due to B. typhosus; in streptococcal, meningococcal and gonococcal infections it is at least as good as sulphanilamide, while it is distinctly superior to sulphanilamide in pneumococcal, staphylococcal and P. pestis infections.

Of the hundreds of aromatic sulphur compounds synthesised and tested, though about seventy are about as active as and twenty-five distinctly superior to sulphanilamide in experimental streptococcal infections in mice, only sulphanilamidopyridine (IX) reigns supreme in its effect in pneumococcal infections. The presence of the heterocyclic ring in this compound gave thus the impetus to search for active compounds among the related heterocyclic derivatives of sulphanilamide. Thus, pyridine, quinoline, acridine, morpholine, piperidine derivatives are being tried. Though the trial cannot be said as yet to be complete, the quinoline, morpholine and piperidine derivatives do not show any promise. The search has been amply rewarded when attention was directed towards the thiazol derivatives by four groups of workers independently—all Ponsbinder and Walter, Toda, Hikawa and So, Lott and Bergeim and Ganapathi and Nandi. 2-N1-sulphanilamidothiazol (X) and the methyl derivative (XI) have been found to be as active as sulphanilamidopyridine (IX) in pneumococcal infections.

Extensive researches with thiadiazol derivative (X) (for which the short name "sulphathiazoil" has been suggested) at this Institute for the past several months, have convinced us that this new drug has a great future. While being far less toxic, it is even more polyvalent in action than sulphanilamidopyridine. In experimental strepto-
coccal infections in mice it is distinctly superior to sulphanilamide. Sokhey and Dikshit have found that it is far superior to sulphanilamidopyridine and almost a specific in experimental plague infections in mice. In staphylococcal infections also, these thiazol derivatives (X and XI) are distinctly superior to sulphanilamidopyridine. While the animal experiments are very encouraging, only extended trials have to pass the final verdict. In the meantime, similar heterocyclic derivatives are being prepared by the author and tested with the hope of obtaining even better compounds.

8. THE FUTURE OF BACTERIAL CHEMOTHERAPY

Whatever be the future of “prontosil”, which has brought about a renaissance in the Chemotherapy of Bacterial Infections, Domagk has indeed earned a place next to Ehrlich. The evolution of this subject, still in the cradle, from “prontosil” to “sulfathiazol” indicates that we are in the right way towards the conquest of the bacterial infections. The results obtained so far give us even the optimism that some day the dream of Ehrlich of “a therapia magna sterilisans”, will materialise at least in the treatment of some bacterial infections. There are many favourable indications for this, e.g., in experimental meningococcal and B. typhosus infections in mice, sulphanilamide can give sufficiently high protection with even one dose of the drug. The achievements so far made are by themselves no means meagre. While Ehrlich met with “one moment” of success after seven years of disappointments, in the present case, in five years have been made conquests of a variety of deadly infections due to at least five types of bacteria. With far more extended trials, we can hope to conquer even the dreaded tuberculosis and detested leprosy.