ACTION OF SULPHANILAMIDE DERIVATIVES IN EXPERIMENTAL STREPTOCOCCAL AND PNEUMOCOCCAL INFECTIONS IN MICE: PART II*

By K. Ganapathi and R. Sanjiva Rao

(From the Haffkine Institute, Parel, Bombay)

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This paper presents the results of our testing thirty-six out of about eighty sulphanilamide derivatives synthesised so far at this Institute[1] in experimental β-hæmolytic streptococcal and pneumococcal (type I) infections in mice. The outstanding therapeutic effects of one of them, 2-N^1-sulphanilamidothiazole (sulphathiazole), in experimental plague,[2] many bacterial and some virus infections[3] have already been described.

The results obtained are also discussed here from the point of view of the relationship of chemical constitution to antibacterial action. For the sake of convenience, in the protocols and discussion, the compounds tested will be referred to by our serial numbers which are given before each compound in brackets.

The following compounds were tested:—

(N^4)-Amino-substituted Sulphanilamide Derivatives:

(11) 4-Sulphonamidobenzenethiocarbamide:
$NH_2 \cdot CS \cdot NH \cdot C_6H_4 \cdot SO_2NH_2$.

(22) N-4-Sulphonamidophenyl-N′-phenylthiocarbamide:
$Ph \cdot NH \cdot CS \cdot NH \cdot C_6H_4 \cdot SO_2NH_2$.

N^1-Sulphanilamidobenzene Derivatives:

(1) 4-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(4')$.

(5) 3-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(3')$.

(6) 2-(N^1-Sulphanilamido)nitrobenzene:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NO_2(2')$.

(20) 4-(N^1-Sulphanilamido)dimethylaniline:
$NH_2 \cdot C_6H_4 \cdot SO_2NH \cdot C_6H_4 \cdot NMe_2(4')$.

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Azo dyes from Sulphapyridine and Sulphathiazole:

(9) 2-N^1-Sulphanilamidopyridine diazotised and coupled with *m*-phenylenediamine.

(10) 2-N^1-Sulphanilamidopyridine diazotised and coupled with 4-aminothiouracil.

(21) 2-N^1-Sulphanilamidothiazole diazotised and coupled with 4-aminothiouracil.

Pyridine Derivative:

(16) 2-(4′-N^1-Sulphanilamidobenzenesulphonamido)pyridine (Formula I).

(*N^4*)-*Amino-substituted Quinoline Derivative*:

(7) 2-(4′-Sulphonamidobenzeneamino)quinoline-3-carboxylic acid (Formula II).

(*N^1*)-*Sulphonamido-substituted Acridine Derivative*:

(8) 2 : 8-Di(N^1-sulphanilamido)acridine.

(*N^4*)-*Amino-substituted Acridine Derivatives*:

(23) N-(9′-Acridyl)sulphanilamide (Formula III, R = H).

(24) 2-[N^4-(9′-Acridyl)sulphanilamido]pyridine (Formula III, R = 2-pyridyl).

(26) 4-[N^4-(9′-Acridyl)sulphanilamido]aniline (Formula III, R = $-C_6H_4\cdot NH_2$).

(27) 4-[N^4-(9′-Acridyl)sulphanilamido]benzenesulphonamide (Formula III, R = $-C_6H_4\cdot SO_2NH_2$).

(28) 4-[N^4-(9′-Acridyl)sulphanilamido]nitrobenzene (Formula III, R = $-C_6H_4\cdot NO_2$).

N^1-Sulphonamido-substituted Guanidine Derivatives:

(56) Sulphanilylguanidine:

$$NH_2\cdot C_6H_4\cdot SO_2N{:}\,C\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}$$

(58) N^4-Sulphanilylsulphanilylguanidine:

$$NH_2\cdot C_6H_4\cdot SO_2NH\cdot C_6H_4\cdot SO_2N{:}\,C\begin{matrix}\diagup NH_2\\ \diagdown NH_2\end{matrix}$$

N^1)-Sulphonamido-substituted Thiazole Derivatives:

(13) 2-N^1-Sulphanilamidothiazole (Formula IV, R = H).

(33) 2-N^1-Sulphanilamido-4-methylthiazole (Formula IV, R = Me).

(45) 2-[4′-N^1-Sulphanilamidobenzenesulphonamido]thiazole (Formula V).

(N^4)-*Amino-substituted Thiazole, Thiazolin & Pseudothiohydantoin Derivatives*:

(40) 2-(4′-Sulphonamidobenzeneamino)thiazole (Formula VI, R = R′ = H).

(37) 2-(4′-Sulphonamidobenzeneamino)-4-methylthiazole-5-ethylcarboxylate (Formula VI, R = Me; R′ = CO_2Et).

(39) 2-(4′-Sulphonamidobenzeneamino)thiazole-4-ethylacetate (Formula VI, R = $-CH_2 \cdot CO_2Et$; R′ = H).

(41) 2-(4′-Sulphonamidobenzeneamino)-4-methylthiazole-5-ethylacetate (Formula VI, R = Me; R′ = $-CH_2 \cdot CO_2Et$).

(34) 2-(4′-Sulphonamidobenzeneimino)-3-allylthiazolin (Formula VII, (R = allyl; R′ = H).

(38) 2-(4′-Sulphonamidobenzeneimino)-3-phenylthiazolin (Formula VII, R = Ph; R′ = H).

(36) 2-(4′-Sulphonamidobenzeneimino)-3-allyl-4-phenylthiazolin (Formula VII, R = allyl; R′ = Ph).

(46) 2-(4′-Sulphonamidobenzeneamino)-5-iodomethyl-Δ^2-thiazolin (Formula VIII).

(44) N^2-4-Sulphonamidophenylpseudothiohydantoin (Formula IX).

(N^1)-*Sulphonamido-substituted Heterocyclic Derivatives*:

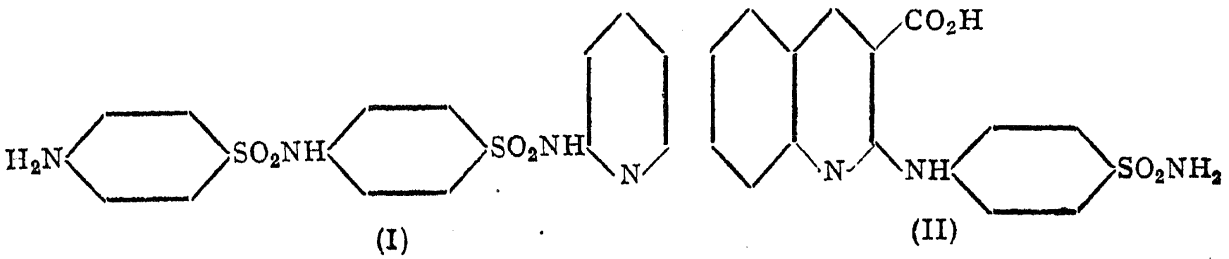
(50) 7-N^1-Sulphanilamidoalloxazine (Formula X).

(51) 2-N^1-Sulphanilamido-5-methyl-1 : 3 : 4-thiodiazole (Formula XI).

(54) 5-N^1-Sulphanilamidobarbituric acid (Formula XII).
(5-N^1-Sulphanilamido-2 : 4 : 6-trioxypyrimidine).

(53) 4-N^1-Sulphanilamidouracil (Formula XIII).

(57) 2-N^1-Sulphanilamido-4-methylpyrimidine (Formula XIV).



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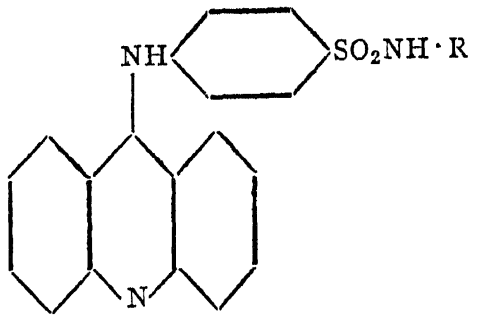

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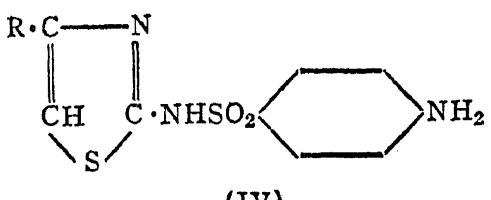

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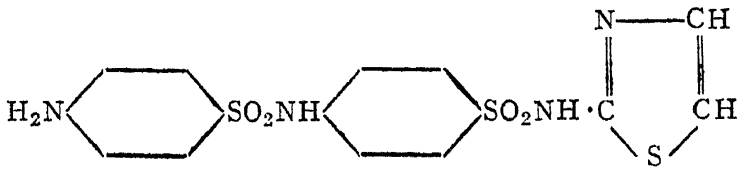

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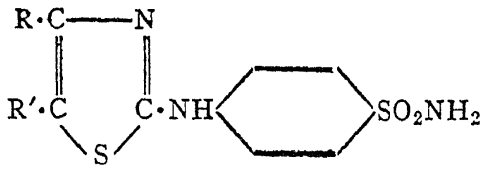

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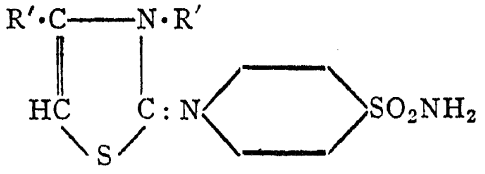

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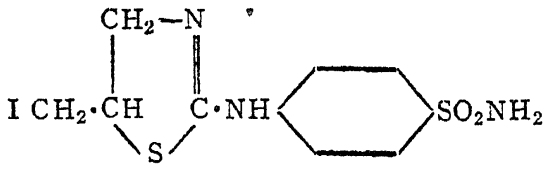

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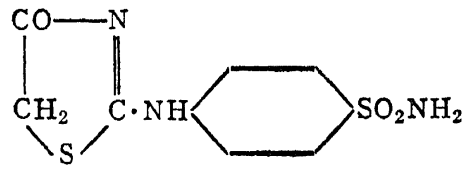

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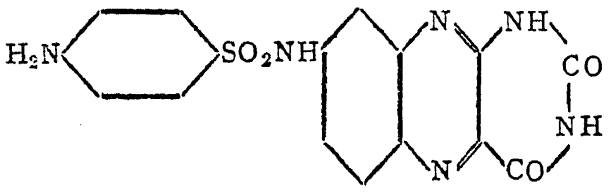

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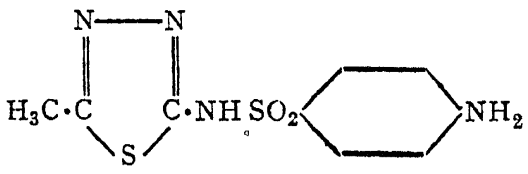

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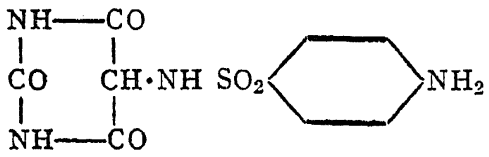

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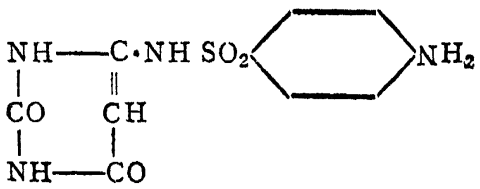

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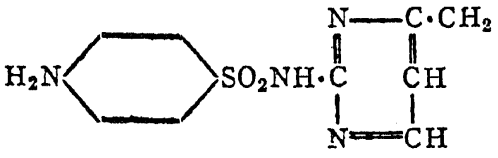

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Experimental Results

The method of testing has been described in detail in a previous publication. 'Richards' strain of β-hæmolytic streptococcus was used, the infecting dose being 0·5 c.c. of a 1 : 6 dilution of a 17-hr. old broth culture. In the case of the pneumococcus (type I), this dose was 0·5 c.c. of 1 : 2000 dilution of a 17-hr. old broth culture. The drugs were administered as suspensions (0·5 c.c.) in gum acacia.

The toxicity of each of the drug was tested first, to be sure that the doses administered were not themselves lethal. Many of the inactive ones were tested in doses as high as 50 mg.

The compounds 7, 8, 11, 21, 26, 27, 28, 34, 36, 37, 38, 39, 40, 41, 44, 46, 50, 53, 54 and 58 were very little or not active against both the infections. The results obtained with the other compounds are given below in the tables.

TABLE I

Therapeutic Effect in Experimental Streptococcal Infections in Mice

Experiment No.	Drug administered	No. of mice used	Dosage in mg.	No. of mice dying on each day after infection							Survivors	Average survival time (days: max. 10)
				1	2	3	4	5	6	7–10		
I	The drugs were administered at the time of infection and repeated 6 hours later and then once a day for 3 days more											
	S.A.	6	10	2	1	1	0	0	0	0	2	3·8
	1	6	5	3	0	0	0	0	1	0	2	4·5
	5	6	5	2	0	0	0	0	0	2	2	5·8
	6	6	5	0	0	1	1	0	3	0	1	5·0
	10	6	10	1	0	0	0	0	0	3	2	6·8
	13	6	10	0	0	0	0	0	0	2	4	9·0
	23	6	10	1	1	1	0	1	0	1	1	4·0
	24	6	10	0	1	1	0	0	2	1	1	5·0
	26	6	10	2	2	2	..	..	..	..	0	1·0
	C.	6	..	5	1	..	..	..	..	..	0	0·2
II	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 3 days											
	20	6	10	2	1	1	0	0	0	0	2	3·8
	45	6	10	2	1	0	0	0	0	0	3	5·1
	C.	6	..	6	..	..	..	..	..	..	0	0·0
III	The drugs were administered as in Experiment II											
	56	10	20	5	2	0	1	0	0	0	2	2·5
	57	10	10	7	0	1	0	0	0	0	2	2·2
	13	10	10	2	0	1	0	0	0	0	7	7·2
	C.	10	..	8	1	1	..	..	..	..	0	0·3

S.A.= Sulphanilamide.

C.= Controls (no treatment given).

TABLE II

Therapeutic Effect in Experimental Pneumococcal Infections in Mice

Experiment No.	Drug administered	No. of mice used	Dosage in mg.	No. of mice dying on each day after infection					Survivors	Average survival time (days: max. 10)
				1	2	3	4	5–10		
I	The drugs were administered at the time of infection and repeated 6 hours later and then once a day for 5 more days									
	S.P.	10	30	0	0	0	3	0	7	7·9
	13	10	30	0	0	1	1	0	8	8·5
	16	10	30	1	6	3	..	..	0	1·2
	24	10	30	2	5	3	..	..	0	1·0
	C.	10	..	7	3	..	..	..	0	0·3
II	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 3 more days									
	20	6	20	3	0	2	0	0	1	2·3
	21	6	20	3	2	1	0	0	0	0·7
	45	6	20	3	2	0	0	1	0	1·0
	C.	6	..	5	1	..	..	..	0	0·2
III	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 2 more days									
	33	12	20	0	0	0	3	1	8	7·8
	51	6	20	0	2	1	1	0	2	4·8
	C.	6	..	4	1	1	..	..	0	0·5
IV	The drugs were administered at the time of infection and repeated 6 hours later and then twice daily for 5 more days									
	57	10	5	0	0	0	1	3	6	8·2
	57	10	10	0	0	0	1	3	6	8·4
	13	10	10	3	1	0	0	3	3	4·8
	13	10	20	0	0	0	0	3	7	8·9
	C.	10	..	9	1	..	..	..	0	0·1

S.P.= Sulphapyridine.
C.= Controls (no treatment given).

It can be seen that the drugs 1, 5, 6, 10, 13, 20, 23, 24, 45 and 57 are at least as active as sulphanilamide against the streptococcus infection in the doses tried. But against pneumococcus infection only 13, 33 and 57 gave a very high and 56 and 51 moderate protection. It is very striking that the pyrimidine derivative, 57, in as low a dose as 5 mg. gives almost the same protection as sulphathiazole, 13, in 20 mg. doses. The rest of the compounds were inactive or only slightly prolonged the lives of the infected animals.

Discussion

The synthesis and study of the sulphanilamide derivatives undertaken, though they have for their primary objective the discovery of drugs superior

to sulphanilamide or sulphapyridine (2-sulphanilamidopyridine), also constitute a systematic attempt to explore the fundamental relationship existing between chemotherapeutic activity and molecular structure. The results presented above have revealed many significant facts in this direction.

In a previous publication,[4] we have produced evidence strongly supporting the hypothesis of Tréfouel *et al.*[5] that the therapeutic activity of 'prontosil' is due to sulphanilamide liberated from it *in vivo* by reduction. To investigate this question further, we tested the three azo dyes, 9, 10 and 21, prepared from sulphapyridine and sulphathiazole. It was intended to see whether the dyes are active in pneumococcus infection since they should owe their activity to the two parent drugs produced *in vivo*. While the dye 21 was almost inactive, dye 10 showed considerable antistreptococcal and very little antipneumococcal activity. Though, superficially, this appears to be argument against the hypothesis of Tréfouel *et al.*,[5] a closer scrutiny reveals the implications of these results. In the dosage used in the experiments the dye 10 produced blood concentrations of about 2 mg. per cent. of sulphapyridine and the dye 21 very little sulphathiazole, while in experiments wherein the drugs sulphapyridine and sulphathiazole themselves were employed, their corresponding blood concentrations were of the order of 10–25 mg. per cent. (unpublished observation). It has been shown by comprehensive experiments by Marshall that a blood concentration of 2–3 mg. per cent. of the above two drugs can give good protection against streptococcal infection, while only 7–9 mg. per cent. of it can do so in pneumococcal infection. Thus the good protection in streptococcal infection and very little protection in pneumococcal infection of the dye 10 and the very feeble activity of the dye 24 are just these to be expected according to the hypothesis of Tréfouel *et al.* It is because of the low blood concentration of sulphanilamide produced following the administration of 'prontosil' that this drug is not at all recommended in the treatment of severe infections. As has been pointed out by us previously,[4] a strong case has yet to be made to recommend the 'prontosils' or other similar azo dyes in preference to sulphanilamide for clinical trials.

A major number of the compounds which possess substituents at the (N^4)-amino radical of sulphanilamide or (N^1)-sulphanilamido derivatives, are all inactive probably because they do not undergo fission at the amino linkage to produce *in vivo* sulphanilamide or sulphanilamido derivatives. The inactive compounds, 11, 22; 7; 26, 27, 28; 34, 36, 37, 38, 39, 40, 41, 44, 46 come under this category. Two of the acridine derivatives, 23 and 24, reported are moderately active in streptococcal and very little active in pneumococcal infections. The activity of these two drugs is quite possibly

due to the liberation from them of the free sulphanilamide and sulphapyridine respectively *in vivo*, since such 9-substituted acridine derivatives are well known to undergo fission at this position. Since the production of adequate blood concentration of the sulphanilamido derivatives from these acridine compounds depends upon the rate of fission mentioned above as well as the degree of absorption in the system of the original acridine complex and since these factors naturally do vary with each compound, we find that of this class of compounds some are active while others are not. The therapeutic activity of the compounds 23 and 24 thus resembles that of N^4-benzylsulphanilamide (' proseptazine ') which owes its activity to sulphanilamide liberated from it *in vivo*.[7] A comparison of the two isomeric thiazole derivatives, sulphathiazole, 13, which is remarkably active and the compound 40 which is absolutely inactive—the difference between the two being the thiazole ring is substituted in the former at the (N^1)-sulphonamide radical and in the latter at the (N^4)-amino radical of sulphanilamide—clearly shows the importance of a free amino group as also the substitution of the specified heterocyclic ring at the sulphonamido group for the production of pronounced therapeutic activity.

Regarding all the compounds considered hitherto which form one group, wherein substituents anticipated to enhance the therapeutic property are introduced at the (N^4)-amino radical of sulphanilamide or sulphanilamido derivatives, we can conclude as follows: the therapeutic activity of these compounds appears to depend upon whether or not they can undergo fission at the (N^4)-amino linkage to yield free sulphanilamide or sulphanilamido derivatives *in vivo* and the degree of therapeutic effect depends upon the effective blood level of the latter produced *in vivo*. This conclusion just fits in with the various theories of the mechanism of action of the sulphanilamides that assign some important biochemical or physico-chemical functions to the free (N^4) amino group in the production of the therapeutic activity. The compounds of this class, whenever active—*e.g.*, ' prontosils ', ' proseptazine ', ' soluseptazine ', the Schiff's bases, etc.,—are less polyvalent in therapeutic action than the parent drugs, evidently because the effective blood levels of the active compounds produced *in vivo* following their administration are so low as to control only mild and not severe infections.[6]

Of the many aliphatic, aromatic and carbocyclic substituents tried at the sulphonamide radical of sulphanilamide, only the benzene derivatives have not decreased the therapeutic activity of sulphanilamide. The three isomeric nitro derivatives, 1, 5 and 6, of N^1-sulphanilamidobenzene were tested in view of the therapeutic significance attached to the nitro group by Rosenthal *et al.*[8] Though these compounds were somewhat more active

than sulphanilamide in streptococcal infection, they were more toxic. Their antipneumococcal effect compared to sulphapyridine or sulphathiazole was negligible. The compound 20 also possessed considerable anti-streptococcal but very little antipneumococcal effect. Taking all the N^1-sulphanilamidobenzene derivatives tested hitherto as a whole, it can now be generalised that though many derivatives are available somewhat superior to sulphanilamide in antistreptococcal effect, they possess no distinct advantages to replace sulphanilamide in practical therapy; further, they are nowhere near in therapeutic effect to sulphapyridine or sulphathiazole in pneumococcal infection.

N^4-sulphanilylsulphanilamide is reported by many groups of workers to be about as active as sulphanilamide. But the corresponding sulphanilyl derivatives of sulphapyridine 16 (formula I) and of sulphathiazole, 45 (formula V) are distinctly less active than the parent compounds. This appears to indicate that the molecular sizes of sulphapyridine and sulphathiazole appear to be the optimum for maximum therapeutic activity. While sulfanilylguanidine, 56, shows some distinct therapeutic activity, the similar sulfanilyl derivative of this compound, 58, is inactive. These instances indicate that the mere multiplication of the sulfanilamido groups in a compound does not help in obtaining mere potent compounds.

The conclusions drawn above together with the fact that sulphapyridine is more polyvalent in therapeutic effect than sulphanilamide, urged us to synthesise and test sulphanilamido derivatives with heterocyclic rings substituted in the sulphonamide radical. The thiazole derivative, 13, thus obtained has proved to be of outstanding value as has been previously reported.[1, 2, 3] We hope to test a series of related derivatives of this compound after our preliminary survey of the different rings is over. The thiodiazole derivative, 51 (formula XI), which very closely resembles the thiazole derivatives, 13 and 33 (formula IV), in molecular structure, appeared to be far less active than the latter in our preliminary experiments. The fact that pyridine and thiazole rings are present in some vitamins and coenzymes, led us to try the alloxazine derivative, 50, this ring system being present in the vitamin, riboflavin (which forms part of Warburg's "yellow enzyme" and also the coenzyme of amino oxidase); but this compound was found to be absolutely inactive. Some derivatives of the pyrimidine group (this ring system being present in vitamin B_1 connected to a thiazole derivative) have already been synthesised and tested. 5-Sulphanilamidobarbituric acid, 54, and 4-N^1-sulphanilamidouracil, 53, have been found to be inactive, while 2-N^1-sulphanilamido-4-methylpyrimidine, 57, is remarkably active. These agree with the results reported by Roblin *et al.*[9] A very detailed study

is being made with the 2-N^1-sulphanilamidopyrimidine derivatives synthesised here and these will be reported in a later communication.

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Summary

The results of testing thirty-six derivatives of sulphanilamide of various groups in experimental β-hæmolytic streptococcal and pneumococcal (type I) infections in mice have been presented. The therapeutic activity of the derivatives with substituents in the (N^4)-amino radical of sulphanilamide or sulphanilamido derivatives, appears to depend upon whether or not they can yield *in vivo* the latter compounds in adequate concentrations. In the attempts to discover highly active compounds, the study of the derivatives of sulphanilamide with suitable heterocyclic rings substituted at the sulphonamide radical have been made. Of the many such compounds so far tested, the sulphanilamidothiazole derivatives, 13 and 33, and the sulphanilamidopyrimidine derivative, 57, have been found to be of outstanding value. The significant relationship existing between the chemical constitution and chemotherapeutic action has been pointed out.

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