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THE SULPHONAMIDES: RELATIVE POTENCIES AND SPECIFICITY OF ACTION

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Woods showed that *p*-aminobenzoic acid antagonized the antibacterial action of the sulphonamides in a competitive manner. He suggested that *p*-aminobenzoic acid was an essential metabolite for bacteria and that the sulphonamides acted by interfering with this metabolite. It has since been shown that *p*-aminobenzoic acid is an essential metabolite for bacteria (see Woods and Nimmo-Smith, 1949) and the view that the sulphonamides act by interfering with the utilization of *p*-aminobenzoic acid has come to be generally accepted. An extension of this theory is that the action of the sulphonamides is " non-specific " and that the sulphonamides have the same relative potencies against different organisms (Hawking and Stewart Lawrence, 1950). These authors use the expression, " sulphanilamide coefficient," to depict the relative activity of a sulphonamide, as though it can be used as a general term that can be applied to the relative potency of a drug against any organism.

The concept of the " non-specificity " of action of the sulphonamides has been able to grow up and persist because most people have only studied the action of one or of a very few sulphonamides against a variety of organisms, or of a large number of sulphonamides against one or a very few organisms. In particular both streptococci and sulphanilimide have not been included in many recent studies. No single paper has been found in which the relative potencies of all the common sulphonamides have been compared *in vitro* against a number of pathogens. It is the purpose of this paper to present such information and to show that there are large differences in the relative potencies of the sulphonamides against different organisms. Similar data on the relative blood-concentrations produced by the sulphonamides in a variety of species have been given by Francis (1949).

METHODS

Falling threefold dilutions of the drugs were prepared, the concentration in the first tube being 1/100. These dilutions were added in 0.2 ml. amounts to 1.8 ml. of medium containing agar, to give slopes containing 1/1,000, 1/3,000, etc., of the drugs. " Lab-Lemco " was used as a basis of the medium for the experiments shown in Table I and beef infusion for those shown in Table II. Evans's peptone was used and horse blood, as it has been shown that horse blood tends to neutralize sulphonamide inhibiting substances, whereas blood from some other species does not (Harper and Cawston, 1945). The blood was used at a concentration of 5 per cent (v/v) and 1 per cent (w/v) glucose was also added to ensure that all organisms would grow well. The agar was

maintained at 70 to 80° C. during distribution, which led to the production of "chocolate" agar. A small series of experiments was carried out in milk containing 1 per cent (w/v) glucose; bromcresol-purple was present in the medium and the results were judged by changes in the colour of the indicator. The results are included in Table I. Broth cultures which were incubated overnight were used for inoculating all media, a loopful of these cultures being streaked on to the slopes or inoculated into the milk.

The experiments shown in Table I were carried out with one batch of Lemco medium and all organisms were included in each of three consecutive tests. In the first series of three tests shown in Table II one batch of medium was used and it was sown with *Str. pyogenes, Str. pneumoniae, Past. septica,* and *Bact. coli.* In a second series of three tests the medium was sown with *Salm. cholera suis, N. gonorrhoea, N. meningitidis,* together with *Str. pneumoniae* to act as a link with the first series. The growths in each tube were recorded as 0, 1, 2, or 3, and similar endpoints were obtained with the respective drugs and organisms in each of the three experiments in the two series. The average growth in tubes 1, 2, etc., in the three tests of each series was calculated. A curve was constructed for each drug over the critical range and the average concentration giving 50 per cent growth found by interpolation. This concentration was expressed in "tube numbers." All averages were then worked out on the basis of "tube numbers," i.e., a log. scale was used and these averages were converted to the actual dilutions shown in Tables I and II. Thus, if 50 per cent growth occurred in tube 3, the concentration would be 1/9,000 (dilution in first tube 1/1,000), whereas if the average result were 3.5 the concentration would be 1/15,590. It will be seen that the average 50 per cent endpoint with all drugs, against *Str. pneumoniae,* was at a dilution of 1/217,700 in the first series of experiments and 1/243,000 in the second. There was just over a twofold difference in the sulphanilamide coefficient with some drugs, but this is less than the threefold difference between two consecutive tubes. These findings indicate the degree of variation and that all the results in Table II can be considered together.

Results

In the following account 4 : 4'diaminodiphenyl sulphone is referred to as sulphone and is included under the general term "sulphonamide." It is not, of course, a sulphonamide, but its action is strongly antagonized by *p*-aminobenzoic acid.

The results in Table II are considered first because they are felt to be more in accord with those obtained by other workers. It will be seen that the organisms can de divided into two distinct groups on the basis of the average "sulphanilamide coefficient" of the heterocyclic drugs obtained when they are used. With streptococci, pneumococci, and gonococci the coefficient varied from 2.3 to 12.6, but with the other organisms from 35 to 139. The compounds can be divided into the non-heterocyclic sulphonamides (sulphanilamide, sulphaguanidine, and sulphone), and the remainder, which are heterocyclic. Sulphone was more active than any other compound against *Str. pneumoniae,* and against *Str. pyogenes,* with the exception of sulphathiazole, which gave particularly good results in this series of experiments. Against all other organisms the non-heterocyclic sulphonamides were less active than the heterocyclic.

If only the heterocyclic sulphonamides are considered it will be noted that they were nearly always placed in the same order of activity : the order shown in Tables I and II, sulphapyridine being the least potent and sulphathiazole the most potent. The exceptions were sulphamezathine against *Str. pyogenes* and sulphadiazine against

TABLE I

THE INHIBITORY ACTION OF THE SULPHONAMIDES *in vitro*: AVERAGE RESULTS OF THREE CONSECUTIVE TESTS

Organism		Dilutions giving 50% inhibition									Sulphanilamide coefficient (A)							
		Sulphanilamide	Sulphaguanidine	Sulphone	Sulphapyridine	Sulphamezathine	Sulphamerazine	Sulphadiazine	Sulphathiazole	Logarithmic average	Sulphaguanidine	Sulphone	Sulphapyridine	Sulphamezathine	Sulphamerazine	Sulphadiazine	Sulphathiazole	Average of heterocyclic sulphonamides (B)
Str. pyogenes (Lancefield Group A)	Tube No.	3.5	3.7	4.7	3.5	3.5	3.9	4.7	4.7	4.0	1.2	3.7	1.0	1.0	1.6	3.7	3.7	2.2
	Dilutions†	15,600	19,400	58,200	15,600	15,600	25,200	58,200	58,200	27,000								
Str. pneumoniae (7465)*	Tube No.	2.9		5.6	4.8	4.0	4.3	4.3	3.5	4.2		19.3	8.0	3.3	4.6	4.6	1.9	4.5
	Dilutions	8,100		156,500	65,000	27,000	37,500	56,600	33,600									
Past. septica (D.B.183)	Tube No.	3.4	4.7	4.6	6.1	6.8	7.4	8.4	6.5	6.0	4.2	3.7	19.4	41.8	77.1	242.3	30.1	82.1
	Dilutions	14,000	58,200	52,200	271,100	585,100	1,080,000	420,800	243,000									
Salm. cholerae suis (Kunzendorf)	Tube No.	1.2	1.7	2.9	3.9	4.5	5.0	5.7	5.6	3.8	1.8	6.8	19.1	38.8	67.5	145.6	109.3	76.1
	Dilutions	1,200	2,200	8,100	22,900	46,700	81,000	174,700	156,600	21,700	(1.7)	(3.4)	(15.6)	(14.0)	(41.9)	(140.3)	(72.6)(C)	
Salm. dublin	Tube No.	0	0.4	0.9	1.9	2.2	2.5	3.2	3.0	1.7	3.0	8.0	25.0	37.0	52.0	106.0	90.0	62.0
	Dilutions	100	300	800	2,500	3,700	5,200	10,600	9,000	2,200	(2.4)	(1.7)	(11.2)	(14.0)	(14.0)	(52.2)	(52.2)(C)	
Logarithmic average	Tube No.	2.2	2.6	3.7	4.0	4.2	4.6	5.2	4.6	3.9	1.6	5.2	7.2	8.9	13.9	26.9	13.9(D)	
	Dilutions	3,747	5,799	19,420	27,000	33,620	52,190	100,900	52,190	25,200								

* National Collection of Type Cultures.

† The denominators of dilutions are shown.

A. The sulphanilamide coefficient is obtained by dividing the denominator of the dilution giving 50% inhibition with sulphanilamide by the denominator of the dilution of any other drug giving 50% inhibition.

B. The average sulphanilamide coefficient of the heterocyclic sulphonamides is obtained by averaging the individual coefficients.

C. Figures in brackets represent the average sulphanilamide coefficients in four series of tests in which the organisms were grown in milk containing 1% glucose: bromcresol-purple was present in the medium and the results were judged by changes in the colour of the indicator.

D. The average sulphanilamide coefficients on the bottom row are obtained from the logarithmic averages.

TABLE II

THE INHIBITORY ACTION OF THE SULPHONAMIDES *in vitro*: AVERAGE RESULTS OF THREE CONSECUTIVE TESTS

Organism		Dilutions giving 50% inhibition									Sulphanilamide coefficient (A)							
		Sulphanilamide	Sulphaguanidine	Sulphone	Sulphapyridine	Sulphamezathine	Sulphamerazine	Sulphadiazine	Sulphathiazole	Log. arithmic average	Sulphaguanidine	Sulphone	Sulphapyridine	Sulphamezathine	Sulphamerazine	Sulphadiazine	Sulphathiazole	Average of heterocyclic sulphonamides (B)
Str. pyogenes (Lancefield Group A)	Tube No.	4.3	4.4	5.6	4.4	3.9	4.3	5.1	6.0	4.8	1.1	4.2	1.1	0.6	1.0	2.4	6.5	2.3
	Dilutions†	37,530	41,890	156,600	41,890	24,180	37,530	90,380	243,000	61,530								
Str. pneumoniae (7465)*	Tube No.	4.4	5.6	7.2	5.3	5.7	5.9	6.1	7.0	5.9	3.7	21.6	2.7	4.2	5.2	6.6	17.4	7.2
	Dilutions	41,890	156,600	907,800	112,600	174,700	217,700	277,100	729,000	217,700								
Str. pneumoniae (7465)	Tube No.	4.2	5.5	7.5	5.7	6.3	5.8	5.5	7.5	6.0	4.1	37.6	5.2	10.1	5.8	4.1	37.6	12.6
	Dilutions	33,620	140,300	1,263,000	174,800	337,800	195,000	140,300	1,263,000	243,000								
Neisseria gonorrhoea (6820)	Tube No.	2.8	3.5	3.8	4.3	4.3	4.5	5.4	5.0	4.2	2.2	3.0	5.3	5.3	6.5	17.4	11.2	9.1
	Dilutions	7,224	15,590	21,670	37,530	37,530	46,770	125,700	81,000	33,620								
Neisseria meningitidis (3372)	Tube No.	3.0	4.3	5.1	5.5	5.4	6.4	7.4	8.0	5.6	4.2	10.1	15.6	13.9	42.0	125.7	243	88.0
	Dilutions	9,000	37,530	90,380	140,300	125,700	377,700	1,131,000	2,187,000	163,600								
Past. septica (D.B. 183)	Tube No.	2.4	3.2	3.4	4.0	5.0	5.2	5.9	6.4	4.4	2.4	3.0	5.8	17.4	21.7	46.8	80.9	34.5
	Dilutions	4,655	11,210	13,960	27,000	81,000	100,900	217,700	377,000	43,770								
S. cholerae suis (Kunzendorf)	Tube No.	2.7	3.5	4.4	6.2	6.2	7.3	7.4	7.8	5.7	2.4	6.5	46.8	46.8	156.6	175.0	271.0	139.2
	Dilutions	6,473	15,590	41,890	302,600	302,600	1,014,000	1,131,000	1,755,000	172,800								
B. coli (Evans 235)	Tube No.	2.9	2.7	4.0	4.9	5.5	6.5	6.5	6.5	4.9	0.8	3.3	9.1	17.4	52.2	52.2	52.2	36.6
	Dilutions	8,063	6,473	27,000	73,620	140,300	420,800	420,800	420,800	75,820								
Logarithmic average	Tube No.	3.3	4.1	5.1	5.0	5.3	5.7	6.2	6.8	5.2	2.3	6.9	6.2	8.6	13.4	23.1	44.8 (C)	
	Dilutions	13,070	30,130	90,380	81,000	112,600	174,800	302,600	585,100	100,900								

* National Collection of Type Cultures.

† The denominators of dilutions are shown.

A. The sulphanilamide coefficient is obtained by dividing the denominator of the dilution giving 50% inhibition with sulphanilamide by the denominator of the dilution of any other drug giving 50% inhibition.

B. The average sulphanilamide coefficient of the heterocyclic sulphonamides is obtained by averaging the individual coefficients.

C. The average sulphanilamide coefficients in the bottom row are obtained from the logarithmic averages.

N. gonorrhoea. There was no difference in the action of sulphamerazine, sulphadiazine, and sulphathiazole against *Bact. coli,* whereas there was a fivefold difference with some other organisms.

A comparison of Tables I and II shows that there were big changes in the order of potency when " Lab-Lemco " was used as a basis for the medium instead of beef infusion. The most striking difference between the two series is that sulphathiazole was much less potent in the first series than in the second. This difference was so striking that the stock solution of sulphathiazole, which had been in the refrigerator since the earlier series of tests, was included on two occasions in the second series; it was as active as the current stock solution of sulphathiazole. It would appear that there was some difference between the media in the two series of experiments that specifically influenced the action of sulphathiazole. The sulphanilamide coefficients, except with sulphathiazole, were much larger against *Past. septica* in the first series of experiments than in the second. It will be seen that the sulphanilamide coefficients obtained in milk (Table I) were consistently lower than in the chocolate agar medium with " Lab-Lemco " as a basis.

It has been shown that the results obtained with the different organisms shown in Table II may be compared, and it will be seen that sulphathiazole was about seven times as active against *Salm. cholerae suis* as against *Str. pyogenes,* but sulphanilamide was about a fifth as active against the former as against the latter organism. As already stated, there is little difference in the relative potencies of the different sulphonamides against streptococci, but the action of the heterocyclic sulphonamides is much better than that of the non-heterocyclic sulphonamides against some of the Gram-negative organisms.

Discussion

The results reported in this paper are supported by those of other workers. Thus Lauger, Suter, and Martin (1944) found that the action of one sulphonamide on a particular micro-organism does not guarantee its action on another. Compounds tested against pneumococci, streptococci, and *Bact. coli* may inhibit one, two, or all three of the micro-organisms. McIntosh and Dyrsdale (1945) described a meningococcus that was sensitive to sulphamezathine and sulphathiazole but not to sulphapyridine. Recently, Schweinburg and Rutenburg (1949) have described six different cases of bacterial infections that proved susceptible to one sulphonamide after failing to respond to another. The bacteria were shown to be susceptible to the effective sulphonamide but not to the others. Schmidt, Sesler, and Hughes (1944) found the sulphapyrimidine drugs to be about equally active against streptococci and Friedländer's bacillus, *Bact. coli,* and shigella species. On the other hand, sulphamezathine was twice as active as sulphadiazine or sulphamerazine against pneumococci and staphylococci.

Wyss, Grubaugh, and Schmelkes (1942) and Havinga, Julius, Veldstra, and Winkler (1946) obtained different relative potencies against the same organism in different media, and a large number of results collected from the literature by Stewart Lawrence and Francis (1952) show that the reported sulphanilamide coefficients of sulphathiazole against *Bact. coli* vary from 25 to 250 with a mean of 53.0, which is very close to the mean of 52.2 obtained in the present study. It should perhaps be stated here that although the figure of 50 is often quoted as the sulphanil-

amide coefficient of sulphathiazole the maximum coefficient observed *in vivo*, after corrections have been made for differences in blood concentrations, is only 13.3.

Schmidt and Sesler (1946) pointed out that the evidence supporting the " non-specific " action of the sulphonamides had never been very conclusive. They showed that with the sulphanilanilides the antagonistic action of *p*-aminobenzoic acid could be demonstrated with *Bact. coli* and Friedländer's bacillus, but not with pneumococci. Like sulphones, the sulphanilanilides are much more effective against Gram-positive than against Gram-negative organisms. Sulphadiazine was only twice as active as sulphanilamide against pneumococci *in vitro*, but 32 times as active against Friedlander's bacillus. Tamura (1944) showed that *p*-aminobenzoic acid did not inhibit the bacteriostatic action of the sulphonamides against *B. tularense*.

Recent work on the mode of action of the sulphonamides has been reviewed by Dible and MacLennan (1951). It is now known that *p*-aminobenzoic acid is concerned in the metabolism of folic acid, and it seems probable that the action of the sulphonamide is primarily directed against the metabolic conversion of *p*-aminobenzoic acid into pteroyl-glutamic acid or possibly into pteroic acid. The fact that two drugs are inhibited by *p*-aminobenzoic acid does not prove that they act in the same way. Thus, the action of a drug as dissimilar from the sulphonamides as proguanil may be inhibited by *p*-aminobenzoic acid (Thurston, 1950), and Yegian and Long (1951) showed that, although *p*-aminosalicylic acid and the sulphonamides are both inhibited by *p*-aminobenzoic acid, tubercle bacilli made resistant to *p*-aminosalicylic acid are not resistant to the sulphonamides or sulphone. They indicate that there may be similar specific differences between the sulphonamides, and this is known to be true (Schweinburg and Rutenburg, 1949).

The position has become very complex, but it may eventually be possible to explain the various specificities in the actions of the sulphonamides. In the meantime it appears that such simple conclusions as those put forward by Bell and Roblin (1942) are not tenable. They state that the more negative the SO_2 group of an N^1-substituted sulphanilamide derivative, the greater is its bacteriostatic potency, which leads them to state that, if the relative electron attracting power of the N^1-substituent is known, it is possible for the first time to predict the bacteriostatic potency of any new sulphanilamide derivative of this type. This is probably true against *Bact. coli* in a defined medium but not against all organisms in that medium or even against one organism in different media.

Summary

1. The relative potencies of the common sulphonamides and 4 : 4′diaminodiphenyl sulphone (sulphone) were compared *in vitro* against a number of pathogens.

2. In the most satisfactory series of experiments (Table II) the average sulphanilamide coefficient of the heterocyclic sulphonamides was only 2.3 against streptococci but 139 against *Salm. cholerae suis* and 36 against *Bact. coli*.

3. Sulphone was more active than any of the sulphonamides, except sometimes sulphathiazole, against streptococci, but it was less active than the heterocyclic sulphonamides against Gram-negative organisms.

4. The significance of these findings is discussed.

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