THE USE OF SYNTHETIC MEDIUM AS AN IN VITRO TEST OF POSSIBLE CHEMOTHERAPEUTIC AGENTS AGAINST GRAM-NEGATIVE BACTERIA

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Various workers, including Long and Bliss (1937, 1940), Lowell, Strauss and Finland (1940), and Lawrence (1940) employed *in vitro* methods in comparing the bacteriostatic and bactericidal activity of sulfonamides. Their studies usually dealt with gram-positive bacteria such as streptococci and pneumococci since gram-negative bacteria were found to be resistant to the earlier known sulfonamides. However, some gram-negative bacteria were studied *in vitro* by Britton (1938), Libby and Joyner (1940), Kempner, Wise, and Schlayer (1940) and Strauss and Finland (1941b), although their experiments involved only the few earlier known sulfonamides.

In our laboratory, gram-negative bacteria representing the groups of bacteria causing cholera, plague, typhoid and dysentery have been used in *in vitro* tests of a number of compounds including the most recently developed sulfonamides. The organisms used in our experiments were: *Vibrio cholera*, *Pasteurella multocida*, *Shigella dysenteriae*, *Eberthella typhosa* and *Salmonella pullorum*. A description of the strains of these bacteria is given in table 1. All of these organisms grew well in synthetic medium described in table 2. This synthetic medium has several advantages over the yeast extract and peptone broth which we employed in earlier studies. Since all of the ingredients were highly purified, successive lots of medium were always similar in nutritive value and equally free of substances which might interfere with sulfonamide activity (as suggested by Chu and Hastings (1938), Woods (1940), Strauss and Finland (1941a) and Goetchius and Lawrence (1944) when crude media constituents were used).

This synthetic medium has been employed over a period of two and one-half years and has been found consistently satisfactory in our tests with each of the gram-negative organisms described in this report.

The compounds to be tested were weighed in such amounts that when dissolved, their concentration was approximately 24 to 25 mg per cent. Water soluble chemicals were dissolved in distilled water. The other chemicals were dissolved in non-toxic quantities (about 3%) of either denatured ethyl alcohol or methyl cellosolve and then diluted with distilled water. The pH was adjusted to 6.8 to 7.0 with sodium hydroxide or hydrochloric acid. Most of the chemical solutions were sterilized by autoclaving for twenty minutes at 15 pounds pressure. Chemicals susceptible to alteration by heat were either Seitz filtered or pasteurized.

4.5 ml of the test compound solution were added to triplicate tubes of 0.5

TABLE 1

Description of Strains of Gram-negative Bacteria Used in In Vitro Tests in Synthetic Medium

DISEASE REPRESENTED	ORGANISM SELECTED	HISTORY
Cholera	*Vibrio cholera*, strain 105	Used in the production of cholera vaccine (avirulent)
Plague	*Pasteurella multocida*, strain 449	Used in production of veterinary antisera (pathogenic for animals but not for man)
Typhoid	*Eberthella typhosa*, strain 45	Used in the production of typhoid vaccine (virulent)
Fowl Pullorum	*Salmonella pullorum*, strain 651	Used in the production of diagnostic antigens (nonpathogenic for man but highly virulent for chickens)
Dysentery	*Shigella dysenteriae*, Strain 150	Used in production of diagnostic antigens (pathogenic for man)

TABLE 2

Synthetic Medium Used in the In Vitro Test of Gram-negative Bacteria

Ingredients A	
Sodium chloride	0.5 gram
Ammonium sulfate	5.0 grams
Potassium phosphate, monobasic	2.0 grams
Asparagine	1.0 gram
**dl*-Tryptophane	0.5 gram
**l(+)Glutamic acid	0.5 gram
† Dextrose	2.0 grams
† Cystine	1.0 mg
† Calcium pantothenate	0.05 mg
† Thiamin	0.25 mg
† Nicotanamide	0.125 mg
† Biotin	0.02 microgram
† Glycine	4.0 mg
Final volume with distilled water	1000.0 ml

* Are each separately ground in mortars with 10 N sodium hydroxide and hot distilled water before being added to Ingredients A.

† Are sterilized by separate Seitz filtrations and are added aseptically to Ingredients A.

Ingredients A and **dl*-Tryptophane and * l(+)Glutamic acid are dissolved in distilled water, the pH adjusted to 6.8 to 7.0 with sodium hydroxide, sterilized by autoclaving at 15 pounds pressure for 20 minutes, and cooled before the other ingredients are added to it.

NOTE: This medium was tubed aseptically in 5 ml aliquots by a means of a Brewer filling machine.

Also, this medium was prepared in concentrated form by diluting the ingredients to a total volume of 100 ml.

ml of concentrated synthetic medium, and two of the tubes were inoculated with 0.2 ml of the 18–24 hour standardized culture. The third tube was saved as an uninoculated control. The tubes were incubated for 48 hours at 37 C and then compared to the growth control for evidence of bacterial growth. When the uninoculated control tube appeared discolored or cloudy, bacterial growth was determined by sub-culturing in synthetic medium, incubating for 48 hours at 37 C and again observing for evidence of bacterial growth. Only the bactericidal end-points were recorded.

If inhibition of the growth of the organisms had been observed, the chemicals were retested to determine the least concentration of the compound necessary to cause bactericidal activity. The tubes were set up in triplicate and diluted serially. Two tubes were inoculated as described below and the remaining uninoculated tube was included as a control.

A description of the strains of gram-negative bacteria used in our experiments is given in table 1. Stock cultures were maintained on brain heart infusion agar consisting of 3.7 per cent brain heart infusion (Difco product) and 1.5 per cent agar. They were incubated at 37 C for 24 hours and then stored in the chill room. Subcultures were made at intervals of not more than 7 days. To prepare the organisms for tests, a loopful of stock culture was transferred to yeast extract peptone broth (1% peptone, 0.5% yeast extract, 0.3% potassium phosphate dibasic). The broth cultures were then incubated at 37 C for 24 hours and 0.2 ml amounts were immediately transferred to 5 ml of our synthetic medium and incubated for 48 hours. 0.2 ml of this culture was transferred to 5 ml of synthetic medium and incubated for 18 to 24 hours. This final culture was used for inoculating the tubes containing chemicals under test. However, it was desirable to use as the test inoculum only the cultures that have been repeatedly transferred in synthetic medium in preference to those that were only two subcultures away from yeast extract peptone broth. The inoculum was made by diluting 18 to 24 hour cultures of the above preparation with synthetic medium until the following readings were obtained on the Lumetron colorimeter, using filter #B530: synthetic medium—80, *Pasteurella multocida*—64 to 68, the other gram-negative organisms tested—72 to 76.

The compounds tested *in vitro* are from the following chemical groups: acid, alcohol, amine and amide, coumarin, ester, ether, guanide, guanidine, hydantoin, imidazole, ketone, melamine, methane, nitrile, oxazolidine, phenol, piperazine, piperidine, pterin, pyridine, quinone and quinoline, sulfonamide, sulfide, sulfone, urea, dyes (acridine, amino derivatives of triphenylmethane, anthraquinone, azine, derivatives of diphenylnaphthylmethane, derivatives of natural organic compounds, dis-azo from diamines, dis-azo and tris-azo, fluorine, hydroxy derivatives of triphenylmethane, mono-azo, nitro and nitroso, oxazine, rhodamine, stilbene, thiazine, thiazole, triazine and miscellaneous), and miscellaneous compounds. However, the results of the active compounds are listed in table 3. 25 mg per cent is the highest concentration of each chemical tested.

TABLE 3

Compounds Showing Bactericidal Activity Against Gram-negative Organisms in Synthetic Medium

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
β-Benzoylacrylic acid	5.8	<0.7	5.8	5.8	11.7
Methylene-bis-gallic acid	23.3	11.7	none	11.7	23.3
Methylene-bis-salicylic acid	5.8	2.9	23.3	5.8	5.8
Methylene-bis-3-hydroxy-2-naphthoic acid	2.9	<0.7	23.3	1.4	<0.7
β-Acetylacrylic acid	none	11.4	none	none	none
Nordihydroguaiaretic acid	3.5				
Tetraacetylnordihydroguaiaretic acid	none	4.2	none	none	16.6
4′-Methoxy-2,4-diaminodiphenylamine Dihydrochloride	59.0				
4′-Hydroxy-4-amino-diphenylamine	41.0				
4′-Ethoxy-4-aminodiphenylamine Hydrochloride	46.1				
β-Hydroxy-β-phenylethyldimethylcetylammonium Bromide	2.7				
Dimethylcetyl-β-hydroxyethyl-ammonium Bromide	5.0	0.2	0.2	0.2	0.2
Dimethyl-hydroxyethyl-*o*-stenolmethyl ammonium Chloride	2.5				
p-Aminodiphenyl	23.3	5.8	23.3	23.3	11.7
p-Phenylenediamine	2.9	<0.7	2.9	1.4	1.4
N-1″-Naphthalene-4″-sulfonic acid-*N*′-naphthonyl-benzidine	none	2.9	none	none	none
4′-Methoxy-2,6-dichloro-aminodiphenylamine	none	11.3	none	none	2.8
iso-eugenol	none	5.8	none	23.3	23.3
p-Aminophenol	<0.7	<0.7	<0.7	<0.7	<0.7
Methylene-bis-4-chloro-resorcinol	5.8	<0.7	2.9	5.8	2.9
Di-isobutyl-resorcinol	11.7	1.4	5.8	23.3	1.4
Chloro-di-isobutyl-resorcinol	5.8	<0.7	5.8	5.8	<0.7
m-Aminophenol	none	1.4	none	none	23.3
o-Aminophenol	2.9	<0.7	2.9	1.4	2.9
1-Phenyl-2(4-pyridyl)acetylene	none	2.9	11.7	none	5.8
1-Methyl-2,4,6-triphenyl-4-pyridol Hydrochloride	none	24.8	24.8	24.8	24.8
2-Methyl-3-amino-1,4-naphthoquinone	10.6				
Dinitrohydroquinone Monoacetate	none	22.5	none	none	none
1,4-Di(4′-methyl-2′-sulfoaniline)-anthraquinone	none	23.3	none	none	none
Potassium Benzoquinone Sulfonate	none	11.7	23.3	none	none
Oxyhydroquinone Triacetate	22.2				
1-Hydroxy-2,4-dianilino-anthraquinone	none	0.5	21.1	none	10.5
Sulfanilamide	none	none	none	none	none
Sulfadiazine	23.3	5.8	23.3	11.7	none
Sulfamethazine	none	<0.7	none	11.7	none
Sulfapyridine	11.7	23.3	none	none	23.3
5-Sulfanilamido-2-aminopyridine	none	22.5	none	none	none
5-Sulfanilamido-2-bromopyridine	11.7	11.7	none	11.7	2.8
5-Sulfanilamido-2-chloropyridine	11.7	11.7	none	22.5	22.5
2-Sulfanilyl-5-aminopyridine	2.8	23.3	none	23.3	23.3
Sulfamerazine	11.7	11.7	11.7	11.7	none

TABLE 3—*Continued*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
2,5-Dinitrosulfanilamide	2.8	1.4	22.5	2.8	2.8
2-Chloro-*N'*-(2-pyrimidyl) sulfanilamide	none	11.2	none	none	none
N'-(2-Hydroxypropyl)sulfanilamide	none	24.3	24.3	none	none
N^4-Sulfanilylsulfanilamide	11.7	none	none	23.4	none
N'-Sodium-*N'*,N^4-disulfanilyl-sulfanilamide	none	23.3	none	none	none
N',*N'*-Di-(2-hydroxyethyl)-N^4-disulfanilylsulfanilamide	none	23.8	23.8	none	23.8
N'-(2-*N*-Morpholinophenyl) sulfanilamide	none	23.3	none	none	none
N'-(3,4-Dimethylbenzoyl) sulfanilamide ("Irgafen")	23.3	23.3	none	11.7	none
N'-Methyldisulfanilamide	none	23.8	none	none	none
N'-2-Hydroxyethyl-N^4-sulfanilylsulfanilamide	none	23.3	none	none	none
N'-(2'-Dimethylaminophenyl) sulfanilamide	none	11.7	none	none	none
N'-(4-Dimethylaminophenyl) sulfanilamide	23.3	23.3	none	23.3	23.3
N'-Phenyl-*N'*-(2-hydroxyethyl) sulfanilamide	23.3	none	none	none	none
N'-(2-Phenylcinchoninyl) sulfanilamide	none	23.3	none	none	none
2,5-Bissulfanilamido-benzene-sulfonic acid	none	23.3	none	none	none
Sulfanilamidothymol	5.8	<0.7	<0.7	5.8	2.9
2-Sulfanilamido-5-chloropyridine	11.7	24.8	24.8	24.8	24.8
2-Sulfanilamido-5-bromopyridine	11.7	11.7	23.3	5.8	23.3
2-Sulfanilamido-5-iodopyridine	5.8	<0.7	none	5.8	1.4
2-Sulfanilamido-5-nitropyridine	5.8	5.8	5.8	11.7	5.8
2-Sulfanilamido-5-aminopyridine	none	11.7	none	none	none
3-Sulfanilamido-pyridine	11.7	2.9	none	11.7	5.8
2-Sulfanilamido-4-methyl-thiazole	1.3	10.6	2.6	2.6	<0.7
6-Sulfanilamido-cinchophen	none	23.3	none	none	none
2-Sulfanilamido-4-methyl-pyrimidine	11.7	11.7	11.7	11.7	none
2-Sulfanilamido-4-methoxy-pyrimidine	none	23.3	none	none	none
2-Sulfanilamido-4-ethoxy-pyrimidine	none	23.3	none	none	none
2-(Sulfanilamido)-4-amino-5-bromopyridine	11.3	2.8	none	22.5	none
2-(Sulfanilamido)-5-bromo-4,6-dimethyl pyrimidine	none	22.5	none	none	none
2-Sulfanilamidopyrazine	11.7	11.7	5.8	11.7	23.3
2-Sulfanilamidothiazole-5-carboxylic acid	none	23.3	none	none	none
2-Sulfanilamido-4-methyl-5-carboxy thiazole	23.3	23.3	11.7	23.3	23.3
2-Sulfanilamido-4-*tert*-butylthiazole	none	5.8	none	none	none
Sulfanilamide Hexylresorcinol	none	<0.7	none	11.7	11.7
2-(*p*-Azido-benzenesulfonamido)-pyridine	none	11.7	none	none	none
p-Nitrobenzenesulfonamide	23.3	2.9	none	none	11.7
N'-Methyl-4-nitrobenzene-sulfonamide	none	5.8	none	none	5.8
4,4'-Diaminodiphenylsulfone-2-sulfonamide	none	23.3	none	none	none

TABLE 3—*Continued*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
2-Aminopyridine-5-azobenzene-*p*-sulfonamide	none	11.7	none	none	none
1-(Sulfanilyl)-3-methyl-5-pyrazolone	none	23.3	none	none	none
N^2-Sulfanilylorthanilamide	23.3	none	none	none	none
2-Sulfanilylhydroquinone	11.7	1.4	2.9	11.7	2.9
N^2,N^8-Bis(acetylsulfanilyl) acriflavine	none	5.8	23.3	23.3	23.3
Sodium *N*-Sulfanilylanthranilate	none	23.3	none	none	none
2-Sulfanilyl-5-aminopyridine	none	23.3	none	23.3	23.3
N^4-Sulfanilylsulfanilyl-anthranilic acid	none	23.8	23.8	none	none
Sodium-*N*-Sulfanilylnaphthionate	none	23.3	none	none	none
Sodium Strepto-*n*-disulfanilyl-sulfanilate	none	23.3	none	none	none
N^2,N^8-Bissulfanilylacriflavine	none	23.3	none	none	none
Disulfanilylbenzamidine	none	23.3	none	none	none
N'-Sulfanilyl-N^3-aminoguanidine	none	23.3	none	none	none
Disulfanilyl-4,4'-diamino-stilbene-2,2'-disulfonic acid	none	23.3	none	none	none
Sodium-*p*-nitrobenzene-sulfonylcyanamide	none	23.3	none	none	none
p-Nitrobenzenesulfonyl-*p*-toluamidine	none	11.7	23.3	none	23.3
p-Amino-β-(*p*-aminophenyl-sulfonyl)-styrene	none	23.3	none	none	none
Bis(3-aminophenylsulfonyl)-2-amino-5-chloropyrimidine	none	23.3	none	none	none
p-(β-Piperidinoethylsulfonyl) aniline	none	11.7	none	none	23.3
p-(β-iodoethylsulfonyl) aniline Hydrochloride	23.3	11.7	23.3	23.3	23.3
p-(β-Diethylaminoethylsulfonyl)aniline	23.3	5.8	23.3	23.3	23.3
p-(β-Carboxyethylsulfonyl)aniline Hydrochloride	none	23.3	none	none	none
β-(*p*-Aminophenylsulfonyl) acrylic acid Hydrochloride	5.8	1.4	none	5.8	2.9
p-(β-Tosylethylsulfonyl)acetanilide	23.3	2.9	none	none	23.3
p-(Acetaminophenylsulfonyl) acetic acid	none	23.9	none	none	none
Promin	none	23.3	none	none	none
Promizole	none	23.3	none	none	23.3
Dipyrazyl-sulfone	none	23.3	none	none	none
Di-*p*-tolylsulfone	none	23.3	none	none	none
p-Aniline-vinyl-sulfone Hydrochloride	23.3	5.8	23.3	23.3	11.7
p,*p'*-Diamidino-diphenylsulfone Dihydrochloride	none	23.3	none	none	none
p,*p'*-Dihydroxy-diphenylsulfone	none	24.3	none	none	24.3
p-Aminodiphenylsulfone	none	11.7	23.3	none	none
p,*o'*-Diaminodiphenylsulfone	none	5.8	none	none	11.7
2-Ethoxy-5-hydroxy-4-aminodiphenyl-sulfone	none	23.3	none	none	none
4-Amino-4'-hydroxy-diphenylsulfone	23.3	2.9	none	23.3	none
4,2'-Diaminodiphenylsulfone	none	23.3	none	none	23.3
4,2',4'-Triaminodiphenylsulfone	23.3	23.3	none	none	23.3
2,2'-Diaminodiphenylsulfone	none	23.3	none	none	23.3
3,4,4'-Triaminodiphenylsulfone	none	23.3	none	none	23.3
2-Chloro-4,4'-diaminodiphenyl-sulfone	11.7	23.3	none	none	11.7

TABLE 3—*Continued*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERAE
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
p,p'-Diaminodiphenylsulfone	none	<0.7	23.3	23.3	23.3
4-Acetylamino-4'-aminodiphenylsulfone	none	23.3	none	none	none
4'-Hydroxy-4-aminodiphenylamine-2-sulfonic acid	none	1.4	5.8	2.9	2.9
Dimethylaminobenzeneazobenzene-2-sulfonic acid	none	none	23.7	none	none
2-Hydrazino-8-hydroxy-6-naphthalene-sulfonic acid	none	11.7	23.3	23.3	23.3
2-Hydrazino-5-hydroxy-7-naphthalene-sulfonic acid	none	2.9	11.7	2.9	5.8
Sodium salt of 2-Butoxypyridine-5-aminomethane-sulfonic acid	none	23.3	none	none	23.3
p-Aminosulfonic acid	none	23.3	none	none	none
Diamylammonium-4-hydroxy-4-nitro-diphenylamine-2-sulfonate	none	23.3	none	none	none
2,2'-Dibenzothiazolyl-disulfide	2.9	1.4	none	2.9	2.9
p-Acetylaminophenyl-*p'*-nitrobenzylsulfide	none	23.3	none	none	none
2,2'-Di-(benzylthiazoyl) disulfide	none	<0.7	none	5.8	none
p,p'-Diamino-diphenyldisulfide	11.7	5.8	11.7	23.3	5.8
p,p'-Diamino-diphenylsulfide	23.3	5.8	11.7	23.3	23.3
Phenylsulfide	none	5.8	none	none	5.8
o,o'-Diamino-diphenyldisulfide	<0.7	<0.7	1.4	<0.7	<0.7
p,p'-Diamino-diphenyldisulfide	11.7	2.9	11.7	11.7	2.9
o,o'-Diacetylamino-diphenyldisulfide	none	2.9	none	none	1.4
Zinc-tris-*p*-chlorophenylol-sulfide	2.9	<0.7	2.9	2.9	<0.7
1-Allyl-4-(*p*-nitrophenyl) thiosemicarbazide	none	23.3	none	none	none
4-Allyl-1-(2-quinolyl) thiosemicarbazide	23.3	<0.7	none	23.3	23.3
Benzothiazole	none	2.9	none	none	23.3
Mercaptobenzothiazole	11.7	<0.7	5.8	none	none
6-Phenylamidazo[2,1-b] thiadiazole Hydrochloride	none	22.5	none	none	22.5
5,6-Diphenylamidazo[2,1-b] thiadiazole Hydrochloride	none	11.7	none	none	none
1-(Amidosulfophenyl)-3-(amidosulfophenyl imino)-5-phenyl-2-pyrrolidone	none	11.7	none	none	none
2-Amino-4-sodium-sulfomethyl-4,5-dihydroimidazole Hydrochloride	none	23.3	none	none	none
2-Methylphenylene-1,4-diurea	23.3	11.7	none	23.3	5.8
Phenylene-1,4-diurea	23.3	11.7	none	23.3	11.7
2,4-Dinitrophenylurethane	none	11.7	none	none	11.7
N-Ethyl-*N'*-dodecylthiourea	none	11.7	none	none	23.3
N-Allyl-*N'*-phenylthiourea	22.5	5.6	11.3	22.5	5.6
Di-2-pyridylthiourea	none	5.6	none	none	22.5
2-Phenylimidazo[1,2-a]pyrimidine Hydrochloride Monohydrate	none	23.3	none	none	none
N-Carbethoxy-*N'*-aminopiperazine Monohydrochloride	none	5.8	none	none	none
N-Carbethoxy-*N*-benzylpiperazine Hydrochloride	none	23.3	none	none	none

TABLE 3-*Continued*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
N-Methylenebis(*N'*-carbethoxypiperazine)	22.5	11.3	none	22.5	11.3
N-Carbethoxy-*N*-[2-(5-chloropyrimidyl)]piperazine	none	23.3	none	none	none
1-Methyl 2,6-diphenyl-4-ethyl-4-piperidol Hydrochloride	21.2				
Isosafrol	23.4	23.4	23.4	23.4	23.4
4,4'-Diaminophenylether	none	11.9	none	none	23.7
3,5-Dimethylpyrazole	none	23.3	none	none	none
2-Sulfanilaminopyrazine	11.7	11.7	5.8	11.7	23.3
4,4'-Diamino-3,3'-dimethyldiphenylmethane	none	23.3	none	none	23.3
p,p'-Diaminodiphenylmethane	none	23.7	23.7	none	none
N,N'-Bis(*p*-aminophenyl) caproamidine	5.8	2.9	2.9	5.8	5.8
N,N'-4,4'-Bisdimethylamino-azobenzene Butyramidine	none	23.3	none	none	23.3
Dibutylacetylbiuret	none	23.3	none	none	none
(Isoamylethylacetyl)biuret	none	23.3	none	none	none
Benzalacetophenone Dibromide	9.0				
Phenyl-mercuric-borate	<0.7	<0.7	<0.7	<0.7	<0.7
3-Acetylcoumarin	none	11.7	none	none	none
4,4'-Diaminodibenzalacetone	none	20.9	none	none	none
ω-Bromo-ω-benzoylacetophenone	4.5	12.0	4.5	11.4	0.4
1,3-Diphenyl-2-(*N*-1,2,3,4-tetrahydroisoquinolyl)propanone	<34.0	<17.0	<30.0	<34.0	<34.0
Dimethylfuran	3.1				
4-Hydroxydesoxybenzoin	22.5	2.8	22.5	22.5	1.4
Bis(2,3,4-trichloro-6-hydroxyphenyl)-methane	<0.8	<0.8	<0.8	<0.8	<0.8
Bis(2-hydroxy-4,5,6-trichlorophenyl) methane	1.6	<0.7	<0.7	<0.7	<0.7
2,2-Dihydroxy-3,3'-5,5'-6,6'-hexabromodiphenylmethane	none	5.8	none	none	11.7
Diphenylsulfoxide	none	23.7	none	none	none
Alloocimene	none	23.3	23.3	23.3	23.3
Acetine blue	18.0	1.4	none	11.7	5.8
Acridine	23.9	11.9	none	23.9	6.0
Acridine orange	2.8	<0.7	11.7	<0.7	<0.7
Acridine red	none	2.9	none	none	none
Acridine yellow	18.9	<0.7	23.3	<0.7	<0.7
Alizarine blue black BG	none	5.8	none	none	none
Alizarine blue S	none	<0.7	none	none	none
Alizarine cyanone green B extra	none	23.3	none	none	none
Alizarin red S	none	1.4	none	none	none
Alizarine yellow GG	none	5.8	none	none	none
Alizarin yellow R	none	1.4	none	none	none
Amethyst violet	1.3	<0.7	23.3	<0.7	<0.7
Aniline green	1.5	<0.7	none	<0.7	<0.7
Auramine 103%	9.5	none	none	none	none
Azure blue	10.8				
Benzyl violet	1.3	<0.7	2.9	<0.7	<0.7
Brilliant cresyl blue	8.9				

TABLE 3-*Continued*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
Brilliant violet	2.2				
Carbol gentian violet	20.7	2.9	none	5.8	2.9
Chlorhydrine blue	22.5				
Chrysaniline yellow	10.0	1.4	23.3	5.8	<0.7
Cresyl violet	10.0				
Dahlia	5.2				
Dahlia methyl green	5.6	<0.7	11.7	<0.7	<0.7
Diazin green	2.7				
2,4-Dibromo-6-carboxy-4′-dimethyl aminoazobenzene	none	5.8	none	none	none
Diguanidine fuchsin carbonate	5.8	5.8	<0.7	5.8	11.7
2,2′-Dihydroxy stilbene	none				
Di-isobutyl resorcinol	11.7	<0.7	11.7	23.3	2.9
Ethyl violet	5.2	<0.7	5.8	<0.7	<0.7
Fuchsin, basic	12.8	2.9	none	2.9	2.9
Fuchsin, new	10.5				
Hematoxylin C. P.	9.5				
Induline scarlet	2.7	<0.7	5.8	1.4	<0.7
2-Isopropoxy-4,6-diamino-s-triazine	none	24.7	none	none	none
Janus black 2	5.4	<0.7	23.3	<0.7	<0.7
Janus green B	2.4	<0.7	2.9	1.5	0.2
Magdale red (true)	18.0	1.4	none	2.9	2.9
Malachite green hydrochloride	2.3				
Malachite green oxalate	4.5	<0.7	none	<0.7	<0.7
Mallophene	none	5.8	none	none	none
Meldola blue	9.5				
Methoxy-6-chloro-9-diethyl-amino-propylaminoacridine Hydrochloride	11.7	2.9	11.7	11.7	5.8
Methyl red amide	none	none	11.7	none	none
Methylene blue U.S.P.	9.5	<0.7	23.3	1.5	<0.7
Methylene green	none	<0.7	none	23.3	1.4
Naphthazurine	1.6	<0.7	none	2.9	1.4
Methylene green B	none	<0.7	none	5.8	<0.7
Methylene violet	6.4				
Methylene yellow	10.4	<0.7	11.7	<0.7	1.4
Methyl violet B	2.5	<0.7	11.7	<0.7	<0.7
Methyl violet 2B	2.8	<0.7	5.8	<0.7	<0.7
Methyl violet 5B	2.7	<0.7	1.4	<0.7	<0.7
Methyl violet 6B	2.6	<0.7	11.7	<0.7	<0.7
Muscarin	21.7	<0.7	none	2.9	5.8
Naphthylene blue R	20.7	<0.7	none	1.4	1.4
New methylene blue N	none	<0.7	none	2.9	1.4
Nigrosine (alcohol soluble)	5.6	1.4	none	11.7	2.9
Orthochrome T	<0.7	<0.7	1.5	<0.7	<0.7
Para fuchsine 100%	20.8	2.9	none	2.9	2.9
Para rosaniline, base	23.5				
Phosphine	11.2	<0.7	11.7	1.4	1.4
Pinacyanole	<0.7	<0.7	1.5	<0.7	<0.7
Propyl red	none	11.9	none	none	none
Pyronin yellowish	2.1	<0.7	11.7	<0.7	<0.7
Rheonine A1	9.9	1.4	none	2.9	2.9
Rosaniline violet	2.9	<0.7	1.4	<0.7	<0.7

TABLE 3—*Concluded*

NAME OF COMPOUND	EBERTHELLA TYPHOSA	PASTEURELLA MULTOCIDA	SALMONELLA PULLORUM	SHIGELLA DYSENTERIAE	VIBRIO CHOLERA
	mg%	*mg%*	*mg%*	*mg%*	*mg%*
Safranin bluish	19.7				
Safranin O	11.0				
Safranin yellow	9.0	<0.7	23.3	1.4	2.9
Sodium *p*-Dimenthylaminoazobenzene-*p'*-carboxylate	none	5.7	none	none	none
Thionin pure	19.0				
Toluidine blue 0	15.8	<0.7	11.7	1.4	<0.7
Toluylene blue	20				
Triguanidine fuchsin carbonate	2.8	2.8	none	2.8	5.6
Victoria blue R	5.5	1.4	23.3	5.8	1.4
Victoria blue 4R	5.3				
Victoria green	2.3				
Wool green S	20.7	11.7	none	11.7	11.7
Total Number Compounds Tested	1030	650	650	650	650
Total Number Active Compounds	139	223	87	115	138
Total Number Inactive Compounds at 25 mg%	891	427	563	535	512

Most of the Grubler dyes were obtained from the University of Wisconsin and the other dyes from National Aniline Company, Eastman Kodak, Company and Hartmann-Leddon Company. The majority of the chemicals tested were prepared by chemists of the Calco, Stamford and Lederle Divisions of The American Cyanamid Company.

Of the active compounds (table 3):

139 were active against *Eberthella typhosa*
223 were active against *Pasteurella multocida*
87 were active against *Salmonella pullorum*
115 were active against *Shigella dysenteriae*
138 were active against *Vibrio cholera*

The number of compounds showing *in vitro* bactericidal activity was about the same with each of the five strains of bacteria. *Pasteurella multocida* was more sensitive to a greater number of the chemicals. The variety of the chemical structures of the compounds reported here make any generalizations as to the relation of the structure to bactericidal activity very difficult. The guanidines, phenols, sulfones and organic acids showed a rather high percentage of activity. In the dye groups, the triphenylmethane, acridine, oxazine, azine, and thiazine groups contained the greatest number of active derivatives. Of greatest importance appeared to be the sulfonamides, whose *in vitro* activity was demonstrated also *in vivo* by Little *et al.* (in press)

The *in vitro* active compounds that showed some *in vivo* protection are summarized: 2-sulfanilamidopyrimidine, 2-sulfanilamidopyrazine, 2-sulfanilamidopyridine and also their heterocyclic substituted chloro-, bromo-, and methyl compounds.

The *in vivo* activity of sulfonamide group was limited to the pyrimidine derivatives. This clear cut distinction was not obtained *in vitro*.

The above results compare favorably with the results of Strauss and Finland (1941) using semi-synthetic medium. They found Sulfadiazine and Sulfathiazole to be bactericidal at 1 to 2 mg per cent against *Escherichia coli* and *Shigella choleraesuis* and 5 mg per cent against *Salmonella enteriditis* and *Salmonella typhimurium*.

SUMMARY AND CONCLUSIONS

A readily reproducible synthetic medium which supported growth of *Vibrio cholera*, *Pasteurella multocida*, *Shigella dysenteriae*, *Eberthella typhosa*, and *Salmonella pullorum* was used over a period of two and a half years in *in vitro* tests of the activity of more than 1000 compounds. Approximately 15 per cent of these chemicals were able to inhibit completely the growth in this medium. Chemicals whose *in vitro* activity was also demonstrated *in vivo* were all recently developed sulfonamides such as 2-sulfanilamidopyrimidine, 2-sulfanilamidopyrazine, 2-sulfanilamidopyridine and their heterocyclic substituted chloro-, bromo-, and methyl compounds.

None of the compounds showing *in vivo* activity as demonstrated by Little *et al.* (in press) failed to show *in vitro* activity.

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REFERENCES

BRITTON, C. J. C. 1938 The effect of sulphonamide compounds on certain bacteria *in vitro*. Brit. J. Exptl. Path., **19**, 1940–143.

CHU, H. I., AND HASTINGS, A. B. 1938 The effect of *para*-amino benzene sulfonamide on the oxygen consumption of tissue and certain pathogenic bacteria. J. Pharm. Exptl. Therap., **63**, 407–413.

GOETCHIUS, G. R., AND LAWRENCE C. A. 1944 The *in vitro* effect of N-substituted *p*-aminobenzoic acid derivatives upon sulfonamides. J. Bact. **48**, 683–687.

KEMPNER, W., WISE, B., AND SCHLAYER, C. 1940 Manometric determination of the effects of various sulfanilamide compounds on *Brucella melitensis*. Am. J. Med. Sci., **200**, 484–492.

LAWRENCE, C. A. 1940 Bacteriostatic actions of three thiazole derivatives of sulfanilamide upon bacteria in broth cultures. Proc. Soc. Exptl. Biol. Med., **43**, 92–97.

LIBBY, R. L. AND JOYNER, A. L. 1940 The action of sulfathiazole on the colon-typhoid-dysenteriae group of organisms. J. Infectious Diseases, **67**, 67–69.

LITTLE, P. A., DEMELLO, G., TANZOLA, J., AND SUBBAROW, Y. 1947 Comparison of mouse-protective tests and chick-protective tests of over 900 chemicals against *Pasteurella multocida*. J. Bact. (in press).

LITTLE, P. A., AND SUBBAROW, Y. 1948 The mouse-protective test as a means of determining the inhibitory effect of chemicals on *Vibrio cholera*. J. of Immunology (in press).

LONG, P. H., AND BLISS, E. A. 1937 *Para*-amino-benzene sulfonamide and its derivatives. Experimental and clinical observations on their use in the treatment of *beta*-hemolytic streptococcic infection: a preliminary report. J. Am. Med. Assoc., **108**, 32–37.

LONG, P. H., AND BLISS, E. A. 1940 Bacteriostatic effects of sulfathiazole upon various microorganisms. Its therapeutic effects in experimental pneumococcal infections. Proc. Soc. Exptl. Biol. Med., **43**, 324–327.

LOWELL, F. C., STRAUSS, E., AND FINLAND, M. 1940 Observations on the susceptibility of pneumococci to sulfapyridine, sulfathiazole, and sulfamethylthiazole. Ann. Int. Med., **14**, 1001–1023.

STRAUSS, E., AND FINLAND, M. 1941a Selective inhibition of sulfonamide drugs by various media. Proc. Soc. Exptl. Biol. Med., **47**, 428–431.

STRAUSS, E., AND FINLAND, M. 1941b Bacteriostatic and bactericidal action of sulfadiazine *in vitro* on gram-negative bacteria. Proc. Soc. Exptl. Biol. Med., **47**, 432–434.

WOODS, D. D. 1940 The relation of *p*-aminobenzoic acid to the mechanism of the action of sulphanilamide. Brit. J. Exptl. Path., **21**, 74–90.