64. Antibacterial Action and Chemical Constitution in Long-chain Aliphatic Bases

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The success of sulphanilamide as a chemotherapeutic agent encourages the investigation of other classes of compound for antibacterial properties. The compounds described in this paper were the long-chain amines, amidines and guanidines which had been shown by King, Lourie & Yorke [1938] to possess remarkable trypanocidal powers. They were chosen because they had a proved biological action, and also formed a convenient series of related compounds in which the effects of changes in constitution on antibacterial action could be observed. These compounds were generously placed at my disposal by Dr Harold King, to whom my grateful thanks are due.

The drugs are listed below. Many of them have powerful antibacterial actions, and the effects of variations in the length of carbon chain, in the end groupings and in the bacteria studied are discussed.

Drugs. These comprised the hydrochlorides of:

1. Diamines, general formula $\text{NH}_2 \cdot (\text{CH}_2)_n \cdot \text{NH}_2$, with $n=2, 6, 14, 16$ and 18 .
2. Diamidines, general formula $\text{NH}_2 \cdot (\text{NH})\text{C} \cdot (\text{CH}_2)_n \cdot \text{C}(\text{NH})\text{NH}_2$, with $n=8, 9, 10, 11, 12, 13, 14, 16$ and stilbene diamidine.
3. Diguandines, general formula $\text{NH}_2 \cdot (\text{NH})\text{C} \cdot \text{NH} \cdot (\text{CH}_2)_n \cdot \text{NH} \cdot \text{C}(\text{NH}) \cdot \text{NH}_2$, with $n=5, 6, 8, 10, 12, 14, 16$ and 18 .
4. Di-isothioureas, general formula $\text{NH}_2 \cdot (\text{NH})\text{C} \cdot \text{S} \cdot (\text{CH}_2)_n \cdot \text{S} \cdot \text{C}(\text{NH})\text{NH}_2$, with $n=4, 6, 8, 11, 12$ and 16 .
5. Di-quaternary ammonium compounds, general formula $\text{N}^+(\text{CH}_3)_3 \cdot (\text{CH}_2)_n \cdot \text{N}^+(\text{CH}_3)_3$, with $n=8$ and 14 .
6. Monoamines, general formula $\text{H} \cdot (\text{CH}_2)_n \cdot \text{NH}_2$, with $n=9, 12, 14, 16$ and 18 .
7. Monoamidines, general formula $\text{H} \cdot (\text{CH}_2)_n \cdot \text{C}(\text{NH}) \cdot \text{NH}_2$, with $n=15$ and 17 .
8. Monoguanidines, general formula $\text{H} \cdot (\text{CH}_2)_n \cdot \text{NH} \cdot \text{C}(\text{NH})\text{NH}_2$, with $n=9, 14, 16$ and 18 .
9. Mono-quaternary ammonium compounds, general formula $\text{H} \cdot (\text{CH}_2)_n \cdot \text{N}^+(\text{CH}_3)_3$, with $n=9$ and 18 .
10. Phenyl diguanide, phenyl guanidine, hexamethylene dipiperide, and octamethylene di-imino ether were inactive at the concentrations tested.

For brevity, in the text, 18-methylene diamine, e.g., is referred to as 18-diamine, or as $n=18$. It should be noted that since, by the above nomenclature, one carbon atom is included in the amidine group, the diamidines have $n+2$ and the monoamidines $n+1$ carbon atoms in an unbroken chain. The 12-diamidine, for example, is therefore compared with the 14-diamine, and the 15-amidine with the 16-amine.

Methods

The activity of the drugs is recorded (Tables 1-4) as the limiting dilution in thousands which prevents visible growth after 18 hr. incubation at 37° in Hartley digest broth, or (when testing the effect of serum) in a mixture of equal parts of inactivated human serum and Hartley broth. *V. cholerae* grew with difficulty in 50% serum broth, so 33% was used for this organism. The drug was added with aseptic precautions from stock dilutions of 1 in 500, 1 in 5000, 1 in 50,000 or 1 in 500,000, as required, and a control tube of medium was always included. The determinations were repeated on either side of the

limiting concentration, to check this up. Altogether more than 15,000 tests were done in this series. The implant was 5 μ l. of a 1 in 200 dilution of overnight culture in 100 μ l. of medium. This heavy inoculum was used to minimize variations due to differences in the implant.

Table 1. *Limiting inhibitory dilutions of drugs in thousands against cocci in broth and 50% serum broth*

Drug	<i>Str. viridans</i>		<i>Str. pyogenes</i>		<i>Pneumococcus</i>		<i>Staph. aureus</i>	
	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum
6-Diamine	0.2	0.1						
14- "	1500	500	200	500	80	300	20	30
16- "	4000	200	400	100	1500	40	100	20
18- "	7000	200	2000	100	2000	30	200	20
8-Diamidine	5	5	20	20	5	5	3	20
9- "	10	5	50	30	15	10	7	40
10- "	20	10	100	50	20	15	20	80
11- "	50	20	80	200	50	20	50	100
12- "	200	20	80	1000	50	20	100	400
13- "	500	100	100	1000	100	50	200	500
14- "	600	100	200	1000	200	50	200	800
16- "	1000	50	300	300	100	50	200	200
Stilbene-diamidine	100	20	150	150	100	20	30	100
5-Diguanidine	1	3	8	2	0.5	1	1.5	10
6- "	3	5	50	10	3	2	3.5	20
8- "	20	30	200	150	30	15	15	70
10- "	100	30	300	300	80	30	80	300
12- "	250	200	400	1500	150	50	700	500
14- "	5000	200	2000	1000	4000	200	2000	500
16- "	2000	200	1000	500	300	150	500	300
18- "	4000	100	2000	200	500	100	500	150
4-Di-isothiouraea	1000	10	500	50	50	15	100	5
6- "	2	5	10	50	10	10	3	7
8- "	1000	100	300	100	150	50	200	<10
11- "	300	100	200	100	400	100	20	10
12- "	200	50	300	150	30	40	10	5
16- "	800	200	1000	80	150	50	100	10
8-Di-quaternary base	0.1	<0.1	0.25	<0.1	<5	<5	<5	5
14- "	15	3	20	150	<5	<5	<5	40
9-Amine	150	70	20	100	40	20	5	20
12- "	1500	40	700	50	7000	10	150	10
14- "	3000	80	500	20	1000	10	150	10
16- "	1000	20	250	20	200	<20	75	<20
18- "	150	20	15	<20	200	20	15	<20
15-Amidine	150	100	500	20	200	15	400	<20
17- "	100	100	75	<20	100	<20	20	<20
9-Guanidine	300	30	100	100	50	20	20	50
14- "	500	50	500	75	200	20	100	30
16- "	800	30	500	50	200	<20	200	20
18- "	150	20	100	20	100	<20	150	<20
9-Quaternary base	10	20	10	100	5	10	2	10
18- "	200	20	150	15	150	20	100	20

Cultures

The main tests were carried out with the following 20 cultures:

Streptococcus viridans, 'B 37' (stock strain) from Dr S. D. Elliott.

Streptococcus pyogenes, group A, type 3, 'Richards' (mouse virulent).

Staphylococcus aureus, 'Paler' (recently isolated).

B. proteus, 'Hunt' (recently isolated).

B. pyocyaneus, 'Scudder' (recently isolated), all from Dr L. Colebrook.

Pneumococcus, type 2 (mouse virulent).

Pasteurella, 265 (mouse virulent), both from Dr van den Ende.

Cl. oedematiens, 'Cossard'.

Vib. septique, 'V.S. 20'.

Cl. welchii, 'Roshier'.

Cl. tertium, 'WPRL', all stock strains from Dr H. J. Parish.

Cl. sporogenes, 'A. 37' (recently isolated), from Prof. A. A. Miles.

Bact. shigae, 'Newcastle' (stock strain).

Bact. flexneri, W (recently isolated).

Bact. sonnei (recently isolated).

Bact. typhosum, Felix type 2 (Vi++).

Bact. paratyphosum B, Felix type H 33 (Vi++).

Bact. typhi-murium (recently isolated).

Bact. enteritidis, 'Dublin' (recently isolated).

Vibrio cholerae, 'Inabe S' (stock strain) from Mr. P. Bruce White.

Some of the results were checked with dysentery cultures kindly supplied by Col. H. T. Findlay.

Table 2. *Limiting inhibitory dilutions of drugs in thousands against anaerobes in broth and 50% serum broth*

Drug	<i>Cl. oedematiens</i>		<i>Vib. septique</i>		<i>Cl. sporogenes</i>		<i>Cl. tertium</i>		<i>Cl. welchii</i>	
	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum
6-Diamine	—	—	—	—	—	—	—	—	—	—
14- "	30	100	30	80	50	150	30	40	10	40
16- "	200	150	200	150	250	250	250	250	150	80
18- "	700	50	400	20	400	40	250	50	500	40
8-Diamidine	5	<5	<5	<5	<5	<5	<5	<5	<5	<5
9- "	10	<5	<5	<5	<5	<5	<5	<5	<5	<5
10- "	15	5	5	<5	<5	<5	<5	<5	<5	<5
11- "	50	7	5	<5	<5	<5	<5	<5	5	<5
12- "	50	15	20	4	20	5	5	<5	5	<5
13- "	50	30	40	5	25	7	25	5	10	5
14- "	100	50	150	10	100	20	60	7	10	10
16- "	200	40	150	20	250	20	100	10	50	20
Stilbene-diamidine	50	7	<20	5	<20	5	<20	<5	<20	<5
5-Diguanidine	—	—	—	—	—	—	—	—	—	—
6- "	—	—	—	—	—	—	—	—	—	—
8- "	5	<5	<5	<5	<5	<5	<5	<5	<5	5
10- "	10	10	20	<5	10	5	20	<5	7	20
12- "	30	20	40	10	30	20	20	15	30	30
14- "	700	150	500	50	600	100	500	15	300	100
16- "	400	150	200	10	500	20	300	20	300	50
18- "	500	50	300	20	600	40	400	15	300	50
4-Di-isothiourea	75	5	20	5	75	4	40	<5	20	4
6- "	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
8- "	75	5	50	5	75	<5	100	<5	50	5
11- "	20	10	10	10	20	5	20	5	10	10
12- "	20	7	20	7	20	<5	10	4	5	5
16- "	50	10	20	5	150	5	40	7	10	5
8-Di-quaternary base	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
14- "	20	7	<5	<5	<5	<5	<5	<5	<5	<5
9-Amine	25	10	35	20	10	10	10	40	10	10
12- "	350	30	150	20	150	20	350	40	200	20
14- "	800	20	400	10	200	20	350	20	200	10
16- "	400	20	600	20	350	<20	350	20	150	<20
18- "	400	<20	150	<20	75	<20	75	20	75	<20
15-Amidine	400	<20	100	<20	200	<20	200	<20	150	<20
17- "	100	<20	75	<20	50	<20	100	<20	100	<20
9-Guanidine	75	20	75	5	75	5	35	<5	20	15
14- "	100	10	100	10	200	<10	100	10	100	10
16- "	50	20	200	<20	100	<20	100	<20	200	<20
18- "	150	<20	20	<20	200	<20	75	<20	150	<20
9-Quaternary base	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
18- "	75	10	150	5	100	5	150	5	100	5

Table 3. *Limiting inhibitory dilutions of drugs in thousands against colon-typhoid organisms in broth and 50% serum broth*

Drug	<i>B. typhosum</i>		<i>B. enteritidis</i>		<i>V. cholerae</i>		<i>B. paratyphosum</i> B		<i>B. typhi-murium</i>	
	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum
6-Diamine	<0.1	0.1	—	—	—	—	—	—	—	—
14- "	20	50	10	20	10	40	10	30	10	20
16- "	100	30	50	30	50	30	50	20	50	20
18- "	100	20	15	4	100	20	35	7	15	5
8-Diamidine	0.8	7.5	<5	<5	<5	<5	<5	<5	<5	<5
9- "	1.2	15	<5	4	<5	<5	<5	<5	<5	<5
10- "	4	50	5	15	<5	7	<5	5	<5	5
11- "	7	35	7	20	<5	10	<5	8	<5	5
12- "	30	100	20	30	10	15	5	15	<5	10
13- "	50	120	20	50	20	30	10	20	5	20
14- "	75	100	30	20	20	15	30	15	10	10
16- "	100	30	30	10	20	15	30	7	20	5
Stilbene-diamidine	60	200	35	40	<20	20	<20	40	<20	40
5-Diguanidine	0.3	2	—	—	—	—	—	—	—	—
6- "	0.5	7	—	—	—	—	—	—	—	—
8- "	5	20	<5	5	<5	<5	<5	<5	<5	<5
10- "	40	200	5	20	5	10	7	10	<5	5
12- "	70	300	20	50	20	20	15	20	5	15
14- "	300	200	150	50	50	20	40	20	25	20
16- "	700	80	400	40	150	15	120	10	100	5
18- "	500	50	300	20	200	20	200	10	150	<10
4-Di-isothiurea	20	15	20	<5	20	5	20	<5	20	5
6- "	0.8	15	<5	<5	<5	<5	<5	<5	<5	<5
8- "	200	10	100	<10	200	<10	100	<10	100	<10
11- "	30	40	25	15	10	15	10	15	15	10
12- "	10	20	10	10	5	7	5	15	10	10
16- "	50	35	25	20	20	20	25	20	30	20
8-Di-quaternary base	<0.1	<0.1	<5	<5	<5	<5	<5	<5	<5	<5
14- "	0.5	4	<5	4	<5	<5	<5	<5	<5	<5
9-Amine	7	7	7	10	5	10	5	10	5	7
12- "	100	10	50	<5	150	10	60	<5	80	5
14- "	120	<10	20	<10	300	10	35	<10	30	<10
16- "	<10	<20	<10	<20	20	<20	<10	<20	<10	<20
18- "	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
15-Amidine	200	<20	50	<20	200	<20	50	<20	40	<20
17- "	<20	<10	<20	<20	20	<20	<20	<20	<20	<20
9-Guanidine	75	20	50	15	20	7	10	10	20	7
14- "	200	10	100	<10	200	<10	40	<10	50	<10
16- "	<20	<10	<20	<10	50	<10	<20	<10	<20	<10
18- "	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
9-Quaternary base	2	5	<5	5	<5	5	<5	<5	<5	<5
8- "	5	<0.5	<5	<5	75	<5	<5	<5	<5	<5

Activity in broth

Sensitivity of bacteria. Bacteria differed greatly in their sensitivity to the drugs (Tables 1-4). For example 18-diamine inhibited *Str. viridans* at 1 in 7,000,000, *Staph. aureus* at 1 in 200,000, *B. enteritidis* at 1 in 15,000 and was inactive against *B. proteus*. In general, if the bacteria are arranged according to their sensitivities, the order in which they fall is roughly the same for all the drugs examined here.

The gram-positive cocci were the most sensitive with *Str. viridans* the most and *Staph. aureus* the least sensitive of the group. The gram-positive anaerobic bacilli were much more resistant than the cocci, but on the whole were more sensitive than the gram-negative bacilli, excepting the dysentery bacteria. *Cl. oedematiens* was the most and *Cl. welchii* the least sensitive of the anaerobes.

The three most sensitive of the gram-negative bacilli were *B. shigae*, pasteurella and *B. flexneri*. *B. typhosum* was the most sensitive and *B. typhi-murium* the most resistant

Table 4. *Limiting inhibitory dilutions of drugs in thousands against dysentery and other gram-negative organisms in broth and 50% serum broth*

Drug	<i>B. shigae</i>		<i>B. flexneri</i>		<i>B. sonnei</i>		<i>Pasteurella</i>		<i>B. proteus</i>		<i>B. pyocyaneus</i>	
	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum	Broth	Serum
6-Diamine	0.1	0.3	—	—	—	—	—	—	—	—	—	—
14- "	20	200	5	60	5	50	60	50	15	20	<10	10
16- "	100	80	50	40	25	20	150	30	15	10	<10	5
18- "	100	20	150	15	100	10	150	20	<20	<5	<20	<5
8-Diamidine	15	200	<5	<5	0.8	5	2	4	<5	<5	<5	<5
9- "	40	400	<5	10	2	7	4	5	<5	<5	<5	<5
10- "	100	1000	10	50	4	20	10	5	<5	<5	<5	5
11- "	350	1000	15	100	15	40	20	20	<5	7	<5	15
12- "	700	2000	40	200	20	80	75	40	<5	15	5	20
13- "	700	3000	75	100	100	100	150	50	5	20	5	20
14- "	300	2000	100	70	100	100	200	80	10	20	10	7
16- "	100	100	75	20	100	20	150	80	20	7	15	<5
Stilbene-diamidine	500	800	35	80	<20	50	80	80	<20	20	<20	7
5-Diguanidine	0.2	1.5	0.15	0.5	0.05	0.6	0.5	1.5	—	—	—	—
6- "	0.5	2	0.2	1	0.1	0.8	2	5	—	—	—	—
8- "	15	100	3	10	1.8	10	5	10	<5	<5	<5	<5
10- "	500	1500	40	120	15	40	60	40	<5	7	<5	10
12- "	2000	4000	160	150	75	150	150	50	5	15	5	15
14- "	3000	5000	500	150	500	200	700	300	20	15	40	15
16- "	700	1000	500	100	350	50	700	200	100	5	75	7
18- "	500	500	400	80	350	40	350	150	75	<10	50	<10
4-Di-isothiurea	10	20	10	7	30	5	50	<5	10	5	<10	<5
6- "	500	5	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
8- "	150	10	100	5	40	<5	100	<10	10	<10	<10	<10
11- "	40	60	40	60	20	40	100	80	<5	7	10	5
12- "	30	60	30	20	7	20	100	30	<5	10	5	7
16- "	75	20	50	20	20	20	200	50	10	15	10	7
8-Di-quaternary base	0.1	0.3	<5	<5	<5	<5	<5	<5	<5	<5	<5	<5
14- "	5	50	<5	10	<5	<5	<5	<5	<5	<5	<5	<5
9-Amine	5	20	5	7	5	5	15	7	<5	5	<5	<5
12- "	100	20	100	7	100	5	200	30	50	<5	15	<5
14- "	100	10	100	<10	20	<10	350	<10	15	<10	10	<10
16- "	<10	<20	<10	<20	<10	<20	150	<20	<10	<20	<10	<20
18- "	<20	<20	<20	<20	<20	<20	<20	<20	<10	<20	<10	<20
15-Amidine	75	<20	100	<20	50	<20	75	<20	50	<20	<10	<20
17- "	<20	<10	<20	<10	<20	<10	50	<10	<20	<20	<20	<20
9-Guanidine	75	50	60	40	30	20	100	50	20	5	20	5
14- "	300	<10	200	<10	100	<10	150	20	30	<10	<10	<10
16- "	<20	<10	<20	<20	<20	<10	100	<10	<20	<10	<20	<10
18- "	<20	<10	<20	<20	<20	<20	<20	<20	<20	<20	<20	<20
9-Quaternary base	10	20	5	<5	<5	<5	30	30	<5	<5	<5	<20
18- "	35	<0.5	10	<5	5	<5	100	<5	10	<5	<5	<5

of the colon-typhoid group. *B. proteus* and *B. pyocyaneus* were the most resistant of all the organisms tested.

Closely related pairs of organisms were similar in behaviour, for example *B. typhosum* and *B. enteritidis*, Dublin (Colon-typhoid sub-group D), and *B. paratyphosum* B, and *B. typhi-murium* (sub-group B).

A few of the drugs differ markedly in their actions towards gram-positive and gram-negative bacteria. For example, the 16- and 18-amines and guanidines were very active against gram-positive, but were inactive against gram-negative bacteria, except *B. cholerae* and *Pasteurella*. These last two were similar also in being much more sensitive than the other gram-negative bacilli to 18-quaternary base. The diamines were much more active against gram-positive than against gram-negative bacteria, both in actual titres and in comparison with the other drugs. The dysentery strains were unusually resistant to the diamines and di-isothiureas, but were sensitive to the more powerfully basic diguanidines and quaternary salts.

Effect of chain length. The activity in all the series of drugs (except the di-isothiourreas) increased with the length of the carbon chain up to $n=14$ to 18 for the disubstituted compounds and up to $n=12$ to 16 for the monosubstituted compounds. The diamine and diamidine series had not reached maximal activity against gram-positive organisms by $n=18$, but with this exception the series had reached a stationary or a declining phase before this point. In the disubstituted series the fall in activity with increasing chain length beyond the optimal was gradual, but in the monosubstituted series the fall was much sharper, especially against gram-negative bacteria. Thus the drugs of the monosubstituted series with $n=16$ or more were inactive against practically all the gram-negative bacteria, although they were potent against the gram-positives.

The diguanidines reached their maximum activity at a shorter chain length than the diamines or diamidines, but the reverse held in the monosubstituted series.

Stilbene-diamidine, which includes two benzene rings, had approximately the activity of the $n=12$ open chain compound.

The rates of change of activity varied greatly in the different series. There was sometimes a 20- to 30-fold increase of titre for an addition of $2n$, as for example from 12- to 14-diguanidine against the cocci, but in general the changes were more gradual.

Drugs of chain length lower than $n=8$ had very slight activity except in the di-isothiourrea series, the activity of which increased and decreased sharply as the series was ascended.

Effect of the terminal group. The effects of mono- or di-substitution, and of changes in the end groups can be seen by comparing compounds of the same chain length. The disubstituted guanidines were far more active than the monosubstituted against all bacteria. In the other series the monosubstituted compounds had, on the whole, a slight advantage, especially against the anaerobes and the colon-typhoid group. The monosubstituted series reached maximum activity at a shorter chain length than the disubstituted, which gave the higher members of the latter a great advantage, especially against gram-negative bacteria.

The di-isothiourreas appeared to be by far the most active at chain lengths of 4 and 6, while the diguanidines were, with few exceptions, the most active from $n=10$ to $n=18$. The advantage of the diguanidines became less as the chain length increased.

The diamines were more active than the diamidines and much more active than the quaternary bases. The activities in the diamine and diamidine series started to decrease at a shorter chain length against gram-negative than against gram-positive bacteria, but the opposite was true in the diguanidine series. The guanidines were the most active in the monosubstituted series, but had not the overwhelming superiority of the diguanidines. The comparison between the strongly basic quaternary bases and the weakly basic amines was incomplete, and a more complete comparison would be interesting. The 8-di-quaternary salt was similar in activity to the 6-diamine. The 9 mono- and the 14-di-quaternary salts were much less active, and the 18-mono- more active than the corresponding amines.

Effect of serum

Table 5 shows how the activities of the drugs against different classes of bacteria were affected by the presence of serum. It has the disadvantage that bacteria behaving differently have been grouped together, but the influence of serum on individual strains can be seen in Tables 1-4.

Besides its direct effect on the drug, serum affects the titres by obscuring small amounts of growth in the opalescent serum, and by its effect on the growth-rate of some bacteria.

Disubstituted drugs. Serum caused an increase in the activity of the lowest members of the disubstituted series, except in the di-isothiourrea group and against *Str. pyogenes*. As the chain length increased, this advantage steadily decreased until at $n=16$ or 18,

Table 5. *Activity of drugs in 50% serum expressed as a percentage of the activity in broth*

Group and 'n'	Cocci	Anaerobes	Dysentery	Rest of gram-negative
Diamine				
-6	40	—	300	—
-14	220	280	900	220
-16	13	80	80	40
-18	5	10	17	14
Diamidines				
-8	220	—	1000	600
-9	190	—	650	600
-10	150	—	650	550
-11	130	—	550	270
-12	400	25	400	230
-13	320	36	210	230
-14	250	18	310	70
-16	65	14	50	30
Stilbene	100	—	200	200
Diguanidines				
-5	300	—	800	500
-6	200	—	600	800
-8	185	—	500	300
-10	140	110	300	220
-12	130	70	150	200
-14	20	17	70	45
-16	40	15	60	11
-18	15	9	40	15
Di-isothiouras				
-4	10	12	90	30
-6	200	—	? 500	? 1000
-8	20	6	8	5
-11	40	60	170	100
-12	60	50	180	180
-16	20	25	55	80
Di-quaternary				
-8	—	—	—	—
-14	—	—	—	900
Amines				
-9	250	150	200	150
-12	5	11	11	10
-14	4	5	—	3
-16	5	4	—	—
-18	11	—	—	—
Amidines				
-15	20	<20	<20	<20
-17	—	—	—	—
Guanidines				
-9	100	25	67	40
-14	15	10	3	<10
-16	8	—	—	—
-18	17	—	—	—
Quaternary salt				
-9	500	—	200	150
-18	11	6	1	< 5

the drug had only a fraction of its activity in broth (Table 5). Thus maximal activity occurred at a shorter chain length in serum than in broth.

Against staphylococcus, *B. shigae* and *B. typhosum* the ratios of serum to broth activities (S/B) varied from 5 to 13 for the shortest chains, falling steadily to unity or less for the longest chains. With *Str. viridans*, pneumococcus, pasteurella and the anaerobes, serum interfered more, so that the values were seldom over unity even for the short chains, and fell further as the chain lengthened. Other organisms were intermediate in their behaviour. The activity against *Str. viridans* was the most depressed of any, possibly owing to the growth stimulation produced by serum on broth cultures of this organism. With *Str. pyogenes*, serum weakened drugs with chain lengths below $n=10$ and above $n=14$, and strengthened the middle members. In the di-isothiouras group the least active compounds were strengthened, and the most active weakened, so that in serum there was a resultant increase of activity with chain length.

Serum activated the diamidine series against *Str. pyogenes* and staphylococcus. On the other hand the diamidines were very much weakened against the anaerobes and the di-isothiouras against staphylococcus. The great sensitivity of *B. shigae* to the diamidines and diguanidines in serum is striking.

Monosubstituted drugs. These were much more strongly inhibited by serum than the corresponding members of the disubstituted series, and the point of serious interference occurred at a shorter chain length. Only in the 9-amine and 9-quaternary salt was an increase in activity in serum observed, and by $n=12$ the activity was only about 10% of the activity in broth. Consequently the higher mono-amines, -amidines and -guanidines were inactive against all organisms except some cocci. 9-Amine and 9-guanidine were equally active against gram-positive, but the guanidine was by far the more potent against gram-negative bacteria.

Titres in serum. It will be seen from Tables 1-4 that the diguanidines came out best of the disubstituted series against all bacteria, especially the gram-negative ones. The 14- and 16-diamines and 16-diamidine also were prominently active. The higher diamines were the most active against *Str. viridans* and anaerobes, the higher diamidines against *Str. pyogenes* and *Staph. aureus*, and the higher diamidines and diguanidines against dysentery.

In the monosubstituted series, the guanidines were best against *Str. pyogenes*, *Staph. aureus* and gram-negative organisms. No compound with chain length above $n=14$ was active against any of these last. The higher amidines were best against *Str. viridans*, and the amines against the anaerobes. 9-Quaternary base was unusually active against *Str. pyogenes*.

Although the activities of some drugs were increased 10-fold against *B. shigae* and *Str. pyogenes*, serum interfered most seriously with the most active drugs, and this limited the peak activity of any series. For example, the activity of 4-di-isothioureia against *Str. viridans* was reduced from 1 in 1,000,000 to 1 in 10,000, and that of 12-amine against pneumococcus from 1 in 7,000,000 to 1 in 10,000.

Serum caused some change in the order of sensitivity of the bacteria. *Str. pyogenes* became the most and pneumococcus the least sensitive of the cocci. *Cl. oedematiens* remained the most sensitive of the anaerobes. *B. shigae* was by far the most sensitive of the gram-negative organisms, and *B. typhosum* next.

Strain variation

The above work had been carried out with one strain only of each species and it was possible that considerable strain variations might occur. To get some idea of possible strain variation, the experiments with some of the drugs were carried out on the following 25 strains of dysentery:

2 of *B. shigae*, 1 of *B. sonnei*, 1 of *B. ambigua* and the following Flexner strains: 3 of V, 2 of W, 1 of X, 2 of Z, 2 of 103, 3 of 170, 2 of 274, 1 of 119, 2 of 288, 2 of 88 and 1 of D.

The variation in the inhibitory titres for the Flexner strains can be seen from Table 6. They are for the most part inconsiderable, especially as the results were not checked as thoroughly as in the main series.

Table 6. *Titres of drugs against dysentery strains*

Drug	Range of titres in broth	Range of titres in serum-broth
18-Diamine	100,000-200,000	20,000
13-Diamidine	50,000-100,000	40,000-100,000
14-Diamidine	50,000-100,000	20,000-100,000
16-Diamidine	75,000-100,000	20,000
14-Diguanidine	200,000-1,000,000	100,000-500,000
16-Diguanidine	200,000-500,000	50,000-200,000
18-Diguanidine	300,000-500,000	50,000-100,000
8-Di-isothioureia	50,000-100,000	20,000
12-Amine	100,000	20,000
14-Amine	100,000-200,000	20,000
15-Amidine	100,000	20,000
14-Guanidine	100,000-200,000	20,000

Sulphanilamide-resistant strains. Three group A streptococcus pyogenes strains, 'Mead', 'Kingsbury', and 'Atkinson', resistant to sulphanilamide, and two other sensitive strains, 'Pickering' and 'Wood' were tested. They were recently isolated from wounds and were kindly supplied by Dr Colebrook. The results (Table 7) show that resistance to sulphanilamide does not affect the sensitivity to the present series of drugs. The titres were not determined as exhaustively as in the main series and the differences were probably not as great as indicated in Table 7.

Table 7. *Titres of sulphanilamide-sensitive and -resistant strains*

Strain	14-Diamidine	14-Diguanidine	14-Guanidine	12-Amine
'Sensitive'				
Richards	200,000	2,000,000	500,000	700,000
Pickering	100,000	1,000,000	—	300,000
Wood	500,000	1,500,000	—	—
'Resistant'				
Mead	400,000	4,000,000	800,000	1,000,000
Kingsbury	100,000	1,500,000	500,000	1,000,000
Atkinson	400,000	2,000,000	800,000	800,000

Effect of repeated subculture on sensitivity

It was noticed that when there was a difference in sensitivity of strains of dysentery organisms, the old stock strains were usually the most resistant, and recently isolated strains the most sensitive. Six recently isolated strains of *B. Flexneri* were subcultured daily in broth thirty times. The titres of twelve of the drugs for the passaged strains were compared with the original titres. The titres for each drug against all the strains were averaged and it was found that the passaged strains were inhibited by different drugs at 1.2-5.0 times the concentration as compared with the original strains. An average of all the results showed the passage strains to be twice as resistant as formerly.

Therapeutic effect

King *et al.* [1938] determined the toxicities of the drugs, which have a mouse-tolerated dose of 1-4 mg. for the lowest members and 0.1-0.5 mg. for the higher members. 18-Amine and 18-guanidine are less toxic than the lower members.

Multiplying the 50% serum titres of the drugs by the mouse-tolerated doses gives a measure of their probable therapeutic efficiencies, so far as these can be deduced from *in vitro* testing. Compounds giving high values are 14-diamine, 14-diamidine and 14-guanidine. 11-Di-isothioureia is the best of its group, especially against gram-negative bacteria; 9- and 12-amines are the best of the monosubstituted compounds.

Dr F. Hawking kindly tested some of these compounds in mice infected intraperitoneally with *Str. pyogenes*, 'Richards'. The drugs were given subcutaneously twice a day for 3 days, and once a day for the next 3 days, in the doses per g. of mouse given below. No significant therapeutic effect was obtained.

	mg.		mg.
13- and 14-diamidines	0.005	9-Amidine	0.05
Stilbene-diamidine	0.01	18-Guanidine	0.05
14-Diamine	0.02	9-Quaternary salt	0.025
14-Diguanidine	0.001		

DISCUSSION

In vitro antibacterial power is the most important single factor in the activity of the chemotherapeutic drugs in use to-day, and the *in vitro* technique, by its speed and precision, is of use in investigating new drugs. It was hoped, by using a large range of bacteria, and several series of related drugs, to discover some of the underlying principles

of drug specificity. This, unfortunately, has not been possible, but the antibacterial action of the present series is worth recording.

The chief feature is the increase of activity with chain length up to a certain point, followed by a decrease beyond that point. The exception to this rule is the curious alternation of compounds with high and low activity in the di-isothiourreas, and the potency of the lowest member. This series differs from the others in having sulphur atoms in the carbon chain. Baker, Harrison & Miller [1941] found that the straight-chain alkyl sulphates had maximum antibacterial activity at 12–16 carbon atoms.

The increase of activity with chain length suggests that surface-active properties may be responsible for the activity of these compounds and that they may act by denaturing some essential protein constituent [Anson, 1939]. It would be interesting to determine the effect of branched chain derivatives, or those having more than two active centres, since alkyl sulphates of this type are very powerfully surface-active [Caryl & Ericks, 1939].

Being basic, the drugs may combine with a bacterial protein, e.g. a nucleoprotein, and render it inert; this is supported by the fact that the most active compounds tend to be the most toxic and the most inhibited by serum. Serum may act by affecting the adsorption of the drug by the bacteria.

The bacteriostatic titres are much lower than the trypanocidal titres, but the variations of activity with chain length are similar in both cases. It is probable that the same mechanism is responsible for both actions of the drugs, and that these may be general protoplasmic poisons.

Taking the results as a whole, the differences in activity caused by interchange of the end groups are not large. The chain length is usually far more important than the end group. The basicity of these groups presumably increases in the following order—di-isothiourreas, amines, amidines, guanidines and quaternary ammonium salts. It does not appear that activity depends on a particular balance between the chain length and the basicity of the end groups. A more complete comparison between the amines and the quaternary salts would be interesting in view of the great difference in basicity between them.

The disubstituted drugs are on the whole more satisfactory than the monosubstituted. The higher members are more active at equal chain length and are far less inhibited by serum, but are more toxic.

One generalization which can be made is that the less strongly basic amines have a relatively stronger action on the gram-positive bacteria while the strongly basic guanidines and quaternary bases have a relatively stronger action on the gram-negative organisms. This is very marked in the dysentery group, against which the diamines and di-isothiourreas are weakly active, and the corresponding amidines, guanidines and quaternary bases relatively stronger. This holds for both the mono- and di-substituted series, and it would appear that increasing the basicity of the end groups is likely to make the compound more active against gram-negative and relatively less active against gram-positive organisms.

If the bacteria are arranged according to sensitivity to the drugs, the order in which they fall is broadly similar to that for the sulphonamides, i.e. those of greatest metabolic activity are the most resistant. Small differences occur, e.g. *Cl. oedematiens* is the most sensitive of the anaerobes to the present series, whereas it is the most resistant to the sulphonamides. The present series too are active in much higher dilution, and are powerfully bacteriostatic in media (broth and serum-broth) in which the sulphonamides have little action. Although the activity of the drugs in broth is considerable, their high toxicity and the interference of serum with the most active are against their chemotherapeutic use, and Dr Hawking's tests show them to be inactive against *Str. pyogenes*. In this they resemble gramicidin [Dubos & Hotchkiss, 1941] which is active in the body

only when applied locally. White, Bratton, Litchfield & Marshall [1941] have found that many substances active *in vitro* are without therapeutic effect. The drugs might be useful for local application against sulphonamide-resistant organisms; and the very sparing solubility of the free bases might confer advantages in the treatment of enteric infections.

SUMMARY

The antibacterial activity of straight-chain ω -mono- and di-amines, amidines, guanidines and quaternary bases in broth increases with chain length up to a maximum and then decreases. The corresponding changes in the di-isothiourreas are irregular.

Gram-positive organisms are on the whole more sensitive than gram-negative, and the bacteria fall roughly in the same order for related drugs.

Serum usually causes an increase in the activity of the shorter-chain and a decrease in that of the longer-chain drugs. This effect, however, varies with the bacteria concerned, and it causes maximum activity in a series to occur at a shorter chain length than in broth.

The drugs are active against sulphanilamide-resistant haemolytic streptococci, but have no therapeutic action when applied systemically.

My thanks are due to Dr Harold King for supplying the drugs, and to Dr Colebrook for the strains, and to both for advice generously given. I am also indebted to Dr A. N. Drury for a supply of human serum, and to Dr F. Hawking for carrying out animal tests.

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