

**ANTIBACTERIAL COLLOIDAL ELECTROLYTES :  
THE POTENTIATION OF THE ACTIVITIES OF MERCURIC-,  
PHENYLMERCURIC- AND SILVER IONS BY A COLLOIDAL  
SULPHONIC ANION**

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THE impairment of the antibacterial activity of inorganic mercuric salts by blood serum, pus and tissue debris has led in recent years to the introduction of salts of phenylmercuric hydroxide, notably the acetate, nitrate and borate<sup>1,2,3,4,5,6</sup>. Armangue and Maestres<sup>7</sup> and later Jensen<sup>8</sup> concluded from work with these organomercuric salts that while the acid radical influences solubility and other physical properties the antibacterial activity is an independent function of the phenylmercuric cation and this activity is not significantly effected by the presence of serum.

Evidence was presented by Süpfle and Miller<sup>9</sup> to show that mercuric ions are adsorbed upon the cortical layer of the bacterial cells thereby poisoning essential enzyme systems ; the union, however, is loose and treated cells can be revived by shaking with a stronger absorbent such as blood charcoal. The superiority of phenylmercuric salts may be associated with the presence of the benzene nucleus which, by conferring lipophilic properties upon the active cation, renders the union of the organo-mercurial with the bacterial envelope less easily reversible.

The enhancement of the antibacterial activity of inorganic mercuric salts by surface active agents was first reported by Hamilton<sup>10</sup> who observed that the activity of potassium mercuric iodide against *Staphylococcus aureus* was increased threefold by the presence of sodium salts of long chain aliphatic carboxylic acids. Later, Hampil<sup>11</sup> showed that certain concentrations of sodium oleate greatly augment the activity of mercuric chloride against this organism. Tobie and Orr<sup>12</sup> found that sodium dioctyl sulphosuccinate strongly potentiates the bactericidal activity of phenylmercuric nitrate and suggested that this was due to the formation of phenylmercuric dioctyl sulphosuccinate although they failed to isolate the compound. The enhancement of activity by surface active agents is not confined to mercurials. Ordal, Wilson and Borg<sup>13</sup> reported that both sodium dioctyl sulphosuccinate and sodium lauryl sulphate increase the activity of 2 : 4-dichlorophenol and ascribed the action to the greater penetrative power of the antibacterial reagent when associated with surface active compounds ; the same effect has been observed<sup>13,14,15</sup> with phenol in the presence of various soaps. In work with cetylpyridinium chloride and sodium tetradecyl sulphate Dyar and Ordal<sup>16</sup> showed that there is much greater adsorption of the former than of the latter upon all bacteria studied ; this accords with the established

## ANTIBACTERIAL COLLOIDAL ELECTROLYTES

fact that cationic soaps are much more lethal to bacteria than the anionic soaps.

Ionic surface active agents are essentially colloidal electrolytes which possess lipophile-hydrophile balance. Colloidal electrolytes possess two properties by virtue of which they influence biological activity, viz., their strong tendency to adsorb at interfaces and their power to form charged hydrated colloidal aggregates (micelles). Micelles, owing to their structure, adsorb ions carrying a charge opposite to that upon the micelle and in addition are able by the process of molecular adsorption to solubilise many organic substances which are normally insoluble in water. Micellar solubilisation is known to facilitate transport of sparingly soluble substances at a rate which may be enormously greater than the normal process of solution and diffusion. These properties of surface active compounds led to an interest in colloidal electrolytes containing, as the constituent cation, an ion of known toxicity to bacteria, viz., the mercuric, phenylmercuric and silver ions. Provided the resulting colloidal electrolyte possessed the requisite physical characteristics it was expected that the colloidal anion would potentiate the lethal action of the metallic or organometallic cation by promoting adsorption of the latter upon the bacterial envelope and that inactivation of the cation by formation of a coagulum with serum electrolytes would be suppressed by the process of micellar solubilisation of the coagulum.

The colloidal acid selected for the present work was dinaphthylmethane disulphonic acid obtained by a Lederer-Manasse condensation of naphthalene-2-sulphonic acid with formaldehyde. The salts of this acid are strongly ionised in aqueous solution; they behave as typical colloidal electrolytes and exhibit the phenomenon of hydrotropy. The standardised solutions used contained 1.0 g. of mercuric-, silver- or phenylmercuric-dinaphthylmethane disulphonates and 19 g. of potassium dinaphthylmethane disulphonate per litre. Comparison has been made between the bacteriostatic activities of these solutions with those of mercuric chloride, silver nitrate and phenylmercuric acetate, these being crystalloid (non-colloidal) electrolytes containing the corresponding antibacterial cations. Three Gram-positive and three Gram-negative test organisms were used, *Staphylococcus aureus* NC7361, *Streptococcus pyogenes* NC2432, *B. subtilis* NC3610, *E. coli* NC86, *Ps. pyocyaneus* NC1999 and *Proteus vulgaris* NC5887. The media were Lab-Lemco broth (B) and broth containing 10 per cent., 50 per cent. and 80 per cent. of normal horse serum (10 per cent., S 50 per cent. S and 80 per cent. S respectively) Tables I and II show the experimentally determined maximum dilutions which completely inhibit visible growth of the organism after 48 hours incubation at 37°C. *p*-Chloro-*m*-xylenol and phenol are included for the purpose of comparison.

In Table II, relating to the silver compounds, are included the experimentally found maximum bacteriostatic dilutions of silver proteinate, silver vitellin and "colloidal silver." It was of interest to include these well known protein silver—silver oxide—silver hydroxide dispersions

since these were specifically introduced as silver compounds which, by virtue of their low content of *ionised* silver, avoid in varying degrees coagulation and consequent inactivation by serum ampholytes.

TABLE I  
MAXIMUM BACTERIOSTATIC DILUTIONS IN MULTIPLES OF 1,000  
(48 hours incubation)

Compound	<i>Staph. aureus</i>	<i>Strep. pyogenes</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>Ps. pyocyaneus</i>	<i>B. Protocus vulgaris</i>
Mercuric chloride; Hg, 74 per cent. :-						
B ... ..	20	20	14	10	10	14
10 per cent. S... ..	20	20	14	14	10	14
50 per cent. S... ..	10	10	8	8	8	8
80 per cent. S... ..	8	8	5	4	2	4
Mercury dinaphthylmethane disulphonate; Hg, 32 per cent. :-						
B ... ..	12	12	8	8	6	8
10 per cent. S... ..	18	18	8	10	8	10
50 per cent. S... ..	18	18	10	10	8	10
80 per cent. S... ..	18	18	10	10	8	10
Phenylmercuric acetate; C <sub>6</sub> H <sub>5</sub> Hg, 82.5 per cent. :-						
B ... ..	400	80	20	20	20	20
10 per cent. S... ..	500	200	40	30	30	30
50 per cent. S... ..	200	200	30	10	20	20
80 per cent. S... ..	100	100	20	8	15	15
Phenylmercuric dinaphthylmethane disulphonate; C <sub>6</sub> H <sub>5</sub> Hg, 56.5 per cent. :-						
B ... ..	250	64	16	10	16	16
10 per cent. S... ..	350	100	25	20	20	20
50 per cent. S... ..	400	120	30	25	30	25
80 per cent. S... ..	400	120	30	25	30	25
<i>p</i> -Chloro- <i>m</i> -xyleneol :-						
B ... ..	7	7	3.5	3	3.5	3.5
10 per cent. S... ..	4	4	2	1	2	2
50 per cent. S... ..	4	4	2	1	2	2
80 per cent. S... ..	4	4	2	1	2	2

It can be seen that the activity of mercury dinaphthylmethane disulphonate in pure broth slightly exceeds that of mercuric chloride per unit of mercuric ion content. While the activity of mercuric chloride falls rapidly as the serum content of the medium rises, that of mercury dinaphthylmethane disulphonate is increased by the presence of all the concentrations of serum employed. In 80 per cent. serum the activity of mercury dinaphthylmethane disulphonate is approximately 5 times as high as that of mercuric chloride per unit of mercuric ion content.

The activity of phenylmercuric dinaphthylmethane disulphonate in broth is of the same order as that of phenylmercuric acetate per unit of phenylmercury content and with both compounds the presence of 10 per cent. serum augments activity. With phenylmercuric acetate, however, as the serum content rises through 50 per cent. to 80 per cent. there is a significant fall in activity; in the case of phenylmercuric dinaphthylmethane disulphonate the initial increase in activity is maintained as the serum content rises to 80 per cent. and at this serum concentration the activity of the compound is of the order of 3 times that of phenylmercuric acetate per unit of phenylmercuric ion content.

ANTIBACTERIAL COLLOIDAL ELECTROLYTES

TABLE II

MAXIMUM BACTERIOSTATIC DILUTIONS IN MULTIPLES OF 1,000  
(48 hours incubation)

Compound	<i>Staph. aureus</i>	<i>Strep. pyogenes</i>	<i>E. coli</i>	<i>B. subtilis</i>	<i>Ps. pyocyaneus</i>	<i>Proteus vulgaris</i>
Silver nitrate; Ag, 60 per cent. completely ionisable :—						
B ... ..	20	24	16	12	18	16
10 per cent. S... ..	10	10	6	6	8	6
50 per cent. S... ..	6	6	4	4	4	4
80 per cent. S... ..	4	4	2	2	2	2
Silver dinaphthylmethane disulphonate; Ag, 31 per cent. completely ionisable :—						
B ... ..	16	20	12	10	12	12
10 per cent. S... ..	20	22	18	12	18	18
50 per cent. S... ..	20	22	18	12	18	18
80 per cent. S... ..	20	22	18	12	18	18
Silver proteinate; Ag, 8 per cent. partly ionisable :—						
B ... ..	10	10	8	6	6	8
10 per cent. S... ..	5	5	4	2	4	4
50 per cent. S... ..	4	4	4	2	4	4
80 per cent. S... ..	4	4	4	2	2	4
Silver vitellin; Ag, 20 per cent. very little ionisable :—						
B ... ..	8	8	6	4	4	6
10 per cent. S... ..	6	6	4	2	4	4
50 per cent. S... ..	4	4	4	2	4	4
80 per cent. S... ..	4	4	4	2	2	4
Colloidal silver; Ag, 70 per cent. none ionisable :—						
B ... ..	8	6	6	3	3	3
10 per cent. S... ..	8	8	6	3	3	3
50 per cent. S... ..	8	8	6	3	3	3
80 per cent. S... ..	6	6	4	2	2	2
Phenol :—						
B ... ..	0.2*	0.2	0.15	0.1	0.15	0.15
10 per cent. S... ..	0.1	0.1	0.1	0.05	0.1	0.1

\* i.e. a dilution of 0.2 × 1000 or 1 : 200.

In pure broth the activity of silver dinaphthylmethane disulphonate is *ca.* twice that of silver nitrate per unit of silver ion content. Whereas, however, the activity of silver nitrate falls very rapidly as serum content rises that of silver dinaphthylmethane disulphonate is increased by the presence of serum up to concentrations of the latter of 80 per cent. in the medium. In 80 per cent. serum silver dinaphthylmethane disulphonate has approximately thirteen times the activity of silver nitrate per unit of silver ion content.

A further point of interest presents itself from Table II with reference to the silver protein derivatives. There is a progressive fall in activity in pure broth from silver proteinate, through silver vitellin to "colloidal silver"; per unit of silver content this progressive fall is exceedingly high. The loss in activity effected by serum also decreases from silver proteinate, through silver vitellin, to "colloidal silver"; although this is the order of increasing total silver content it is the order of decreasing ionisable silver content. By comparison with silver nitrate it is evident that the loss in activity of the silver protein compounds and also the

inhibition of the deactivating effect of serum is due to the replacement of ionisable by non-ionisable silver. In the case of the colloidal electrolyte, silver dinaphthylmethane disulphonate, the high activity must be associated with the ionic nature of the whole of the silver content and the maintenance of this activity in the presence of serum due to the action of the polyvalent hydrated anionic micelle.

#### THE *IN VITRO* REACTION OF MERCURIC-, PHENYLMERCURIC- AND SILVER DINAPHTHYLMETHANE DISULPHONATES WITH SERUM AMPHOLYTES

The loss in activity of mercuric chloride, silver nitrate and phenylmercuric acetate effected by serum and the maintenance of the activity of mercuric-, silver- and phenylmercuric dinaphthylmethane disulphonates in serum can be correlated with the precipitin reactions of the compounds with serum ampholytes. The reagent in 1 : 1,000 dilution was added to a 1 per cent. aqueous solution of the ampholyte, followed by an excess of the reagent in order to determine whether the precipitate (if formed) was redissolved. The results are recorded in Table III.

TABLE III

<i>Compound</i>	<i>Gelatin</i>	<i>Albumen</i>	<i>Casein (Sodium)</i>	<i>Horse Serum</i>
Mercuric chloride... ..	O/i	nil	nil	P/i
Mercury dinaphthylmethane disulphonate ...	nil	nil	nil	nil
Phenylmercuric acetate ... ..	P/i	P/i	P/i	P/i
Phenylmercuric dinaphthylmethane disulphonate	O/s	nil	nil	nil
Silver nitrate ... ..	O/i	P/s	P/i	P/i
Silver dinaphthylmethane disulphonate ...	P/s	O/s	O/s	O/s

nil=no precipitate or opalescence; O=no precipitate but definite opalescence; P=precipitate; /s=soluble in excess of reagent; /i=insoluble in excess of reagent.

#### TOXICITY

The oral toxicities of the compounds were determined in order to ascertain whether the colloidal anion possessed any potentiating effect upon the lethal action of the mercuric, phenylmercuric and silver cations. The compounds were given in aqueous solution to 20 g. mice by stomach tube under light ether—oxygen—carbon dioxide anaesthesia in closely spaced doses. Groups of 4 mice were used for each dose, approximately 40 mice being used for the determination of each toxicity range; the animals were observed for 14 days after the dose.

It is evident that mercuric-, phenylmercuric- and silver- dinaphthylmethane disulphonate are slightly more toxic per unit of active cation content than the corresponding crystalloid electrolytes: this is to be expected from the enhanced adsorption capacities of the compounds. It is of passing interest, however, that the oral toxicities of phenylmercuric acetate and phenylmercuric dinaphthylmethane disulphonate are little more than that of "colloidal silver 70 per cent." and less than that of

## ANTIBACTERIAL COLLOIDAL ELECTROLYTES

silver nitrate both of which compounds are widely used in clinical practice. It would accordingly appear that as far as these two phenylmercuric compounds are concerned the general prejudice against organic mercurials is not founded upon fact.

TABLE IV  
ORAL TOXICITIES

<i>Compound</i>	<i>LD0</i>	<i>LD50</i>	<i>LD100</i>
Mercuric chloride ... ..	7 mg./kg.	10 mg./kg.	12.5 mg./kg.
Mercuric dinaphthylmethane disulphonate ... ..	20	30	40
Phenylmercuric acetate ... ..	50	70	80
Phenylmercuric dinaphthylmethane disulphonate ... ..	50	70	80
Silver nitrate ... ..	30	50	65
Silver dinaphthylmethane disulphonate ... ..	25	50	75
Colloidal silver (70 per cent. Ag) ... ..	50	100	150
Potassium dinaphthylmethane disulphonate ... ..	950	1250	1450

### SUMMARY

1. The potentiating effect of surface active agents upon the antibacterial activity of mercurial compounds has led to an interest in colloidal electrolytes which contain, as the constituent cation, an ion of known high antibacterial activity.

2. The colloidal acid selected was dinaphthylmethane disulphonic acid. The mercuric-, phenylmercuric- and silver-salts of this acid have been prepared and their activities against 3 Gram-positive and 3 Gram-negative organisms compared with those of corresponding crystalloid (non-colloid) salts containing the corresponding cations, viz. mercuric chloride, phenylmercuric acetate and silver nitrate. It was expected that the colloidal anion would potentiate the lethal action of the metallic or organometallic cation by promoting adsorption of the latter upon the bacterial envelope and that inactivation of the cation by formation of a coagulum with serum electrolytes would be suppressed by the process of micellar solubilisation of the coagulum.

3. It has been shown that in pure broth the colloidal anion effects a definite, although small, increase in activity of mercuric and silver ions; the bacteriostatic activity of the lipophile phenylmercuric ion is not altered. In broth containing serum concentrations approaching 80 per cent., however, the activities of mercuric-, phenylmercuric- and silver

dinaphthylmethane disulphonates are of the order of 5, 3 and 13 times as high as those of mercuric chloride, phenylmercuric acetate and silver nitrate per unit of active cation content. These results have been correlated with the precipitin reactions of the compounds with horse serum and other protein ampholytes.

4. The acute oral toxicities of the compounds have been recorded.

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