THE ANTAGONISM OF THE ANTIBACTERIAL ACTION OF MERCURY COMPOUNDS

PART III. THE EFFECTS OF CERTAIN SULPHYDRYL COMPOUNDS ON *E. coli* I

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The antibacterial activity of cysteine, glutathione, thioglycollate, dimercaprol and horse serum to *E. coli* I has been studied. Incorporation of these materials into solid media has little adverse effect upon the number of cells multiplying on it, but in the diluents used for preparing dilutions for counting the effect is more marked. Dimercaprol produces the largest reduction in the number of cells subsequently multiplying. Dimercaprol and thioglycollate are believed to exert their effect by retarding the metabolic processes. Concentrations of the materials suitable for quantitative work involving viable counts have been determined.

SEVERAL workers[1–6] have demonstrated an inhibitory action of cysteine on *E. coli*, especially in a chemically defined medium; the concentration of cysteine responsible being variously reported between 0·002 and 0·2 per cent. The inhibitory action has been ascribed to a prolongation of the lag phase[4,8] when *E. coli* is grown aerobically in chemically defined medium, but does not occur when grown "semi-aerobically"[4] or in broth cultures[8]. Italian workers[6] showed cysteine to cause a temporary bacteriostasis of *E. coli*, the amount and duration of suppression depending on the concentration of cysteine.

Indian workers[2] reported that 1 in 5×10^4 of glutathione had some inhibitory effect upon the growth of *E. coli* whereas Pratt[7] reported that it exhibited a stimulating effect on the growth of this organism, in a chemically defined medium. In a later communication[8], however, it was stated to have little effect on the growth of the organism.

Dubnoff[5] stated that concentrations of about 0·1 mg./ml. of thioglycollic acid were toxic to "wild" types of *E. coli* but Sykes and others[9], in experiments with a variety of organisms, demonstrated that growth retardation was apparent only in concentrations of thioglycollate above 0·5 per cent.

Renoux and Roux[10] reported the bacteriostatic concentration of dimercaprol to *E. coli* was greater than 25 μg./ml., and Berry and Jensen[11] showed that a concentration of 1 in 27·5 showed no bacteriostatic effect on this organism.

An account of the various specific and non-specific factors present in serum which can react with bacteria *in vitro* is given by Wilson and Miles[12].

In view of the various and often controversial findings on the effects of the sulphydryl compounds on *E. coli* it was deemed necessary to determine

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whether these compounds exhibited any antibacterial activity towards the strain used in this work. It may be seen that any effect of the compounds on bacteria may be due to the compound *per se* or to a change in the oxidation-reduction potential produced by the compound.

Experimental and Results

Five substances were selected as possible antagonists of the antibacterial action of mercuric chloride[13]; cysteine hydrochloride, glutathione and thioglycollic acid (monothiols), dimercaprol (a dithiol) and normal horse serum. The first four substances are more or less pure compounds containing a known proportion of sulphydryl, whilst horse serum is a natural product having a small and presumably variable sulphydryl content.

Solutions of cysteine hydrochloride, glutathione and thioglycollic acid were adjusted to pH 7·0 with sodium hydroxide solution; dimercaprol solutions were not adjusted. Solutions were sterilised by filtration with the exception of thioglycollate which was autoclaved[14]. Dilutions of horse serum were aseptically prepared. Solutions of the sulphydryl compounds were prepared on the day of use and assayed by titration with potassium iodate[15].

The bacteriostatic activity of the antagonists towards *E. coli* I was determined by both the liquid and solid dilution methods.

After incubation, crystals of (presumably) cystine were visible at the base of all tubes of liquid medium containing 0·2 per cent or more of cysteine; in the solid medium containing the same concentrations, the cystine was in the form of fine particles dispersed throughout the medium.

Plates containing 0·05 per cent or more of dimercaprol became heavily clouded with oxidised dimercaprol during the overdrying process; this made the surface of the medium rather water-repellant, the drops of culture medium added as inocula being poorly absorbed and tending to coalesce. With 0·25 per cent or more of dimercaprol the solid medium was so opaque as to make it difficult to see some surface colonies, and growth in the liquid medium could not be determined even with the aid of transmitted light. Berry and Jensen[11] overcame this difficulty by determining the pH of the medium; they showed that growth in peptone water containing dimercaprol was accompanied by a pH shift towards alkalinity, but in the experiments recorded here, recourse was made to a biochemical test (indole production with Ehrlich's rosindole reagent). Control experiments showed that the dimercaprol did not interfere with the sensitivity of the test.

The results of these experiments showed that the antagonists were not bacteriostatic to *E. coli* I in the following concentrations: cysteine, dimercaprol and glutathione 0·5 per cent, thioglycollate 1 per cent and horse serum 50 per cent. (It is possible, however, that with the lower concentrations of cysteine and dimercaprol, because of their ease of oxidation, it was the bacteriostatic activity of the oxidised form which was being determined.)

Effect of the Antagonists upon the Viable Count of a Suspension of E. coli *I*

The possible effects of the proposed antagonists were investigated on this organism by means of two sets of experiments. The first consisted of a comparison of the viable counts obtained on peptone agar medium containing various concentrations of the antagonists; the second was similar but the antagonists were incorporated into the dilution blanks and the counts on plain peptone agar were compared. Samples of the final mixture for plating were allowed to stand at room temperature for

TABLE I

EFFECT OF THE ANTAGONISTS ON THE VIABLE COUNT OF *E. coli* I. MEAN NUMBER OF COLONIES DEVELOPING PER DROP AREA IN A SURFACE-VIABLE COUNT

	Concentration of antagonist, per cent					
Horse serum	20	15	10	5	2·5	0
Antagonist in plate	24·24	24·40	23·00	24·06	24·14	23·54
„ „ diluent	25·76	25·97	25·74	26·19	27·21	20·43
As above, after standing ½ hr	27·46	26·55	26·81	26·12	27·04	13·70
Cysteine	0·25	0·20	0·15	0·10	0·05	0
In plate	25·98	26·04	26·48	26·06	26·14	25·48
In diluent	20·75	19·20	21·22	20·03	19·87	17·48
„ „ after ½ hr	19·40	19·10	19·50	20·10	19·70	15·95
Glutathione	0·25	0·20	0·15	0·10	0·05	0
In plate	26·40	25·58	26·32	25·44	25·32	25·38
In diluent	25·50	24·82	25·65	25·65	24·63	24·60
„ „ after ½ hr	24·90	24·50	25·85	24·50	24·10	21·60
Thioglycollate	0·25	0·20	0·15	0·10	0·05	0
In plate	25·12	26·76	27·20	25·54	25·98	25·10
In diluent	20·70	24·48	25·58	26·33	26·48	25·87
„ „ after ½ hr	15·90	19·80	21·10	24·10	26·80	24·40
Dimercaprol	0·20	0·15	0·10	0·05	0·01	0
In plate	23·66	23·34	22·16	23·68	24·24	22·72
In diluent	8·62	9·83	12·03	18·77	20·60	20·28
„ „ after ½ hr	1·40	4·60	8·88	20·70	20·90	18·71

half an hour and the resultant counts compared with those plated immediately after preparation. Each set of experiments was performed over five days, one antagonist being investigated each day. A suspension of *E. coli* I was prepared and adjusted to contain approximately 2×10^9 viable organisms per ml. This suspension was stored at 4° when not in use. The counting method was similar to that described by Miles and Misra[16]. The results for the two series of experiments are shown in Table I, where the mean number of colonies developing per drop area has been calculated from at least 10 replicate counts.

The colonies developing on medium containing 0·25 or 0·20 per cent and even 0·1 per cent of thioglycollate were much smaller than those developing on plain peptone agar plates; this has been interpreted as due to a possible retarding of the metabolic processes of the bacterial cells caused by the high concentration of thioglycollate. A similar reduction in colony size was observed with medium containing 0·15 per cent or more of dimercaprol. Difficulty was encountered with overdried plates containing 0·1 per cent or more of dimercaprol as the insoluble oxidation

product made absorption of the drops unsatisfactory. These concentrations of dimercaprol resulted, after incubation, in a medium too opaque to allow counting of the colonies by the usual illuminated counting box and recourse was made to illumination by obliquely reflected light. This was not particularly satisfactory and it was felt that there was a possibility of much larger counting errors.

In the experiments where the antagonists were incorporated into the diluents, the drop volumes of the serum dilutions differed markedly from those of water, due primarily to the difference in density, and the counts were adjusted to take this into account. Concentrations of 0·20 and 0·15 per cent of dimercaprol appeared to exert a strong retardation of the growth processes of the cells as, in many cases, colonies took 18–24 hours to become visible and were much smaller than those developed on the control plates.

Discussion

As already noted, the experiments were not all carried out at the same time and hence comparison of the results between the antagonists is not valid, neither is that between the two series. The results within a series for a particular antagonist may however be compared.

Incorporation of the antagonists into the medium had little effect upon the number of organisms subsequently growing upon it. A comparison of the highest and lowest mean counts by Student's t test for any dilution of a particular antagonist revealed no significant differences ($P = 0{\cdot}95$).

With the antagonists incorporated into the dilution blanks the results were as follows. In all dilutions of serum the mean count was significantly greater than that obtained using water as the diluent, but there was no significant difference between the counts with the different dilutions. A comparison of the mean counts of samples plated immediately with those allowed to stand for half an hour before plating revealed reasonable maintenance of viability but showed a significant increase with the 20 per cent serum dilution and a marked decrease in the case of the aqueous control. This material has the disadvantages of frothing and allowance must be made for its drop volume being different from that of water.

Cysteine and gluthathione in concentrations up to 0·25 per cent appear to have little effect upon the viability of *E. coli* I. With cysteine a comparison of means in both the dilutions plated immediately and those allowed to stand showed that those containing cysteine were not significantly different from each other. No significant difference was apparent between the mean colony counts from any concentration of glutathione tested whether plated immediately or allowed to stand.

The use of a 0·25 per cent thioglycollate solution as the diluent resulted in a significant reduction in viability, even when the dilution was plated immediately after preparation. In dilutions allowed to stand before plating a significant reduction in viability occurred with all concentrations above 0·1 per cent. Prolonged contact with 0·1 per cent or more of thioglycollate causes a reduction in the number of viable cells, and those still viable multiply more slowly than usual.

Dimercaprol in concentrations above 0·1 per cent causes a marked reduction in the viable count of the organism, and this reduction was even more pronounced in the case of those dilutions not plated immediately. High concentrations probably exert their effect by retarding the metabolic processes.

In all cases the aqueous controls showed reduced viability if not plated immediately.

From these experiments the following concentrations of the proposed antagonists appear to be suitable for quantitative work involving viable counts of *E. coli* I:

Normal horse serum	up to 20	per cent			
Cysteine	„ „ 0·25	„	„	(about	15 mM)
Glutathione	„ „ 0·25	„	„	(„	8 mM)
Thioglycollate	„ „ 0·1	„	„	(„	10 mM)
Dimercaprol	„ „ 0·05	„	„	(„	5 mM)

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