STUDIES ON SALT ACTION

IX. THE ADDITIVE AND ANTAGONISTIC EFFECTS OF SODIUM AND CALCIUM CHLORIDES UPON THE VIABILITY OF BACT. COLI

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In the immediately preceding paper of this series (Winslow and Falk, 1923) we have presented in some detail the results of our studies upon the influence of two salts with a common anion, one with a typical monovalent and one with a typical divalent cation, upon the viability of *Bact. coli* in water. It was there shown that at any pH value 0.725 M NaCl and 0.435 M $CaCl_2$ solutions are toxic to this organism and that 0.145 $CaCl_2$ is toxic in an alkaline solution (pH 8.0), its toxicity here being due to the fact that $CaCl_2$ under such conditions inhibits the ability of the bacteria to regulate the reaction of the solution.

Over four years ago (Winslow and Falk, 1918) we presented a preliminary report which indicated that in a solution containing sodium and calcium salts in appropriate proportions an antagonistic action may be manifest which tends to protect the bacteria against the toxic action which would be exerted by each salt if present alone. The experiments here reported deal with this question, the technique being the same as that reported in the paper first cited (Winslow and Falk, 1923).

A solution of calcium chloride of 0.145 M strength, the strength at which toxic action first appears in an unadjusted alkaline solution, was chosen for the study of antagonism and in table 1 are presented the results of 21 tests in solutions of calcium chloride of this strength plus various concentrations of sodium chloride. The solution was made up alkaline (about pH 8.0) but was not adjusted thereafter and changes in reaction were not recorded.

In this table we have followed the procedure employed in the previous paper and have drawn a heavy rule to indicate the time after which less than approximately two-thirds of the initial bacterial population are still viable. Table 1 shows that a mixture of NaCl and $CaCl_2$ is moderately toxic when the two are present in equal proportion and each in 0.145 molar ("isotonic") concentration, only 38 per cent of the bacteria surviving six hours or longer. (This strain of *Bact. coli* survives in nearly undiminished numbers—89 per cent—for nine hours' in pure, distilled water.) It shows, further, that the toxic effects of the

TABLE 1

Viability of Bact. coli in solutions containing sodium chloride + calcium chloride

Per cent surviving

	PER CENT ALIVE				
NaCl + $CaCl_2$	1 + 1	2 + 1	3 + 1	4 + 1	5 + 1
Tonicity*	2	3	4	5	6
1	114	65	124	90	165
3	80	52	55	110	89
6	38	27	38	104	44
9	41	28	40	117	30
24 hours	35	28	22	90	0+
Number of experiments	8	3	3	4	3

* 1 tonicity = 0.145 M.

salts are additive, since solutions which contain the same quantity of $CaCl_2$ (0.145 mol per liter) but two and three times as much NaCl (2 and 3 $\times$ 0.145 mols per liter) are increasingly toxic. When 4 $\times$ 0.145 molar concentration of NaCl is added to 1 $\times$ 0.145 molar concentration of $CaCl_2$, however, the toxicity instead of increasing further with the increase in total concentration of the solution diminishes very markedly. In this 5 "isotonic" solution over 100 per cent of the initial number of bacteria are still alive after nine hours as compared with 28 to 41 per cent for a similar incubation period in solutions containing the same quantity of $CaCl_2$ but lesser quantities of NaCl. Solutions containing 6 × 0.145 mols per liter (5 Na + 1 Ca) become more toxic again but remain less toxic than 3(2 Na + 1 Ca) or 4(3 Na + 1 Ca) times isotonic solutions up to six hours' exposure. In other words, we may state from these experiments, that the toxic influences of NaCl and $CaCl_2$ upon the viability of *Bact. coli* in water are additive up to a certain ratio of Na:Ca after which they become antagonistic.

The additive and antagonistic influences of NaCl and $CaCl_2$ are summarized in somewhat more convenient form in table 2 and in figure 1 where they are presented with the results from

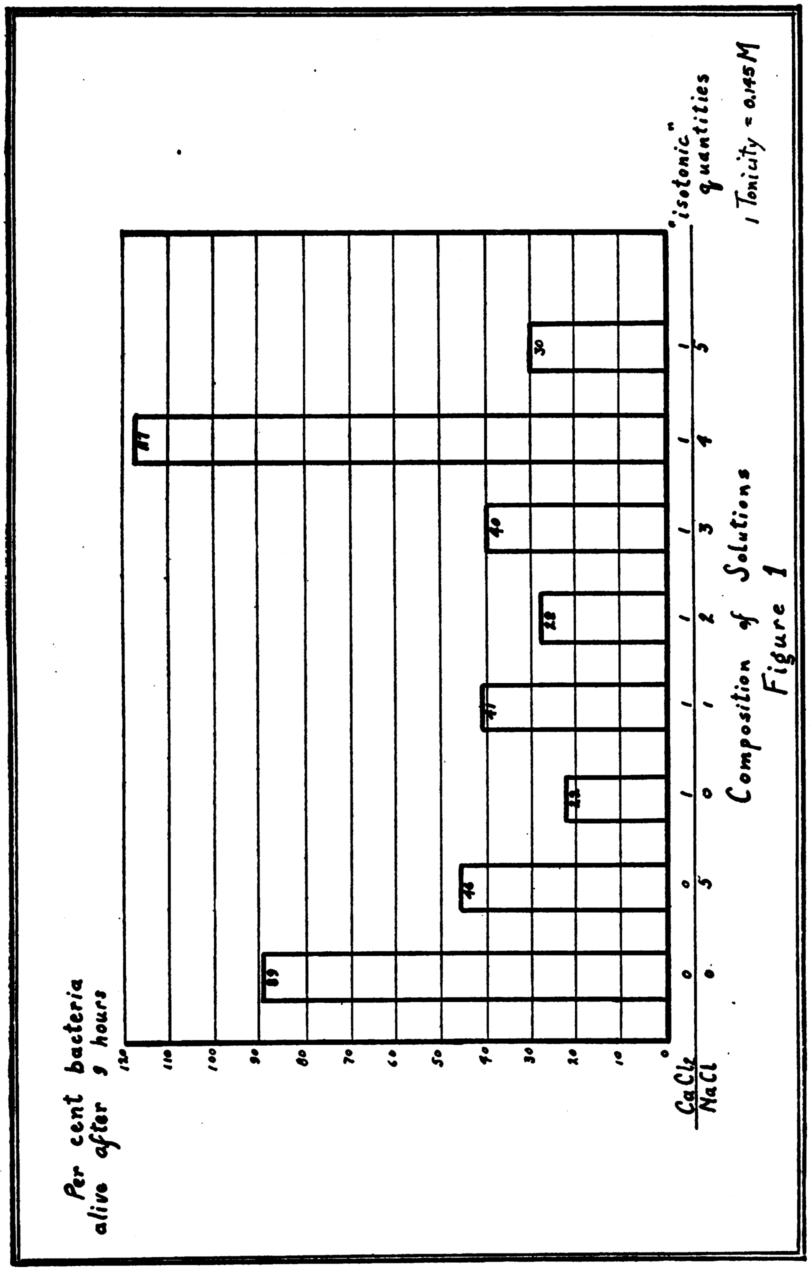
TABLE 2

Viability of Bact. coli in solutions of NaCl and $CaCl_2$, singly and in combinations

TOTAL ISOTONIC CONCENTRATION*	TOTAL MOLAR CONCENTRATION	PER CENT BACTERIA ALIVE AFTER NINE HOURS IN				NUMBER OF EXPERIMENTS AVERAGED		
		Pure NaCl	Pure $CaCl_2$	NaCl + $CaCl_2$	Ratio Na:Ca	NaCl	$CaCl_2$	NaCl + $CaCl_2$
0	0	89	89	89		11	11	11
1	0.145	82	22			16	32	
2	0.290			41	1:1			8
3	0.435	55	0+	28	2:1	7	9	3
4	0.580			40	3:1			3
5	0.725	46	0+	117	4:1	18	10	4
6	0.870	33		30	5:1	5		3

* 1 tonicity = 0.145 M.

comparable experiments with the pure salts used singly. They indicate clearly that in appropriate proportion (4:1), NaCl and $CaCl_2$ are mutually antagonistic to each other with respect to toxicity towards *Bact. coli.* In this proportion and in an absolute concentration in which NaCl by itself is moderately toxic (46 per cent of the bacteria surviving nine hours at 37°C.) and in which $CaCl_2$ is highly toxic (5 iso $CaCl_2$ often produces sterility in nine hours and uniformly reduces the concentration of viable bacteria by more than 99.5 per cent) the mixture of NaCl and $CaCl_2$ permits the bacteria to survive in undiminished numbers for nine to twenty-four hours. Indeed, after nine hours there were still 117 per cent of the bacteria alive as compared to 89 per cent in pure water.


Per cent bacteria
alive after 9 hours
CaCl2
NaCl
"isotonic" quantities
1 Tonicity = 0.145M
Composition of Solutions
Figure 1

In the experiments presented thus far no attempt was made to modify or control the pH of the solutions. In a preliminary publication (Falk, 1920) and in the preceding paper in this Journal (Winslow and Falk, 1923) we have indicated certain findings in regard to the influence of hydrogen-ion concentration upon the action of these salts. We have shown that the viability of *Bact. coli* in pure solutions of water, of NaCl and of $CaCl_2$ is greatest at pH 6.0 to 6.5 and diminishes rapidly at a smaller pH and more slowly at a greater pH. The results of 19 experiments on the influence of pH (maintained by repeated readjustment)

TABLE 3

Viability of Bact. coli in solutions containing sodium chloride + calcium chloride

	PER CENT ALIVE						
NaCl + $CaCl_2$	5 + 1 (tonicity = 6)*						
pH	4.0	5.0	6.0	6.5	7.0	7.5	8.0
1	0+	74	88	106	98	92	90
3	0+	86	82	100	84	80	75
6	0+	62	73	92	71	57	54
9	0+	0.2	71	62	50	45	41
24 hours	0+	0+	24	21	13	1	9
Number of experiments	1	1	2	3	6	2	4

* 1 tonicity = 0.145 M.

upon viability in solutions of the two salts, NaCl and $CaCl_2$, are summarized in tabular form in table 3, while in table 4 the nine-hour results are compared with those obtained after the same period in previous experiments by using the same concentration of the salts separately. In the preceding paper of this series we have described in detail the method of adjusting the pH immediately after the bacterial inoculum is added to the test solution and the methods of readjusting the pH if it varied from the stated value by more than 0.2 at stated intervals during the incubation period.

Table 4 and figure 2 bring out the exceedingly interesting fact that at reactions of pH 7.0 and all more acid reactions the effect of NaCl and $CaCl_2$ is additive, the mixture being more toxic than either salt alone. At regulated alkaline reactions on the other hand (comparable to the experiments with unadjusted reaction reported in tables 1 to 3) the two salts antagonize each other, the mixture being less toxic than either salt alone and markedly less toxic than the added toxicities of the two salts used singly. In addition, it is evident from figure 2 that the toxicity of NaCl, at the strength here studied, appears only at pH values below 6.0 or above 7.0; that of $CaCl_2$ only above 7.0. It appears also that NaCl *narrows* and $CaCl_2$ *widens* the zone for hydrogen-ion tolerance of *Bact. coli* and

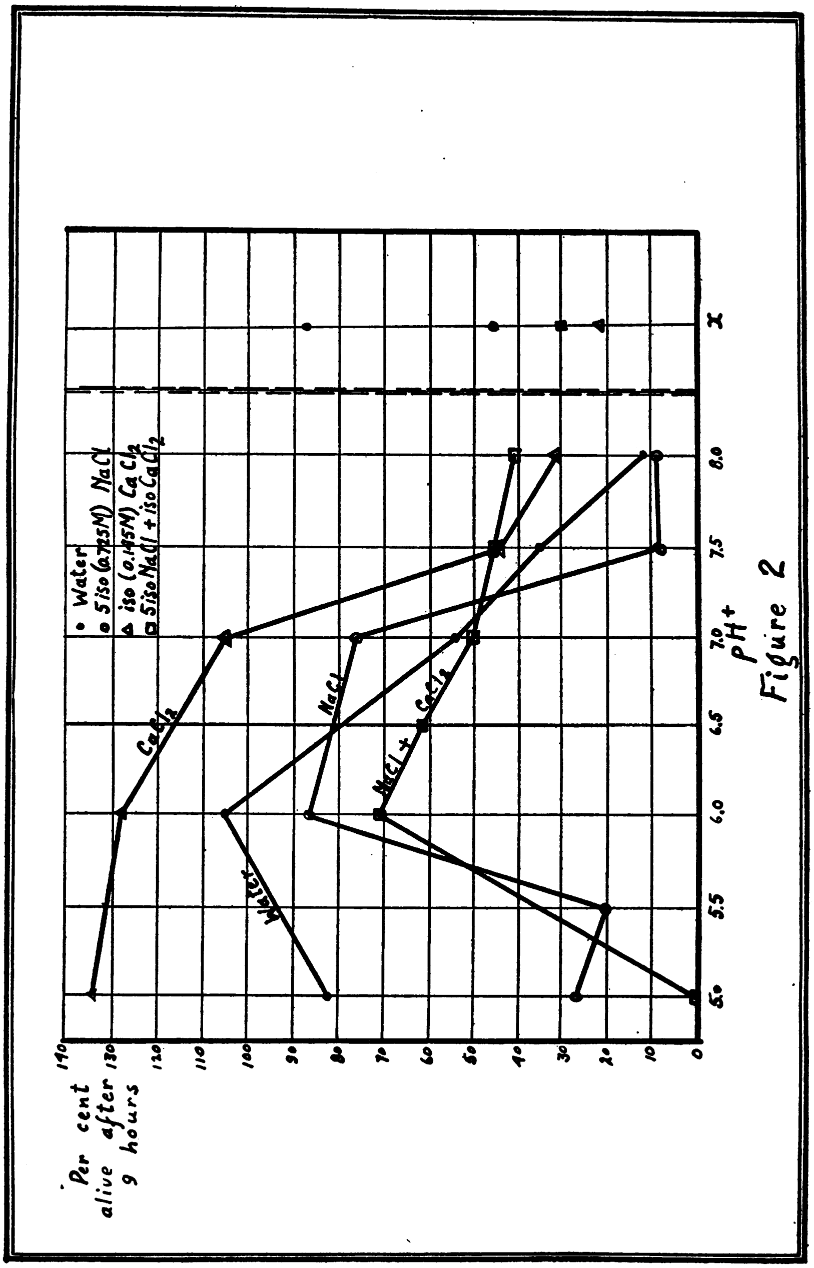
TABLE 4

Effects on viability of Bact. coli of sodium and calcium chlorides after nine hours' exposure

	PER CENT BACTERIA ALIVE				
pH	5.0	6.0	7.0	7.5	8.0
Water	82	106	54	35	12
0.725 M NaCl	27	87	76	8	9
0.145 M $CaCl_2$	134	128	106	44	31
0.725 M NaCl + 0.145 M $CaCl_2$	0.2	71	50	45	41

that effects of the salts which are clearly evident in more acid solutions tend to disappear (approach the pure water curve) at the same pH (7.0 to 7.5). Holm and Sherman (1921) and Sherman and Holm (1922) have reported that salts which accelerate growth of *Bact. coli* seem to widen and salts which inhibit growth seem to narrow the zone for H-ion tolerance. It is significant to observe that these effects upon growth are confirmed for viability.

In the preceding paper in this Journal we have called attention to the fact that in water and saline suspensions of *Bact. coli*—regardless of the initial pH—the hydrogen-ion concentration tends to shift to the neutral zone 7.0 to 7.4. This observation has also been reported by Eggerth and Bellows (1922). It is


Per cent alive after 9 hours
• Water
○ 5 iso (0.725M) NaCl
△ iso (0.145M) CaCl2
□ 5 iso NaCl + iso CaCl2
CaCl2
NaCl
Water
NaCl + CaCl2
pH+
Figure 2

interesting to call attention to the fact that all four curves in figure 2 approach uniform values in this pH zone of maximum physiological buffer. The significance of this observation is being studied in our laboratory.

These results, taken together with those reported in the immediately preceding paper seem to us to warrant the conclusion that the toxic effects exerted by salts may be of two distinct sorts. Very high concentrations of salts appear to exert a toxic effect which is apparent at all reactions and is additive when sodium and calcium chlorides are mixed. At a lower concentration (0.145 M) calcium chloride exerts a different influence, manifest only in alkaline solutions and due to an inhibition of the power of the bacteria to reduce the alkalinity of the solution in which they are suspended. It is this latter type of toxic influence which is antagonized by sodium chloride and in alkaline solution the mixture of these salts in the proportion of 5 parts NaCl to 1 part $CaCl_2$ is more favorable to viability than even distilled water.

The mechanism of ionic and molecular effects upon bacteria has been discussed by one of us (Falk, 1923) at greater length than is possible here. It is being further studied in this laboratory and will be treated more fully in later papers in this series.

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