THE SPECIFIC POTENCY OF CERTAIN CATIONS WITH REFERENCE TO THEIR EFFECT ON BACTERIAL VIABILITY[1]

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OBJECT OF STUDY

In previous contributions from this laboratory (Winslow and Hotchkiss, 1922; Hotchkiss, 1923; Winslow and Falk, 1923a; Shaughnessy and Winslow, 1927; Winslow and Dolloff, 1928; Fabian and Winslow, 1929) we have brought forward evidence to show that cations exert a highly characteristic effect upon bacterial viability.

The fact that a low concentration of a given salt stimulates biological action and a higher concentration inhibits it has been shown by numerous observers and in general all the studies have indicated much the same relative potencies of the various cations. Among the most important work along this line may be mentioned that of Lipman (1909) on the effect of NaCl, KCl, $MgCl_2$ and $CaCl_2$ upon ammonification by *B. subtilis*, of Brown and Hitchcock (1917) on nitrification in soils and of Brooks (1919, 1920, 1921) on carbon dioxide production by *B. subtilis*. Brown and Hitchcock (1917) present excellent curves for the influence of NaCl Na_2SO_4, $MgSO_4$, $CaCO_3$, $NaHCO_3$, Na_2CO_3 and $CaCO_3$ upon nitrification in soils. Here, however, calcium was least potent of the cations studied, in direct contrast with results in simpler media.

Brooks gives excellent curves for NaCl, KCl and $CaCl_2$ (1919) for $MgCl_2$ (1920) and for La $(NO_3)_3$ (1921) all showing stimulation of carbon dioxide production by low concentrations and inhibition

[1] Based on a thesis presented by the junior author in partial fulfilment of the requirements for the degree of Doctor of Philosophy in Yale University.

by higher concentrations. Branham (1929) presents similar data for yeast.

In the first papers of our own series (Winslow and Hotchkiss, 1922; Hotchkiss, 1923) it was demonstrated that a wide variety of cations stimulate bacterial growth in low concentration and inhibit it in high concentration. Winslow and Dolloff (1928) showed that the efficiency of each cation (both in the stimulating and in the toxic range) may be expressed by a characteristic constant and that mixtures of the chlorides of sodium, potassium and magnesium exhibit exactly the effects which would be predicated if their components acted in a purely additive fashion. Fabian and Winslow (1929) found that the sodium ion exerted its characteristic effect in combination with a wide variety of anions including the hydroxyl ion, the result being determined by the combination of two factors,—concentration of sodium and pH.

It seems reasonable to conclude from these results and those of other workers that all cations exert upon bacterial viability a certain influence (aside from other more specific influences) which is qualitatively the same. The quantitative effect of different cations varies very widely but each has a specific efficiency, both as regards stimulation and inhibition. This characteristic, we propose to designate as "specific potency." The effect of mixtures of salts appears to be determined (aside from differences in pH) largely by the arithmetical sum of their specific potencies.

The present study was designed to test this postulate of specific potency by a careful study of salts and salt mixtures involving a larger group of cations than those reported upon by Winslow and Dolloff (1928).

TECHNIQUE

The organism used in these studies was the same strain of *Escherichia coli* (*communis* type) used in all the previous work of this laboratory. It was originally isolated from water in 1916 and is unusually well-adapted to such studies because it maintains itself in distilled water in almost undiminished numbers for a

period of 24 hours or more. The stock culture was maintained on nutrient agar with occasional passages through nutrient broth.

For the actual study of viability we used Dolloff's synthetic medium. This medium (Dolloff, 1926) consists of 5 grams of recrystallized ammonium tartrate, 5 grams of Pfanstiehl lactose and 0.02 gram of dibasic ammonium phosphate in a liter of water. Our stock solution was made up two and a half times this strength and sterilized at 15 pounds pressure for 20 minutes,—to be later added to the salt solutions to produce the standard concentration of the final medium.

The Dolloff medium was selected after a preliminary study in which it was compared with distilled water, used by Winslow and Falk (1923a) and with 1 per cent peptone water (Difco peptone) used by Hotchkiss (1923). It was expected that the activity of such salts as calcium might be very different in the different media since even so low a concentration as 0.005 M $CaCl_2$ formed a distinct precipitate in the Dolloff medium.

In distilled water there were on the average 11 million bacteria per cubic centimeter alive at the end of 48 hours, in Dolloff medium, 99 millions and in peptone-water, 161 millions. The quantitative effect of the salts tested (NaCl and $CaCl_2$) was to a slight degree affected by the medium, the nutrient materials present in the more complex media exerting a protective effect. Thus, maximum stimulation with NaCl was apparent at a concentration of 0.05 M in distilled water at 0.08 M in Dolloff medium and at 0.1 M in peptone-water. Marked toxicity appeared at 0.25 M in distilled water, and at 0.5 M in the other media. With $CaCl_2$, however, the effect was even less, optima so far as distilled water and Dolloff medium were concerned, being between 0.005 and 0.008 M in both cases and marked toxicity appearing at 0.01 M. In peptone-water, the toxic effect of $CaCl_2$ was markedly lowered, being insignificant even at 0.1 M. So far as the Dolloff medium was concerned, it seemed clear that such precipitation as occurred did not seriously affect the relative potency of the salts and this medium was therefore used in all succeeding work. In the inhibitive range many salts are rendered far less active in a peptone medium (Winslow and Dolloff, 1928) so that our results cannot be directly compared with those of Hotchkiss.

The salts used were all chlorides and were Baker Analyzed products. They were made up in convenient concentrations with sterile distilled water and stored in glass-stoppered bottles. All glassware, except that used for plating, was Pyrex and was allowed to stand at least twenty-four hours in cleaning solution, rinsed in hot water and in distilled water and sterilized at 180°C. for two hours.

In making our tests, the organism was grown for twenty-four hours on nutrient agar, washed from the slant with distilled water and then washed three times by centrifugalization. A suspension of the organism was then made up to contain approximately ten million organisms per cubic centimeter. Nine cubic centimeters of the test solution, containing a mixture of Dolloff medium and salt solution adjusted to give the desired final concentration, were inoculated with 1 cc. of this bacterial suspension, so that the initial concentration of organisms at the beginning of an experiment was about one million per cubic centimeter.

The suspensions thus prepared were incubated for 44 to 48 hours at 37°C., when plates were made in triplicate and colonies counted after 48 hours at 37°C.

The incubation period of 48 hours was selected after considerable preliminary experimentation with eight different salts. In these early studies the original suspension contained 20 to 50 million bacteria per cubic centimeter. In the Dolloff medium without added salts, the number rose to over 200 million after 24 to 48 hours and then fell to some 80 million after 144 hours. In favorable salt solutions the numbers rose to perhaps double their respective salt-free controls, while in unfavorable solutions the numbers fell off rapidly, in some cases reaching sterility after 48 hours. Stimulating effects were manifest in about the same degree at all the different time intervals (24, 48, 72, 96 and 144 hours); but slightly toxic salt concentrations tended to lose their inhibitive power after 48 hours, perhaps as a result of adaptation of the organisms to their menstruum. This phenomenon was marked in 0.05 M $CaCl_2$, 0.25 $MgCl_2$ 0.1 M LiCl and 0.0005 M $ZnCl_2$. For this reason, 48 hours was chosen as our standard test period since at this time the salt effects were most sharply contrasted.

Hydrogen ion determinations were made both before and after incubation by the electrometric method, using a Leeds and Northrup student's potentiometer with quinhydrone electrodes. Differences in reaction were not important under the conditions of this study. The Dolloff medium without added salts had a pH of 5.5 and remained at about that level. With the added salts the pH was a little higher, lying in the range 5.5 to 6.3 in 72 out of 80 experiments at the beginning and varying somewhat more widely at the end. Variations in bacterial numbers were not, however, correlated with differences in hydrogen ion concentration.

TABLE 1

Effect of various dilutions of NaCl upon viability of Es. coli in Dolloff medium

NaCl MOLALITY	BACTERIA IN MILLIONS PER CUBIC CENTIMETER									AVERAGE	PER CENT SURVIVAL AS COMPARED WITH SALT-FREE CONTROL	pH
1.0	0	0	0	0	0	0	0	0	0	0	0	5.1–5.6
0.5	59	26	30	4	75	65	3	28	28	42	37	5.4–5.7
0.25			129	154	146	79	130	104	161	129	115	5.5–6.1
0.10	142	108	164	172	147	182	147	110	202	153	137	5.5–6.4
0.08	119	164	195	191	182	149	179	133	240	172	154	5.5–6.8
0.05	97	87	226	224	81	115	151	92	191	140	125	5.5–6.9
0.01			178	173	231	74	134	82	125	142	127	5.5–6.6
0.005			202	166		66	132	79	114	126	113	5.6–6.8
0	67	87	185	157	119	136	95	49	114	112		5.5–5.5

EFFECTS OF THE NINE CATIONS STUDIED

The type of results obtained may be indicated by a single complete protocol presented in table 1. It will be noted that the number of duplicate determinations made at a particular dilution varied from 6 to 9 in this particular case. With many dilutions of other salts the number of duplicate determinations ran up to 10, 11 or 12. As in most bacteriological work, the variation between series is considerable but the general uniformity of the average results indicates that these random errors were reasonably well eliminated by the number of series averaged.

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