THE ENDOGENOUS RESPIRATION OF BACILLUS CEREUS

II. The Effect of Salts on the Rate of Absorption of Oxygen

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I. INTRODUCTION

This paper deals with the action of salts on respiration in the absence of substrate—the endogenous respiration of Stier and Stannard (1935). The use of substrates which permit cell division has been avoided because salts influence cell multiplication, creating difficulties in interpreting the results in terms of the effect of the salts upon respiration. Moreover, measurements of gaseous exchange in the presence of substrate may give a misleading picture of the respiratory activity of a bacterial suspension owing to the existence of concurrent anabolic reactions, even where the number of cells does not change, as in the case of washed cells (Giesberger, 1936; and Barker, 1936). The effects of the components of the buffer mixture were first assessed, since they were expected to be similar to those of other salts. Thus, the investigation was begun by studying the effect of salts in unbuffered solutions without substrate, and the data so obtained were applied to the interpretation of experiments in which the effect of salts was studied in buffered solutions.

II. EXPERIMENTAL PROCEDURE

The comparison of the effects caused by a variety of salts necessitated the preparation of a series of cultures of the same physiological age, washed so as to remove external nutrients. Suspensions of these cells were mixed with salt solutions, and the consumption of oxygen was then followed in manometers.

The conditions under which the cells were grown have been given elsewhere (Ingram, 1939). It was found that cells 24 hours old at 25°C. had a large endogenous respiration, maintained at a constant level for several hours after the preparation of a suspension; and as it was desirable to have some constant value, to which the rates of respiration in salt solutions might be referred, cultures were incubated for 24 hours in these experiments. The growth was washed in the centrifuge, once in distilled water and then twice in distilled water or in buffer solution as the case required. The washed suspensions were shaken and filtered, and diluted to a fixed turbidity, corresponding to 10 mgm. dry weight of cells per milliliter, checked by determining the dry weight of a sample of the suspension after drying at 105°C. for 24 hours. The diluted suspension was then aerated for 15 minutes, after which portions of 1.5 ml. were measured into the flasks of Barcroft manometers and 1.5 ml. of salt solution was added to each sample. Each manometer flask thus contained a given number of cells in a given volume of salt solution. The rates at which the treated samples took up oxygen were then measured at 25°C. (Ingram, 1939).

Solutions were made in distilled water, containing about 30 parts per million of copper. The salts used were "Analar" reagents, except for the chlorides of lithium and cerium which were of "technical" purity.

III. EXPERIMENTAL DATA

Preliminary experiments

At the outset, it was necessary to decide whether the action of salts depended on the amount of salt present in relation to the number of bacteria, or on the concentration of the salt. Normal experiments would not have distinguished between these two possibilities, for equal numbers of cells were always added to the same volume of each salt solution. Special experiments were therefore carried out, with suspensions of cell-content 2.5,5 and 10 mgm. dry weight per milliliter. It was found that the rate of oxygen uptake by each of these three suspensions was reduced in the same proportion after the addition of 0.6 M sodium chlo-

ride. Thus it was presumed that the rate of endogenous respiration in a salt solution was determined solely by the concentration of salt present, and that it was legitimate to array rates of respiration against molar concentrations of salts in the suspensions.

All the data were handled in this way. The rates of oxygen absorption were steady rates attained about 100 minutes after the addition of the salts to the freshly-prepared bacterial suspension; they are expressed as percentages of those in the absence of added salts, that is, of the rates in distilled water or in buffer solution. Measurements of the rate of respiration in a salt solution were reproduced to within 5 per cent in duplicate experiments, when calculated as percentages of the rate in the absence of salt. The variation was due to differences in the susceptibility of the bacteria to salt solutions, rather than to errors in the measurement of the respiration.

The action of salts on respiration was found to be reversible. For example, addition of 1.0 M sodium sulphate to an aqueous suspension reduced the rate of respiration to one-tenth of the initial value; yet when the cells were centrifuged out after one hour in sodium sulphate and washed and re-suspended in distilled water, the rate of respiration returned to its initial value within 20 minutes. It appears that salts do no permanent injury to the respiratory system.

Data obtained with unbuffered suspensions

All the salts investigated behaved in the same way; in low concentrations they increased the rate of uptake of oxygen by *Bacillus cereus* and in high concentrations they reduced it.

The rates of endogenous respiration in the presence of small amounts of a number of salts are given in table 1. The range of concentration, over which stimulation occurred, was lower, the greater the valency of the cation. The concentrations of the chlorides of 1-, 2- and 3-valent cations which maintained the rate of respiration at the same value as in distilled water were roughly 0.1 M, 0.01 M, and 0.0001 M respectively. On the other hand, sodium chloride, sulphate, and citrate, exerted a stimulating action at concentrations less than 0.2, 0.2, and 0.05 M respec-

tively; these concentrations are so much more nearly alike that it may be supposed that the nature of the anion was of minor importance compared with that of the cation.

In table 1 are included a series of measurements made upon cells suspended in lactose solutions. These were made in order to determine the extent to which the osmotic pressure of the solution might affect the respiration of cells suspended in it. This sugar is not attacked by the strain of *B. cereus* used in these experiments. The rate of respiration was shown to be practically

TABLE 1

The increased rate of respiration in the presence of low concentrations of salts

SALT	NUMBER OF EXPERIMENTS	MOLARITY OF SALT PRESENT								
		0.0005	0.001	0.002	0.005	0.01	0.02	0.05	0.1	0.2
Lactose	2			102	105	98	98	99		92
NaCl	5			120			110	104		103
KCl	3						106			106
LiCl	3					128	116	115	119	83
NH_4Cl	2					104		113		94
Na_2SO_4	2					109		107		105
$Na_3C_6H_5O_7$	4		119			150		104	62	
$CaCl_2$	4	119		112	108		97	92		80
$MgCl_2$	1							101		
$CeCl_3$	2	Concentration		0.00005	0.00015	0.0005		0.006	0.01	
		Respiration		104	102	81		63	43	
		pH		5.66	5.58	5.30		5.06	4.60	

independent of lactose concentration up to 0.2 M, which indicates that salts do not increase respiration by changing osmotic pressure.

The data relating to the chloride of a trivalent metal, cerium chloride, are included in table 1. They are not directly comparable with those for other salts by virtue of the acidity developed in the solutions. (It would have been possible to overcome this condition by the addition of alkali along with complex ions to keep the trivalent hydroxide in solution, but the interpretation

of the data appertaining to such solutions would have been difficult.) It would appear that the chloride of the trivalent metal was a more potent inhibitor than those of mono- or di-valent metals, but the concentrations of hydrogen-ions in the solutions were such that it was impossible to ascribe the decrease in respiration to the salt alone.

A study of the inhibition of respiration under the influence of more concentrated salt solutions showed that, above a certain limit of concentration, the rates of absorption of oxygen frequently approximated to those given by the equation

$$\log r = P - Q.c. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

(where r is the rate of uptake of oxygen, c the concentration of salt in the suspension, and P and Q are constants for a given salt). By plotting $\log r$ against c a linear relation was obtained, whether r was measured in absolute or in relative units, for multiplication of the value of r merely changed the value of P. Thus, in the figures, the percentage rates of respiration (referred to a suspension without salt) are plotted on a logarithmic ordinate, against the salt concentration on a linear abscissa. (With this arrangement, the value of Q is given by the slope of the line $\log r/c$, and the value of P by the intercept of the line on the ordinate axis. Q is a measure of the proportion in which respiration is decreased by a given increase in salt concentration. P corresponds to the logarithm of a fictitious rate of respiration in the absence of salt, which is usually higher than that actually observed, since the rate of respiration passes through a maximum value at low salt concentrations; this portion of the respiration/concentration relation is not shown in figures 1 and 2, which portray only that range of salt concentrations over which equation (1) has been found to hold good.)

Figure 1 shows how closely the respiration obeyed equation (1) within the appropriate range of concentration. Roughly speaking, equation (1) was valid directly there was any considerable inhibition of respiration. Figure 1 shows, in addition, that the value of Q increased with the cations of alkali metals in the order K+, Na+, Li+, the values being 0.32, 0.68 and 0.90 respec-

tively. Anomalous data were obtained with ammonium chloride. At concentrations less than 0.4 M the inhibition was about the same as that of potassium chloride, but it increased very rapidly at concentrations approaching 1.0 M. This was probably caused by the increased acidity (pH = 5.3 in 1.0 M solution). The action of salts of divalent cations is demonstrated by magnesium and calcium chlorides. These salts were more powerful

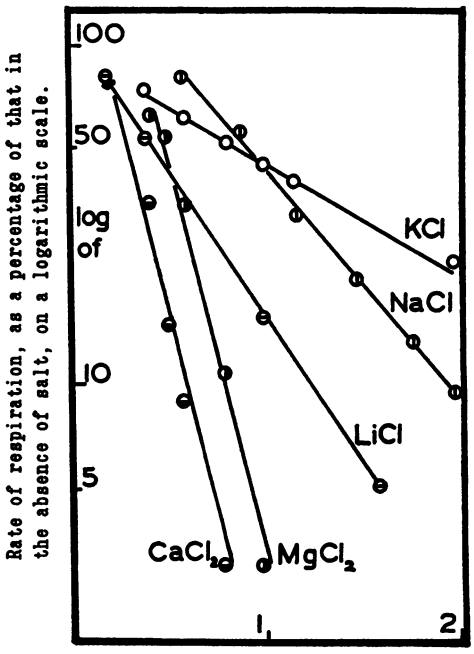

FIG. 1. THE INHIBITION OF RESPIRATION BY CONCENTRATED SOLUTIONS OF METALLIC CHLORIDES

inhibitors of respiration than those of the alkali-metals. Magnesium chloride was found to be slightly less potent than calcium chloride, although the associated values of Q and hence the slopes of the lines in figure 2 were about the same (2.24 and 2.37 respectively). The way in which the inhibition of respiration varied among sodium salts containing different anions may be seen in figure 2. The degree of inhibition caused by a given

concentration of a sodium salt increased with the valency of the anion. The values of Q for the chloride, sulphate, and citrate, calculated from figure 2, are 0.68, 1.27, and 1.58 respectively.

Mixtures of sodium and calcium chlorides were observed to give rise to effects similar to those caused by these salts taken separately. Three combinations of these salts are possible, where (a) both are present in stimulating concentrations, (b) one

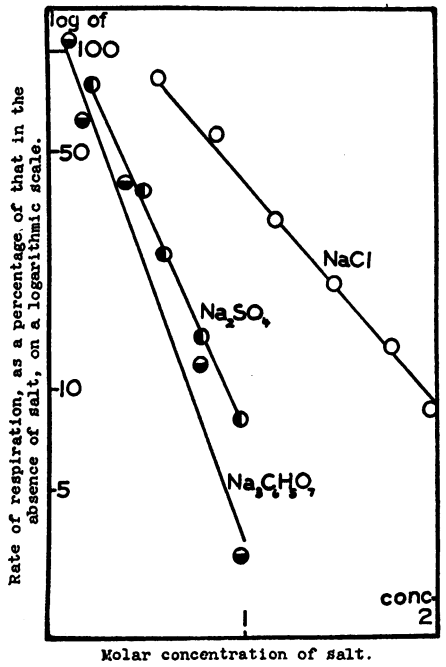

FIG. 2. THE RATE OF RESPIRATION IN CONCENTRATED SOLUTIONS OF DIFFERENT SODIUM SALTS

is present in stimulating, the other in inhibitory concentration, and (c) where both are present in inhibitory concentrations; these three possibilities are represented under corresponding letters in table 2. In dilute mixtures (a) there was slightly greater respiration than in either of the components alone, the greatest respiration occurring in a solution with a Na+/Ca++ ratio of 10:1. Wilson (1922) has shown, correspondingly, that Ringer's solution lowers the viability of bacteria less than

physiological saline. A similar state of affairs was found in case (b) where the weakly inhibitory action 0.02 M $CaCl_2$ was converted into a considerable stimulation of respiration by the presence of nine times its concentration (0.18 M) of NaCl. But in mixtures with more calcium, the rate of respiration was lower than in either salt alone, and this was even more striking when both salts were present in inhibitory concentration.

TABLE 2

The rates of respiration in unbuffered mixtures of sodium and calcium chlorides

TOTAL MOLARITY	NaCl	$CaCl_2$	$\frac{Na+}{Ca++}$	RESPIRATION	EQUIV. CONC. NaCl*	CORRESP. TO EQUIV. CONC. NaCl†
	M	*M*				
(a) 0.03	0.03	0.0005	60:1	127	0.03	108
	0.03	0.001	30:1	137	0.033	107
	0.03	0.003	10:1	139	0.04	106
(b) 0.02	0.18	0.02	9:1	117	0.24	103
	0.16	0.04	4:1	87	0.28	90
	0.1	0.1	1:1	14	0.40	70
(c) 1.0	0.9	0.1	9:1	30	1.2	30
	0.8	0.2	4:1	10	1.4	25
	0.5	0.5	1:1	<1	2.0	8

*† The figures in these columns represent (*) a concentration = (the concentration of NaCl present + 3 times that of the $CaCl_2$ present) and (†) the respiration corresponding to this concentration of sodium chloride.

Data obtained in buffered solutions

The concentrations of hydrogen ions were not the same in suspensions containing different neutral salts because different salts affect the buffering action of a bacterial suspension in different ways (Shaughnessy and Falk, 1924). Divalent cations prevented the production of alkaline substances more than monovalent cations, giving more acid suspensions. To compare the effects of salts on respiration at a more nearly constant pH, experiments were carried out in buffered solutions.

It was found that a mixture of disodium hydrogen and potassium dihydrogen phosphates was satisfactory, if the total con-

centration was small. The cations present behaved as in other salts. The pH of an aqueous suspension of *B. cereus* remained at about 6.15 during the period of constant respiration, and the rate of respiration in phosphate buffer at this pH was some 3 per cent greater than that in distilled water. The total concentration of Na+ and K+ in this buffer was about 0.03 M, so that the increase of respiration corresponded with that obtained with other salts (cf. table 1). In buffer solution of pH 6.0, containing 0.028 M K+ and 0.01 M Na+ (Sørensen, 1909) the rate of respiration was 3 per cent lower than in buffer of pH 6.15; this drop in respiration is consequent upon the small movement in the acid direction away from the pH of optimum respiration (about 6.5). Thus, the rate of respiration in buffer at pH 6.0 was the same as that in distilled water, and the experiments were carried out at pH 6.0 so as to make the data in buffered solutions numerically comparable with those for unbuffered solutions.

The data derived from this series of experiments are set out in table 3, which shows that in buffered suspensions the general effect of salts was the same as in unbuffered suspension, with slight changes in the magnitude of the induced phenomena. Thus, comparison of the data given in tables 1 and 3 for sodium chloride, and sodium sulphate shows that in the case of each salt, low concentrations brought about a greater stimulation of respiration in the presence of the buffer than in the unbuffered suspensions. The stimulating action of low concentrations of a salt thus appears to have been increased by greater acidity.

Two salts were used which were found to cause a marked inhibition of respiration, even at low concentrations; these were sodium nitrite and potassium cyanide. The data are given in table 3. Those relating to the nitrite solutions show that there was a stimulation of respiration in the most dilute solutions, of the same order as that observed with other salts of alkali metals, the toxic effects of the anion becoming apparent only at higher concentrations. The data relating to potassium cyanide are only approximate, because hydrolysis of this salt was accelerated by reaction between potash and the hydrogen cyanide from the gas phase in the manometer flasks; this gave rates of respiration

which increased with time, as the inhibition was reversible. The data are calculated from the initial rates of respiration, and are more reliable the more concentrated the solution to which they refer.

The rates of uptake of oxygen, in buffered suspensions containing considerable concentrations of sodium chloride, did not yield a straight line when log r was plotted against c. The respiration was more strongly inhibited at high salt concentra-

TABLE 3

The rate of respiration in salt solutions buffered at about pH 6.0, in 0.033 M phosphate

SALT	NUMBER OF EXPERIMENTS	MOLARITY OF SALT PRESENT											
		0.0005	0.002	0.005	0.01	0.02	0.05	0.067	0.2	0.5	0.6	1.0	2.0
NaCl	5		117	126	122	112	111		105		86	50	3
KCl	2			114	104	104	98		94		67	45	
NaCl + KCl (1:1)	2			124	115	109	107		100		80	47	
$NaNO_3$	2			119	114	106		103	79		35	3	
KNO_3	2			115	109	108		103	96		57	32	
$NaNO_3$ + KNO_3 (1:1)	2			119	114	107		96	95		38	7	
Na_2SO_4	2	128	131	130		116	106		105	92			
$MgSO_4$	2	128	124	115		105	93		83	79			
$MgCl_2$	2	117	120	113		101	97		85				
$NaNO_2$	2	124		97	64	3	0						
KCN	2	75	39	32		20	20						

tions than in the unbuffered solutions (cf. fig. 1 and table 3). This was probably because the addition of salts to a phosphate buffer causes the pH to change in the acid direction (Green, 1933), and change of pH to values more acid than 6 contributes to the lowering of respiration with *B. cereus*.

Observations made in buffered solutions are a little difficult to interpret, as the buffer salts might be expected to exert antagonistic influences on other ions added to the solutions. Thus, the relatively greater increase in respiration caused by sodium or

potassium chloride when in buffered solution might have been attributed to a disturbance of the Na+/K+ ratio following the addition of Na+ or K+; but as the two chlorides behaved similarly, this did not seem likely. This was borne out by the further observation that addition of a mixture of sodium and potassium chlorides caused alterations in the rate of respiration, intermediate between those caused by either salt added separately. Mixtures of sodium and potassium nitrates behaved in the same way, (with the exception of one anomalous experiment in a mixture of 0.067 M total concentration, giving a low average), confirming the observations in solutions of the mixed chlorides (cf. table 3).

IV. DISCUSSION

The data presented in section III justify the conclusion that all salts act similarly, the differences lying in the concentration of each salt needed to produce a given effect. Winslow and Dolloff (1928) reached the same conclusion from studies of the viability of *E. coli*. It is therefore unlikely that the increased respiration in the dilute salt solutions resulted from the replacement of ions leached from the cells during washing. The two salts found to influence the respiration of *B. cereus* in an unusual manner are both known to stand in special relation to cellular respiration. Sodium nitrite, especially in acid solutions, causes a reduction in the activity of dehydrogenase systems (Quastel and Wooldridge 1927). This has been interpreted as the result of a reaction between nitrous acid and the amino-groups of the dehydrogenases (Myrbäck, 1926). Potassium cyanide inhibits the cytochrome system (Keilin, 1929) and the present writer found on spectroscopic examination that *B. cereus* contains cytochrome. About 20 per cent of the respiration in *B. cereus* appears to be cyanide-stable.

Cerium chloride in rather low concentrations was found to inhibit respiration, but in this case the inhibition may probably be attributed in part to the high acidity of the solution as well as to the polyvalent nature of the cerium ion, except in the most dilute solutions. The effects of dilute solutions of sodium, calcium and cerium chlorides in increasing the endogenous

respiration of *B. cereus* were the same at concentrations roughly in the ratio of 1000:100:1. Buffering of the suspensions at a slightly more acid pH made no appreciable difference; the stimulating action of the salts was perhaps increased a little. Further, it was shown that the stimulating effects of different sodium salts were much the same, so that the anion of the salt can be little concerned in causing increased respiration.

Nicolai (1926) observed an increase in the rate of uptake of oxygen by different bacterial species in buffered glucose solutions in the presence of salts. He interpreted his data in terms of osmotic pressures of the salt solutions, and decided that for salts of alkali metals the optimum respiration occurred at an osmotic pressure 1.5 times that of blood with *Escherichia coli*, and 1.0 times that of blood with staphylococci. He observed, however, that calcium and magnesium chlorides in equivalent concentrations depressed the rate of respiration of the staphylococci, and this leads one to doubt the validity of the interpretation which he placed upon his results. The experiments in which lactose was added to suspensions of *B. cereus* showed that the endogenous respiration must be independent of osmotic pressures up to 10 atmospheres, and that the effects of dilute salt solutions cannot be attributed to osmotic causes.

Fabian and Winslow (1927), after an examination of the viability of *E. coli* in the presence of different sodium salts, concluded that the changes in viability were accounted for by differences in pH and in Na+ concentration between the different solutions, the action of the anions of the salts being negligible. This conclusion is similar to that reached from a study of the respiration of *B. cereus*. The absence of any effect due to the anions, and the ratios 1000:100:1 between equivalent concentrations of 1, 2 and 3-valent cations, recall the similar relations found when salts precipitate electro-negative colloids. Winslow, Falk and Caulfield (1923) have shown that the cell of *B. cereus* is electro-negative at any pH between 3 and 10, but this does not prove that the proteins concerned in respiration are electro-negative. It is hard to imagine how flocculation of a protein could be directly related to increased rate of respiration; never-

theless, one must suppose that the same property of the cations is concerned in the two processes.

The inhibitory powers of metallic cations, measured by the values of Q in the equation

$$\log r = P - Q.c. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

differed in accordance with a Hofmeister series, the anomalous behaviour of NH_4+ being explained by the acidity of its solutions. The ions Na+ and Ca++ or Mg++ were equally potent in concentrations roughly as 3:1; it is not possible to form a reliable estimate of the inhibition caused by trivalent cations. The inhibitory powers of anions are also difficult to decide. For example, at a given molarity of salt the concentration of sodium-ion is approximately three times in sodium citrate, and in sodium sulphate twice that in sodium chloride solution; thus solutions of these salts may be compared on the basis of equal content of Na+ by dividing the appropriate values of Q ($\log r/c$) by 1, 2 and 3. This gives Q the values of 0.68, 0.63 and 0.53 for the chloride, sulphate, and citrate, the differences between these values being due to the different anions. Thus, substitution of chloride by sulphate, or of sulphate by citrate, results in a reduction of the value of Q, despite the fact that under the conditions of comparison a given concentration of chloride would be replaced by only half that concentration of sulphate, or one-third that concentration of citrate. Increasing the valency of the anion of a salt thus reduces the potency of the cation as an inhibitor, and in this sense anions and cations may be termed antagonistic. At the same time, a change in the valency of the cation results in an increase in the value of Q greater than the decrease resulting from a corresponding difference in the valencies of a pair of anions, so that the inhibition due to salt is determined mainly by the cation.

The data obtained by Brooks (1919, 1920), measuring the inhibition of respiration by *Bacillus subtilis* in concentrated salt solutions, may be shown to accord with an equation similar to (1). The relative potencies of Na+ and Ca++ or Mg++ were about 1:3 in her experiments, and the effects of K+ were

less than those of Na+. Her data from solutions containing La+++ agree closely with those for *B. cereus* in solutions containing Ce+++, and were presumably equally influenced by changes in pH. The figures obtained by Rubinstein (1932) with *Sarcina lutea* may also be arranged in a similar manner, although with *S. lutea* the changes brought about by salt require much higher concentrations than with the bacilli. The rates of oxygen uptake by *E. coli*, measured by Nicolai (1926), conformed to the requirements of equation (1) in solutions of moderate concentration. In the most concentrated solutions the rates of respiration were lower than those which would be in agreement with equation (1); but these experiment were carried out in the presence of phosphate buffer, and the enhanced action of high concentrations of the salts is similar to that observed in buffered suspensions of *B. cereus*, but greater in degree as the respiration of *E. coli* is more sensitive to acidity than that of *B. cereus*. It would seem that equation (1) may be widely applicable. A similar relation describes the changes in the solubility of proteins in concentrated solutions of salts. The equation is

$$\log S = b - K.u. \quad \ldots\ldots\ldots\ldots\ldots\ldots \quad (2)$$

where S is the solubility of the protein, u the ionic strength of the salt solution ($u = \frac{1}{2} \Sigma mv^2$ for all the ions present where m and v represent the molarity and valency of a single ion), and b and K are constants for a given salt and a given protein (Cohn 1932).

Antagonistic action between pairs of cations was negligible. Antagonism between Na+ and Ca++ implies the *reduction* of the inhibitory action of their solutions by the presence of the other ion: in the experiments of table 2 the inhibitory action of the ions was *increased* when they were in combination. Winslow and Haywood (1931) have shown that changes in the viability of *E. coli* in salt mixtures can be accounted for by assigning to each cation a "specific potency." In reducing the respiration of *B. cereus*, calcium chloride is roughly three times as potent as sodium chloride, so that in terms of specific potency a solution containing xM NaCl + yM $CaCl_2$ is equivalent to a solution

containing $(x + 3y)M$ NaCl. Table 2 includes values for respiration in salt mixtures, calculated on this basis. They indicate approximately the degree to which respiration was inhibited in the various solutions. The rates of respiration in solutions with much calcium were usually lower than those indicated by calculation, perhaps because the specific potency assigned to calcium was low. (In this investigation the specific potency depended on concentration, being nearer to 10 than to 3 for calcium in dilute solutions): the calculation completely failed to indicate the changes in dilute solutions, and it appears that in them the specific potency concept was not applicable. It is clear, however, that the effects of concentrated solutions can be fairly satisfactorily accounted for in terms of the individual effects of the constituent salts. This emphasises the contention of Winslow and Haywood that the antagonisms between ions in their action on higher organisms do not exist when the same ions act on bacteria.

V. SUMMARY

From the results of manometric measurements of the rate of uptake of oxygen by cells of *Bacillus cereus* suspended in salt solutions, the following conclusions may be drawn.

1. The important factor in the determination of rate of respiration is the concentration of salt present.

2. All salts increase the rate of respiration when present in low concentrations, and decrease it in higher concentrations, unless special specific toxic properties of certain ions intervene.

3. This action of salts cannot be attributed to their ability to change the osmotic properties of the environment of the cells.

4. The increased respiration by unbuffered dilute saline suspensions is brought about by the cation of the salt: the salts of mono-, di- and tri-valent cations are equally effective at concentrations roughly in the ratio (1000:100:1).

5. With buffered suspensions of pH 6, this stimulating action of salts is increased slightly, probably because of the rather greater acidity of the buffered suspensions.

6. In unbuffered suspensions with high concentrations of salt, the rate of respiration is reduced according to the equation

$$\log r = P - Q.c.$$

7. Comparison of the inhibitory powers of different salts suggests that the inhibition of respiration is probably caused by the cations, and not by the anions.

8. There are no antagonistic effects between the ions Na+ and K+, and there is slight interaction between Na+ and Ca++, but only in dilute solutions.

9. With suspensions in phosphate buffer solution, initially of pH 6, the rates of respiration are lower in the presence of high concentrations of salts than those which would agree with the equation given in paragraph (6) above: this probably arises from an interaction between the salts and the buffer, which makes the suspensions considerably more acid than pH 6.

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