SALT DESIDERATUM OF VIBRIO COSTICOLUS, AN OBLIGATE HALOPHILIC BACTERIUM

II. EFFECT OF SALTS ON THE OXIDATION OF GLUCOSE

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Information is meager concerning the influence of salts on the metabolic processes of halophiles. In fact, reports of the work of Robinson et al. (1952) are the only recent studies in this field. Robinson (1952) showed that there was an optimum concentration of sodium chloride for maximum activity of lactic acid dehydrogenase. While studying the effect of salt on Micrococcus halodenitrificans, Robinson and Gibbons (1952) found that different optimal concentrations of salt were required for maximum nitrogen production and oxygen uptake.

This paper presents data on the effects of various concentrations of salts and nonelectrolytes on the oxidation of glucose by Vibrio costicolus.

MATERIALS AND METHODS

The oxidation of glucose by Vibrio costicolus was studied using conventional manometric techniques. The total volume of fluid in each flask was 3.2 ml.

Flask constants. The Bunsen coefficients of oxygen are quite different for the extremes of salt concentration used in these studies and will change the flask constants slightly. This influence of salt concentration on oxygen solubility (data from National Research Council, 1928) was taken into consideration when the usual calculations were carried out.

Cell suspension. The suspension of cells was prepared by harvesting the surface growth from a solid nutrient medium composed of trypsinase 1 per cent, sodium chloride 1 m, and agar 2 per cent. The surface of the medium was inoculated by adding 1 ml of a 24 hour broth culture. After 24 hours' incubation the growth was suspended in 1 m sodium chloride, filtered through cheesecloth, washed twice with 1 m sodium chloride, and re-suspended in 1 m sodium chloride. This stock suspension of cells was adjusted to a concentration which, when diluted 100 times, would give an optical density of 0.070 to 0.090 on a Klett-Summerson photoelectric colorimeter with a blue (400 to 465 μ) filter. It was divided into as many aliquots as salts or concentrations to be used. The 1 m sodium chloride in these aliquots was replaced by an equal quantity of the salt or concentration desired. One ml of each suspension was added to its assigned flask or flasks and the necessary total volume of fluid made up by adding the proper quantity of the same salt and same concentration.

Salt solutions. All salt solutions were prepared in the same manner as described previously (Flannery et al., 1952) so that when the final volume of 3.2 ml was obtained in the flask the desired concentration of the salt also was obtained. All solutions were adjusted to pH 7.2 and were 0.1 m in phosphate buffer.

The experiments were carried out at 32 C for 2 to 3 hours.

RESULTS

Oxidation of glucose with varying concentrations of sodium chloride. The results illustrated in figure 1 show that the oxidation of glucose by V. costicolus increases as the sodium chloride concentration increases. At 1.2 m sodium chloride maximum oxygen uptake occurs, and as the concentration of sodium chloride continues to increase above 1.2 m the consumption declines. Since sodium chloride yields 2 ions, the particle concentration allowing maximum oxygen uptake is calculated to be 2.4 (2 times 1.2; Flannery et al., 1952). Using concentrations of 1, 2, and 3 m sodium chloride at 2 speeds of shaking, the possibility of oxygen diffusion as a limiting factor was eliminated.

Oxidation of glucose with substitution salts. Data in figure 2 show how cation substitution (potassium chloride) and anion substitution (sodium sulfate) influence glucose oxidation when the substrate is 2 μm of glucose. Except for sodium fluoride all the other substitution salts (mag-
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Figure 1. The oxidation of 2 micromoles of glucose in 0 to 3.6 M concentrations of sodium chloride by *Vibrio costicolus*. The substrate was added at 30 minutes.

Figure 2. The effect of cation and anion substitution salts on the oxidation of 2 μM of glucose by *Vibrio costicolus*. The substrate was added at 30 minutes. Curve A represents oxygen uptake with the substitution salt alone, curve B is for the substitution salt plus 0.2 M sodium chloride, and curve C is the endogenous curve.
nesium chloride, lithium chloride, potassium nitrate, sodium nitrate, sodium bromide, sodium iodide, sodium molybdate, and a sodium phosphate mixture) allowed uptake in a similar manner to that shown in figure 2.

Oxidation of glucose with nonelectrolytes substituted for salts. Glucose and arabinose were selected as substitutes for various concentrations of salts. Glucose is fermented by *V. costicolus* with the production of acid but no gas, whereas presence of relatively small amounts of an electrolyte stimulates the oxygen uptake (figure 3).

**DISCUSSION**

The importance of particle concentration to *V. costicolus* suggested in previous studies by Flannery *et al.* (1952) is substantiated by the studies on glucose oxidation (figure 1). Not only does it increase to a point and then decrease as the concentration of an electrolyte rises, but it shows the same changes with increasing concentrations of nonelectrolytes. Particle concentration is important to this organism because:

1. a particle concentration of 2.4 gave optimum total growth measured by growth density studies,
2. a particle concentration of 2.4 for sodium chloride gave optimum glucose oxidation, and
3. a particle concentration of 2 gave optimum glucose oxidation with nonelectrolytes.

Finally, it can been seen in figure 1 how the endogenous curve gradually rises as the optimum sodium chloride concentration is approached and falls again after the optimum concentration is exceeded. This also suggests an important relationship between particle concentration and the organism. As yet the effect on the endogenous oxygen uptake is unexplained.

The data presented in figure 2 illustrate the stimulatory effect of the cation (sodium), whereas the anion (chloride) has little or no stimulatory effect. This was found to hold whether considering growth density or oxygen consumption. Note in figure 2 that when the sodium ion is retained (sodium sulfate, curve A) and the chloride ion (sodium chloride) is added (curve B), the glucose oxidation is lowered. Apparently the particle concentration was already at an optimum, and the addition of the chloride ion did nothing toward stimulating the oxidation of glucose. In the part of figure 2 depicting retention of the chloride ion (potassium chloride, curve A) and addition of the sodium ion (curve B), the reverse is true. It might seem that an antagonism is in effect here except that the same results were obtained with almost all the substitution salts. The substitution salts allow oxidation to continue. When the sodium ion is retained, the addition of small amounts of sodium chloride does not stimulate the rate of glucose oxidation, but when the chloride ion is retained, the addition of small amounts of sodium chloride does stimulate the rate of oxygen consumption. The fact that other salts can be substituted for sodium chloride in glucose

![Figure 3. The oxidation of 20 micromoles of glucose in 2 M arabinose by *Vibrio costicolus*. The substrate was added at 30 minutes. Curve A represents oxygen uptake with arabinose alone, and curve B is for arabinose plus 0.2 M sodium chloride. Curve C is the endogenous curve.](http://jb.asm.org/)

arabinose is not attacked by the organism. Since arabinose is not oxidized by *V. costicolus*, it is a good nonelectrolytic compound to use in place of sodium chloride. Twenty μM of glucose for substrate were added to each flask in the arabinose studies. Similar results to those given in figure 1 were obtained for both carbohydrates. A major difference was that maximum oxygen uptake was attained at 2 M concentration of the nonelectrolytes. Since the carbohydrate does not ionize in solution, the particle concentration allowing maximum oxygen uptake is 2 (2 times 1). Although oxygen uptake can continue in the absence of salt when sufficient carbohydrate concentration is present, it has been noted that the
oxidation studies as well as growth density studies also suggests the operation of a nonspecific particle concentration requirement.

It is interesting to speculate on how particle concentration might influence halophiles. Stuart (1940) found that the oxygen tension of the medium, influenced by the sodium concentration present in that medium, was important to the red pigmented halophiles. However, Flannery (1953) in oxygen depletion studies found that oxygen tension of the medium had little effect on the 48 hour growth of *V. costicola*. Robinson (1952) believed that oxygen tension of the medium was not important to halophiles. Robinson *et al.* (1952), studying electrophoretic mobility and activity of cell-free extracts, eliminated respectively the lipoidal barrier theory and the possibility that the protein constituents of the protoplasm of halophiles might be different from that of nonhalophiles. They did suggest, however, an "energy mechanism" to explain the membrane character of halophiles. They performed inhibition studies which suggested that a coenzyme mechanism might have a dominant role in halophilism.

The fact that nonelectrolytes provide an optimum particle concentration for maximum glucose oxidation raises the question as to what influence this nonspecific particle concentration has on the medium that might be important to halophiles. Nonelectrolytes have been used extensively by investigators to control osmotic pressure. To suggest that the osmotic pressure of the medium is the only important factor influencing halophiles would be erroneous, for an examination of figure 3 shows that small amounts of electrolytes greatly stimulate the oxidation of glucose allowed by nonelectrolytes. Nonelectrolytes will not allow growth of *V. costicola*, and thereby a simple osmotic pressure explanation for halophilism will not suffice. Similar observations on the effect of nonelectrolytes on other organisms have been made. Johnson and Gray (1949) found that sugar solutions caused a depression of luminescence. Loeb (1912) and Thompson (1942), while discussing the importance of osmotic pressure to certain higher marine organisms, reported that sugar solutions isotonic with sea water were not satisfactory. There seemed to be some electrolyte requirement.

It appears that at least two systems influenced by particle concentration are in operation—a cell reproduction system and a respiratory system.

The nonelectrolytes allow respiration to continue but do not permit cell reproduction. It seems likely that not only is a certain particle concentration required to obtain optimum osmotic pressure but there is also an electrolyte requirement: the optimum osmotic pressure required for respiration and in part for cell multiplication, and the electrolyte concentration required for some mechanism of cell multiplication and stimulatory to respiration.

These observations appear to be consistent for *V. costicola*, but to assume that the effect of the environment is the same on all halophiles would perhaps be misleading. The effect may be the same, but until similar studies have been made on other halophiles and until an explanation is found for the different characteristics of the red pigmented halophiles and some of the others, such a conclusion cannot be drawn.

**SUMMARY AND CONCLUSIONS**

The oxidation of glucose by *Vibrio costicola*, an obligate halophile, is dependent upon salt concentration. Maximum oxygen consumption was obtained at 1.2 m sodium chloride. Other salts (sodium sulfate, sodium molybdate, a sodium phosphate mixture, sodium bromide, sodium iodide, sodium nitrate, magnesium chloride, potassium chloride, potassium nitrate, and lithium chloride) can replace sodium chloride and will allow the oxidation of glucose to continue.

Nonelectrolytes (arabinose and glucose) also will replace sodium chloride and will allow glucose oxidation to continue although the presence of small amounts of electrolytes with the nonelectrolyte has a stimulatory effect on the oxygen consumption.

The results obtained confirm the observation that the cation (sodium) has a more stimulatory effect upon the oxygen consumption than does the anion (chloride).

**REFERENCES**


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