THE WATER RELATIONS OF GROWTH AND RESPIRATION OF *SALMONELLA ORANIENBURG* AT 30°C

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Summary

The influence of water activity (a_w) on growth, respiration, and Na and K content of cells during respiration has been studied for *Salmonella oranienburg*. Sucrose, glucose, glycerol, NaCl, and KCl were added to control a_w. The organism grew in a glucose-inorganic salts medium at 0·97 a_w when any of these solutes was used to adjust a_w, but at 0·96 a_w only when glycerol was employed. Respiration was not inhibited in glycerol-adjusted solutions at a_w's at which the rate was very low in other solutes. When a_w was controlled by sucrose or glucose, cells oxidizing glucose accumulated K but not Na. Accumulation of K was greatest at 0·975 a_w. In solutions of NaCl, accumulation of K was small and in glycerol solutions it was absent. The differences between glycerol and the other solutes tested are discussed.

I. INTRODUCTION

It has been shown previously (Christian 1955) that the water relations for growth of *Salmonella oranienburg* in a simple, defined medium are similar whether the water activity (a_w) is adjusted by addition of sucrose or by addition of a mixture of three electrolytes. This paper reports the effects of additional a_w-adjusting solutes on growth, respiration, and the changes in internal Na and K levels which accompany respiration.

Other workers have related the respiration rate of bacteria to the concentration of various ions or salts, but no attempt appears to have been made to relate respiration rate to the activity of the solvent.

II. METHODS

The organism was a strain of S. *oranienburg* used previously in nutrition studies (Christian 1955). Growth rates were recorded and the a_w controlled by the methods described by Scott (1953). The a_w's of glycerol solutions were computed from the data of Scatchard, Hamer, and Wood (1938). The growth medium was a glucose-mineral salts (G.S.) solution (Christian 1955) and the a_w was adjusted to the required levels by addition of sucrose, glucose, glycerol, or a mixture of NaCl, KCl, and Na_2SO_4 in the molal ratio 5 : 3 : 2.

Oxygen uptake was followed in Warburg manometers at 30°C. Cells, grown as described below, were suspended in M/10 KH_2PO_4-Na_2HPO_4 buffer of the desired pH. The contents of the flasks were M/10 glucose (0·1 ml in

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side-arm), cell suspension (1·0 ml), stock solution of a_w-adjusting solute (as required), and glass-distilled water to 3 ml. The centre well contained 0·2 ml of 20 per cent. KOH. The component stock solutions were added by volume, and from a knowledge of their dry weights and densities, the final concentration of each solute was calculated on a molal basis, and hence the total a_w of the mixture determined (Scott 1953). These calculations were checked by isopiestic equilibration and by freezing point determinations.

Readings were taken at 5 min intervals for about 90 min. Oxygen uptake was linear with time and rates were expressed as percentages of the rate at 0·999 a_w. The mean dry wt. of cells per flask was 4 mg.

To study the effect of a_w on the Na and K content of cells of *S. oranienburg*, 250-ml conical flasks containing the same proportions of the constituents used

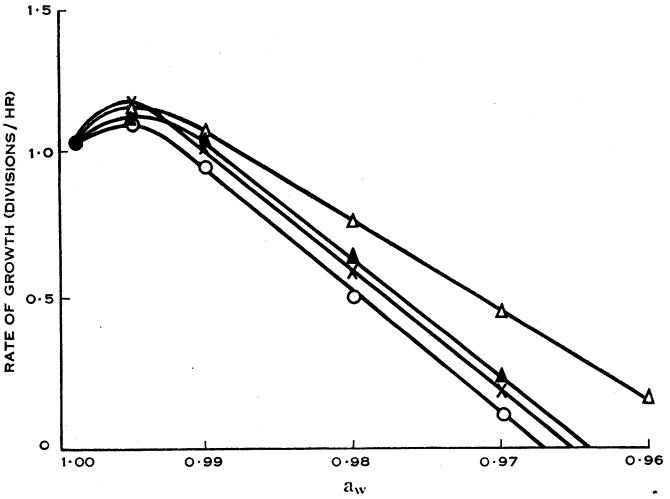

Fig. 1.—Relation between growth rate and a_w for *S. oranienburg* in glucose-salts medium using four methods for controlling a_w. ○ NaCl-KCl-Na_2SO_4 mixture in ratio of 5 : 3 : 2 moles. × Sucrose. ▲ Glucose. △ Glycerol.

in respiration experiments but with a total volume of 15 ml were shaken at 30°C. Duplicate 5 ml samples were taken after 60 min incubation, centrifuged, and the supernatant removed with a capillary pipette. The deposited cells were resuspended in 3 ml of glass-distilled water, steamed for 30 min, and cooled. One ml of N/10 HCl was added and the suspension diluted and analysed for Na and K in an E.E.L. flame photometer. The final concentrations of HCl and of Cl^- ions were below the threshold values for interference with Na and K readings in this instrument (Collins and Polkinhorne 1952). The extraction procedure gave reproducible estimates which were within 5 per cent. of those obtained by ashing the cells. As the cells were not washed prior to analysis, the Na and K values found included contributions from the suspending fluid between the deposited cells. Na and K contents were expressed as μmoles per 100 mg dry wt.

III. Results

(*a*) *Growth*

The rates of growth of S. *oranienburg* in G.S. medium in which a_w was controlled by addition of sucrose, glucose, glycerol, and the triple salt mixture are shown in Figure 1. There was no difference at high a_w's between the rates of growth in media adjusted with sucrose, glucose, or the mixture of salts, but at low a_w's, the salts were the most inhibitory of these and glucose the least. However, media adjusted with these solutes all supported growth at 0·97 but not at 0·96 a_w. When NaCl and KCl were tested individually as a_w-adjusting solutes the rates of growth at 0·98 a_w were similar to that found with the triple salt mixture, as were the lower limits of a_w supporting growth. Gly-

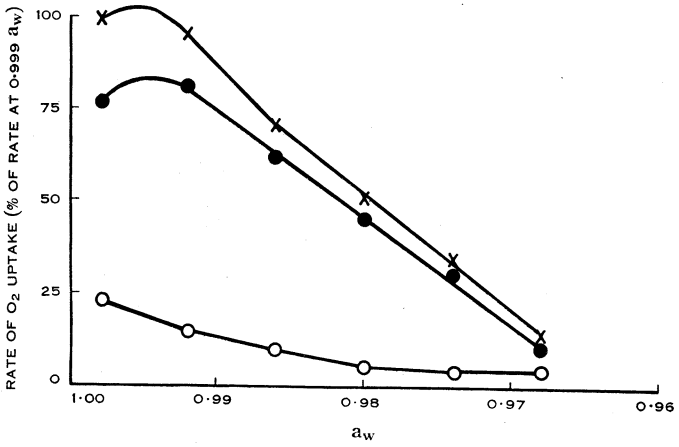

Fig. 2.—Relation between respiration rate and a_w for unwashed suspensions of S. *oranienburg* in which a_w was controlled with added NaCl. × With glucose as substrate (exogenous). ○ Without substrate (endogenous). ● Exogenous rates corrected for endogenous values.

cerol was much less inhibitory than the other solutes at high concentrations and growth was recorded at 0·96 but not at 0·95 a_w. At low a_w's lag periods were much longer when sucrose, glucose, or glycerol were the adjusting solutes than when the electrolytes were used.

(*b*) *Respiration*

(i) *Influence of Growth Medium, Age of Cells, and pH.*—In preliminary experiments respiration rates were compared for cells grown for 6 and 17 hr at 37°C in either nutrient broth (0·999 a_w) or brain-heart-broth (0·993 a_w). Using glucose as substrate comparisons were made in NaCl-adjusted solutions at several a_w's between 0·999 and 0·97. The general trend of the results was similar for cells grown for both periods in both media although the respiration

of cells grown for 17 hr in brain-heart-broth showed somewhat less inhibition at a_w's below the optimum. Accordingly cells grown for 17 hr in this medium were used in subsequent experiments.

The rate of oxygen uptake was virtually constant in M/30 phosphate buffer within the initial pH range of 5·8-7·4 in the presence and absence of substrate at 0·999 and 0·98 a_w. In subsequent experiments the initial pH of the controls (0·999 a_w) was 6·9-7·0 and the lowest initial value recorded in any concentrated solution was 6·2.

(ii) *Exogenous and Endogenous Respiration.*—The rate of endogenous respiration of unwashed cells of *S. oranienburg* was 20-25 per cent. of the respiration rate with glucose as substrate at 0·999 a_w. The influence of a_w on

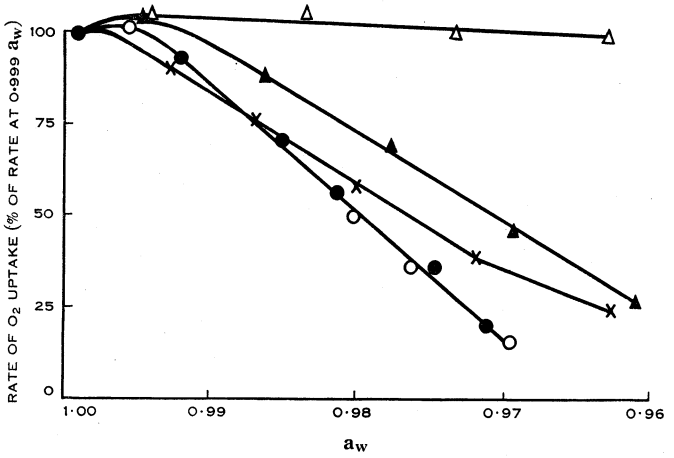

Fig. 3.—Relation between respiration rate and a_w for washed suspensions of *S. oranienburg* using five solutes to control a_w. Substrate : glucose. ○ NaCl. ● KCl. × Sucrose. ▲ Glucose. △ Glycerol.

endogenous and exogenous (glucose) respiration of unwashed cells is shown in Figure 2. Subtraction of endogenous from exogenous rates gave a curve which was a linear function of a_w at a_w's below 0·992. When cells were washed once in 50 volumes of M/10 phosphate buffer, endogenous respiration fell to less than 5 per cent. of the exogenous rate at 0·999 a_w and exogenous results were similar to those shown in the corrected curve in Figure 2. In later experiments suspensions were washed once in this way and the results were not corrected for endogenous activity.

(iii) *Influence of* a_w.—The relationship between rate of oxygen consumption and a_w for washed cells of *S. oranienburg* is shown in Figure 3. The substrate was glucose and the a_w was adjusted over the range of 0·999-0·96 by addition of the single solutes sucrose, glucose, glycerol, NaCl, and KCl. Re-

sults are the means of at least two experiments. The control rate was always about 400 μl per hr per flask, a Q_{O_2} of 100.

There was no appreciable difference in the rates recorded when NaCl or KCl was the adjusting solute, and the curve was almost identical with that for growth rate against a_w in salt-adjusted synthetic medium when growth was plotted as a percentage of the rate at 0·999 a_w. Glucose and sucrose permitted somewhat higher respiration rates at low a_w's than did the electrolyte solutions. Respiration rates remained essentially constant as a_w was lowered to 0·96 with glycerol. Glycerol and sucrose were both tested as respiratory substrates for *S. oranienburg*. With sucrose, oxygen uptake was the same as the endogenous rate, and with glycerol the rate was the same as with glucose as substrate both at 0·999 a_w and when adjusted to 0·98 a_w with NaCl.

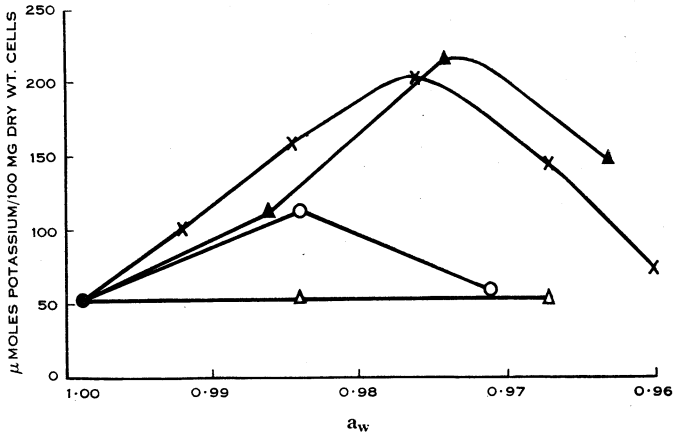

Fig. 4.—Relation between potassium content of cells and a_w for washed suspensions of *S. oranienburg* after 60 min incubation at 30°C using four solutes to control a_w. Substrate : glucose. ○ NaCl. × Sucrose. ▲ Glucose. △ Glycerol.

(iv) *Occurrence of Lag.*—Lag periods of up to 30 min occurred between addition of substrate and commencement of oxygen uptake when a_w was adjusted to low values with NaCl or KCl. Little or no such lag was observed when sucrose, glucose, or glycerol was used.

(*c*) *Na and K Content of Cells*

The cells used in the experiments contained initially 34·2 μmoles K and 58·3 μmoles Na per 100 mg dry wt., a K/Na ratio of 0·59. As the molal ratio K/Na of the growth medium, brain-heart-broth, was about 0·1, and that of the washing and suspending buffer about 0·2, these cells, in common with many others, accumulate K in preference to Na.

When cells were washed in buffer and suspended in water no leakage of Na or K was observed over a 3-hr period of shaking at 30°C. As these

results were obtained from analyses of supernatants taken at intervals, they did not exclude the possibility of an immediate change when the cells were first suspended. With glucose as substrate cells incubated at 0·999 a_w in phosphate buffer (M/30, pH 6·9) for 60 min showed an increase in K content to 53·7 μmoles and a fall in Na content to 44·9 μmoles, the K/Na ratio thereby being increased to 1·2. Uptake of K was initially linear with time and gradually approached a maximum after about 90 min. Cells suspended in buffer without substrate showed no change in K or Na contents.

Slight loss of Na was observed after incubation with substrate as a_w was reduced towards 0·96 by addition of sucrose, glucose, glycerol, or KCl, although the loss was usually less than that found at 0·999 a_w. The high concentrations of Na in the supernatant when a_w was adjusted to low levels with NaCl prevented accurate measurements of the Na content of cells so treated.

The K content of cells incubated with substrate in sucrose- or glucose-adjusted solutions increased rapidly as a_w was reduced to about 0·975 and then fell towards the control value at about 0·96 a_w (Fig. 4). When glycerol was used to adjust a_w the final K content was about the same at all a_w's tested as in the control at 0·999. An initial increase in K similar to that in glucose-adjusted solutions occurred with NaCl, but this fell again as a_w was reduced to 0·97. Of the non-electrolytes used to adjust a_w, only sucrose contributed significant amounts of K to the incubation mixture. At 0·967 a_w it increased the total K concentration by 5 per cent. K content was not recorded in concentrated KCl solutions.

As the final Na content of cells was about the same at all a_w's in sucrose-, glucose-, and glycerol-adjusted solutions the shapes of the curves for K/Na ratio, K + Na content, and K content against a_w were similar.

Comparable experiments in M/300 buffer (i.e. one-tenth the external concentrations of Na and K used previously) showed the same trends with the various adjusting solutes. In this dilute buffer, however, an actual loss of K from the cells occurred at 0·999 a_w. This loss was substrate dependent and a function of time. Similar losses of K occurred in the glycerol-adjusted solutions at all a_w's. This fall to a new level was apparently related to the very low external K concentration rather than to a_w, since very small increases in the external concentrations of K greatly reduced the loss.

IV. Discussion

When considered in relation to their effects on growth the results of the experiments reported here agree with the conclusions of previous papers (Christian and Scott 1953; Scott 1953; Christian 1955) that the influence of concentrated solutions of normally non-toxic substances is due to their effects on the availability of water.

The respiration data do not conform to such a simple view. For all solutes except glycerol the similarity between the effects of a_w on growth and respiration was close enough to suggest that a_w influences growth mainly through its effect on respiration. The complete absence of respiratory inhibition in

glycerol solutions which markedly inhibited growth does not, however, support such an explanation.

It is, perhaps, more likely that the intracellular loci concerned with growth are different from, or additional to, those concerned only with respiration. Differences in the permeability of membranes at these various loci may then account for some of the observed differences in the effects produced by different solutes. It may be that such differences in permeability are connected with the duration of the observed lag periods. In the measurements of rates of growth the longest lag periods were observed when sugars were the predominant solutes, but the longest lag periods in respiration studies were always associated with electrolyte solutions.

When it occurs, intracellular accumulation of potassium is dependent on respiration, but the greatest accumulation of this cation occurs when the respiration is about one-half the maximum rate. It is difficult to account for this. It is possible that further reduction in respiration rates beyond this point is unable to support a rate of uptake of potassium sufficiently in excess of outward leakage to maintain a high steady state level within the cell. Separate measurements of the inward and outward movement of potassium would clearly be valuable.

Cells immersed in solutions of reduced a_w may reduce their internal a_w to approach osmotic equilibrium with the external solution. Accumulation of solutes or loss of water, or both, would result in such a change. It is of some interest, therefore, to consider the extent to which the observed potassium accumulation may have contributed to such a reduction in the mean intracellular a_w. The maximum accumulation was an increase from 50 to 200 μmoles of K/100 mg dry wt. of cells, when the external a_w was reduced from 0·999 to 0·975. Assuming that the intracellular water is three times the dry wt., this additional 150 μmoles would be dissolved in 300 mg of water. This is equivalent to 0·5m potassium. If it is further assumed that an equal number of univalent anions were accumulated, the reduction in the internal a_w would be about 0·016, which is somewhat less than the 0·024 by which the external a_w was reduced. If, in fact, the internal a_w has followed the external a_w fairly closely it is evident that this would involve either loss of water or accumulation of other solutes.

For cells in glycerol solutions potassium was not accumulated during respiration. It is clear, therefore, that high accumulation of potassium is not vitally concerned in the metabolism of the organism at low a_w's. Whether or not solutes other than potassium are accumulated from glycerol solutions is a question inviting further study. The penetration into the cell of glycerol itself, or a derivative, may be regarded as likely in view of the high lipoid solubility attributed to glycerol. If cells of *S. oranienburg* are, in fact, freely permeable to glycerol, the rise in intracellular glycerol concentration following exposure to concentrated solutions of this substance would increase the concentration of oxidizable substrate available within the cell. Such an increase in substrate concentration may counteract the tendency for respiration to be decreased by the reduced a_w's. An adequate explanation of the differences between glycerol

and other solutes must, however, await further experiments including studies with other organisms unable to utilize glycerol and with cell-free respiratory systems.

V. References

CHRISTIAN, J. H. B. (1955).—The influence of nutrition on the water relations of *Salmonella oranienburg*. *Aust. J. Biol. Sci.* **8**: 75-82.

CHRISTIAN, J. H. B., and SCOTT, W. J. (1953).—Water relations of salmonellae at 30°C. *Aust. J. Biol. Sci.* **6**: 565-73.

COLLINS, G. C., and POLKINHORNE, H. (1952).—An investigation of anionic interference in the determination of small quantities of potassium and sodium with a new flame photometer. *Analyst* **77**: 430-6.

SCATCHARD, G., HAMER, W. J., and WOOD, S. E. (1938).—Isotonic solutions. I. The chemical potential of water in aqueous solutions of sodium chloride, potassium chloride, sulfuric acid, sucrose, urea, and glycerol at 25°. *J. Amer. Chem. Soc.* **60**: 3061-70.

SCOTT, W. J. (1953).—Water relations of *Staphylococcus aureus* at 30°C. *Aust. J. Biol. Sci.* **6**: 549-64.