

THE INFLUENCE OF SODIUM CHLORIDE AND TEMPERATURE ON THE ENDOGENOUS RESPIRATION OF *B. CEREUS*

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I

INTRODUCTION

Analysis of the temperature coefficients of chemical reactions has in certain cases thrown light on the mechanism of the reaction. Attempts have been made to interpret the action of temperature on biological reactions in the same way. Crozier (1924-25) has emphasized the importance of master reactions in controlling the temperature coefficient, while Bělehrádek (1935) has stressed the part played by diffusion, and the viscosity of the medium in which the reaction occurs.

The present paper describes experiments in which the rate of respiration by *B. cereus* has been measured at temperatures within the range 11 to 27°C., at different pHs, and in different concentrations of sodium chloride. The data are then interpreted in terms of the hypotheses advanced by Crozier and Bělehrádek.

II

EXPERIMENTAL PROCEDURE

The material and methods were the same as those used in previous studies on this organism (Ingram 1939 *a* and *b*). Buffered suspensions of washed cells of *B. cereus* were mixed with an equal volume of distilled water or salt solution and the rates at which oxygen was consumed by these mixtures were measured in Barcroft differential manometers. The manometers were placed in thermostats, where their temperature was maintained to $\pm 0.05^\circ\text{C}$.

Two methods were used, for the comparison of the rates of oxygen consumption at different temperatures with those at 25°C. The first method, employed in the experiments at pH 6.0, involved the preparation of two comparable series of suspensions; these were compared at 25°C. as a check on their similarity, after which one series was kept at this temperature, and the other was placed in a thermostat at some other temperature for comparison with the standards. These measurements were in some cases made up to 15 hours after the preparation of the suspensions, by which time the exponential decline of the rate of respiration (with time) was well under way (Ingram, 1939 *a*).

Stier and Stannard (1937) have shown that the temperature coefficient of respiration by bakers' yeast does not change when the exponential decline of respiration sets in.

The possibility that *B. cereus* might behave differently was tested by the use of a second method of comparison. One series of suspensions was employed during the period of constant respiration, and alternated between the reference temperature of 25°C. and the temperature of the investigation. Observations were made at pH 6.5 and 7.5 over a considerable range of temperature, following this procedure. This second method has the defect that there may be an appreciable lag in the adjustment of the protoplasm to change of temperature. It was found, however, to give results similar to those obtained by the first method, when 10 minutes were allowed for adjustment to take place.

III

Experimental Data

The equation used by Arrhenius (1889) to express the effect of temperature on the velocity of a chemical reaction is

$$\ln. \frac{k_1}{k_2} = \frac{\mu}{R} \left(\frac{1}{T_2} - \frac{1}{T_1} \right) \quad (1)$$

where k_1 and k_2 are the velocity constants at absolute temperatures T_1 and T_2 respectively, R is the gas constant, and μ is a constant characteristic of the reaction which measures the mean energy required to activate the reacting molecules. When applied to a biological reaction proceeding at a constant rate, this equation may be transformed into

$$\mu = 4.6 \frac{\log r_1 - \log r_2}{\left(\frac{1}{T_2} - \frac{1}{T_1} \right)} \text{ calories} \quad (2)$$

where r_1 and r_2 are the rates of the reaction at absolute temperatures T_1 and T_2 . Thus if equation (1) is valid, one should obtain a straight line of slope $\mu/4.6$ by plotting $\log r$ against $1/T$.

The data obtained at pH 7.5 and at pH 6.5, over the temperature range 11–27°C., are set out in this way in Figs. 1 and 2 respectively. These figures show that at pH 7.5 or at pH 6.5 the addition of sodium chloride in concentrations between 0.6 M and 1.8 M reduced the rate of respiration considerably, but that the value of μ was not changed. Fig. 3 gives the data obtained over a more restricted range of temperature, 20–25°C., at pH 6.0. In the presence of low concentrations of sodium chloride the rate of respiration was raised, and the value of μ was increased at the same time.

The rates of respiration given on Figs. 1–3 are referred to a common basis, which is the rate of respiration in distilled water at 25°C. taken as 100 units. This corresponds to a Q_{O_2} of about 20. It should be remarked that the

addition of salts to suspensions in phosphate buffers causes them to become slightly more acid, so that the pHs given are only approximate. This has been discussed in an earlier paper (Ingram, 1939 *b*).

The data of Figs. 1 and 2 indicate that the value of μ was probably less the higher the temperature. This was particularly evident at low temperatures. There was no evidence to suggest that the fall was discontinuous. The change in the value of μ with temperature makes it impossible to compare the various values, except when they are calculated over the same interval of temperature. Thus, in order to compare the effect of temperature at pH 6 over the range 20 to 25°C. with that at pH 6.5 and 7.5 over the range 11 to 27°C., it is necessary to resort to some coefficient which has a unique value over a wide range of temperature.

Bělehrádek (1935) has shown that the rate of many biological reactions may be related to temperature by means of the equation

$$r = a \cdot t^b \quad (3)$$

$$\text{i.e. } \log r = \log a + b \cdot \log t \quad (4)$$

where r is the rate of the reaction at a temperature of $t^\circ\text{C}$. and a and b are constants. This equation may be applied over a wide range of temperature with a single value of b ,

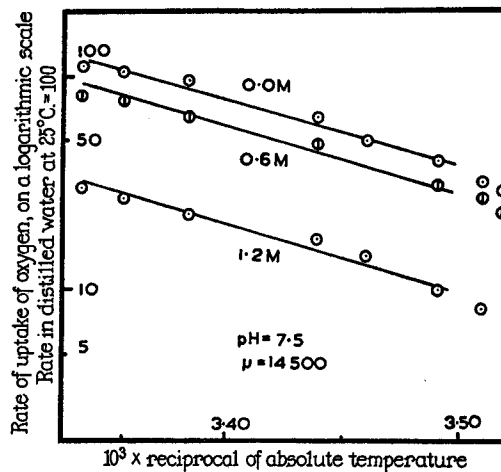


FIG. 1. The rates of respiration at pH 7.5, in the presence of various concentrations of sodium chloride.

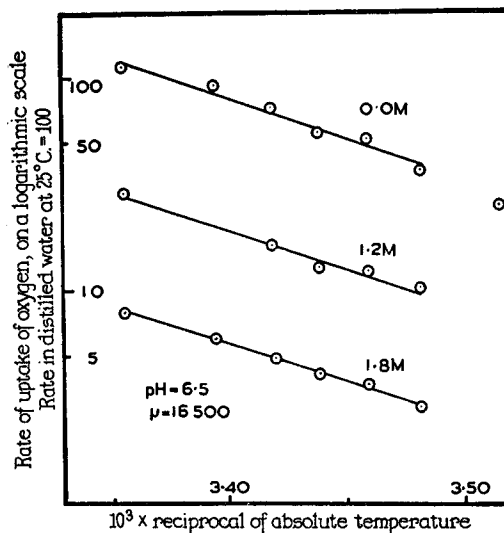


FIG. 2. The rates of respiration at pH 6.5, in the presence of various concentrations of sodium chloride.

and it is therefore suitable for comparing the data referring to pH 6.0 with those obtained at pH 6.5 and 7.5. This is done in Fig. 4 by plotting $\log r$

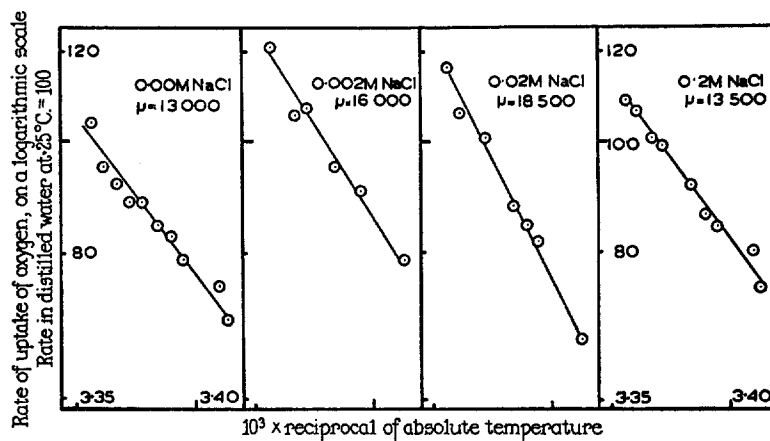


FIG. 3. The rates of respiration at pH 6.0, in the presence of various concentrations of sodium chloride.

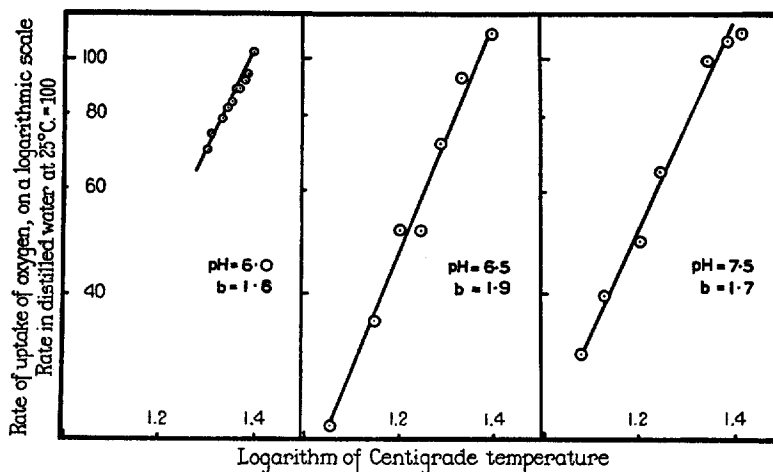


FIG. 4. The rates of respiration in buffer solution only, at different pHs

against $\log t$. The slope of each line represents the value of b . Fig. 4 shows that the temperature coefficient was not much altered by change of pH, but that it was greatest at pH 6.5. The rate of respiration was also greatest at this pH, which is near to the pH of optimum respiration for *B. cereus* (Ingram, 1939*b*).

IV

DISCUSSION

The data presented in the previous section make it clear that the alterations in the temperature coefficient of respiration which are caused by the presence of sodium chloride are related to the effect of the salt on respiration. If the concentration of salt present is greater than 0.2 M the rate of respiration is reduced, and the temperature coefficient is not changed; with concentrations less than 0.2 M the rate of respiration is increased, and the temperature coefficient is raised. Moreover, it appears that if the rate of respiration is varied by alteration of pH, the temperature coefficient is higher, the greater the rate of respiration; in this case, however, the change in temperature coefficient is smaller than that brought about by the addition of small amounts of sodium chloride.

Investigations of this subject are so few that it is impossible to obtain confirmation of these results from earlier studies within the realm of bacterial physiology, but such data as are available from other fields agree fairly well with those above. From the data of Martin (1904) referring to the beat frequency of terrapin heart in potassium chloride solutions, Bělehrádek (1935) decided that the temperature coefficient passed through a maximum, and then declined again with increasing salt concentration. As the most concentrated solution corresponded to about 0.04 M KCl, this behavior is in agreement with that reported at pH 6.0 in section III of the present communication, so far as the relation towards the salt is concerned; but the increase in the temperature coefficient was much greater than has been observed with respiring *B. cereus*. Sherwood and Fulmer (1926) showed for the growth of yeast in a synthetic medium, that the value of μ was increased only slightly, by the presence of a little ammonium chloride in some of the nutrient solutions. The recent work of Bodine and Thompson (1935) is also relevant. These workers have presented measurements of the rate of consumption of oxygen by *Melanoplus* eggs of different ages. They found that the Q_{10} of this process varied with the age of the eggs, but that dehydration caused no further change, even when the eggs had collapsed through loss of water. The dehydration was effected by concentrated Belar solutions, so that the conclusion to be drawn from their experiments is similar to that drawn below, from the experiments with strong salt solutions set out in section III above. Finally, Clark (1921) found that the Q_{10} of the frequency of heart beat in *Rana* was independent of pH above 15°C. At lower temperatures the rate of beat, and the Q_{10} , were higher at pH 8 than at pH 9.

Bělehrádek (1935)¹ believes that both the presence of electrolytes and change in the water content of protoplasm result in changes of protoplasmic viscosity, and thus in the temperature coefficients of all metabolic processes. This view has been criticized by Stiles (1930) on the grounds that viscosity changes need not be reflected in rates of metabolism. It is extremely unlikely that the viscosity of the protoplasm of *B. cereus* was unaffected either by change of pH over the range 6.0 to 7.5 or by addition of sodium chloride in concentrations up to 1.8 M within this pH range, especially in view of the marked diminution in the volume of the cells in salt solutions. Cells of *B. cereus* shrink to less than 80 per cent of their initial volume on suspension in 2 M sodium chloride (writer's unpublished observation). The viscosity of proteins is markedly reduced by the presence of salts (Kruyt and Lier, 1929), except near the isoelectric point where it is very susceptible to fluctuations of pH (Pauli and Matula, 1933) and it is probable that electrolytes bring about similar changes in protoplasm. Chambers and Reznikoff (1925-28) showed that sodium salts reduce the viscosity of protoplasm, and Jacobs (1922) and Prát (1926) have shown that small amounts of acids produce a similar effect. Thus adopting the view that a decrease in protoplasmic viscosity results in decreased temperature coefficients, one would expect salts or acidity to reduce them considerably. The evidence shows that this does not occur, so that it is improbable that the viscosity of the protoplasm determines the temperature coefficient of respiration in *B. cereus*.

The values of μ calculated from Figs. 1-3 approximate to the value 16,700 calories, which is believed by Crozier (1924-25) to characterize processes controlled by an iron dehydrogenation. It is not desirable to deduce the changes of μ with temperature from these figures, as there is no evidence to suggest that there are critical temperatures at which sudden changes occur, such as have been postulated by Crozier and Navez (1930-31). The values of μ calculated from Figs. 1-3 are averages over the range 13 to 27°C., and observations at lower temperatures were disregarded as being obviously inconsistent with these values. It is probable that in the present case a dehydrogenation is involved, for the inhibition of respiration in *B. cereus* by salts is due to inhibition of the dehydrogenases which limit respiration (Ingram, 1938, *cf.* also Quastel and Wooldridge, 1927). Further, when it is remembered that the increase in the temperature coefficient in dilute salt solutions is associated with an increase in the rate of metabolism, the master reaction concept may be seen to provide a reason-

¹ Bělehrádek, J., Temperature and living matter, *Protoplasma Monograph No. 8*, Berlin, Gebrüder Borntraeger, 1935, 69.

able explanation of the phenomena. The inhibition observed when salts are added is that of a limiting reaction, and this is likely to remain limiting if its rate is lowered. The constancy of the temperature coefficient during inhibition of respiration may thus be regarded as the result of the slowing of a reaction, already the slowest.

It might further be supposed that the alteration on stimulation could represent the beginning of a change-over to a new master reaction. Such a plan would be unsatisfactory from a quantitative point of view. Burton (1937) has demonstrated that very large changes in the relative rates of reactions are necessary before one can become "master" over others in the determination of a temperature coefficient. In the present experiments the temperature coefficient changed appreciably with an increase of less than 20 per cent in the rate of respiration.

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V

SUMMARY

Measurements were made of the rate of consumption of oxygen by suspensions of *B. cereus*, in sodium chloride solutions of concentration up to 1.8 M and over a range of pH from 6.0 to 7.5. It was found:

1. That the temperature coefficient was independent of the presence of sodium chloride in concentrations between 0.2 and 1.8 M, although the rate of respiration was lowered considerably under these conditions.
2. That in the presence of concentrations of sodium chloride less than 0.2 M, the rate of respiration was increased, and so was the temperature coefficient.
3. That small changes in the temperature coefficient occurred when the pH was changed. The temperature coefficient was higher the higher the rate of respiration.

These data may be more readily interpreted by the hypothesis that the temperature coefficient is controlled by some master reaction, than by that which supposes that the temperature coefficient is determined by protoplasmic viscosity.

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