TEMPERATURE ACTIVATION OF CERTAIN RESPIRATORY ENZYMES OF STENOTHERMOPHILIC BACTERIA

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I. INTRODUCTION

A review of the literature on thermobiosis reveals many casual observations of the phenomenon, but very few experimental studies which contribute to our knowledge of the mechanism involved (5). In addition to the ability of microorganisms to grow at elevated temperatures, an equally fundamental problem is found in the inability of a group of bacteria, designated as "stenothermal thermophiles" or "obligate thermophiles," to metabolize and reproduce at temperatures which are suitable for most other forms of life. A representative number of these thermophilic bacteria, also used in the present study, were examined earlier and found to exhibit a temperature range for growth of 38–75°C. (4). The effect of a large variety of environmental factors (such as nutrient and nutrilite supply, inhibitors, oxygen and carbon dioxide supply, oxidation-reduction potential of the medium, relative hydration of the medium, and pH of the medium) was studied, using the classical methods for measuring growth, as well as a manometric measurement of oxygen consumption. Above 38°C. the stenothermophilic bacteria exhibited very definite requirements with regard to these environmental factors; below 38°C. no combination of conditions could induce these organisms to initiate growth.

References made by several workers to the inactivation of microbial enzymes by low temperatures (3) and to deficiencies, both qualitative and quantitative, in the respiratory mechanism of thermophiles (9, 11) have no supporting experimental data. The absence of growth and proliferation in stenothermophilic cultures below 38°C., however, may be related to the failure of one or more steps in the metastable chain of exothermal and synthetic reactions, so interlinked that the retardation of any single reaction might prevent completely the functioning of others and thus make growth impossible. The temperature range for the respiratory processes, including all chemical processes by which energy is made available to the cell, has been found to be considerably wider than that of growth processes in many organisms. Studies of the activity of bacterial respiratory enzymes as a function of temperature have been limited to organisms with relatively low minimum temperatures for growth.

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In view of the intimate relationship between growth and respiration, this investigation of temperature activation of the respiratory system of a group of bacteria with a very high minimum growth temperature was undertaken with the purpose of (*a*) determining whether the respiratory system of these thermophiles functions at a temperature below the lower limit of growth, (*b*) comparing the energies of activation of the complete respiratory system and its enzymic components, and (*c*) comparing activation energies above and below the critical temperature for growth.

II. EXPERIMENTAL

(*a*) *Bacterial Cultures.*—Five cultures of stenothermophilic bacteria were selected for study. Obligate thermophilic cultures Nos. 4 and 5 were obtained from the Research Laboratories of the National Canners Association labelled F.S. Nos. 1620 and 4102, respectively. Cultures 1, 2, and 3 were soil isolations. All organisms were facultatively anaerobic, Gram-positive sporulating rods, belonging to the genus *Bacillus*. No taxonomic study was made beyond routine observations necessary to establish the individuality of the cultures in question. These organisms were designated as "stenothermophiles" because of their inability to proliferate at, or below, 37°C.

(*b*) *Preparation of Bacterial Suspensions.*—The organisms were grown in 1 liter Erlenmeyer flasks, each containing 250 ml. of sterile Bacto-nutrient broth of pH 7.0. Each flask received a 25 ml. inoculum of a 22 to 24 hour nutrient broth culture. The flasks were incubated at 55°C. in a humidified incubator for 22 to 24 hours. Incubation was never permitted to exceed 24 hours. By this procedure the resulting cultures were essentially free of spores. Cells were sedimented by centrifugation at room temperature and washed three times by suspending them in distilled water and centrifuging. The low cell yield per unit of medium made it unsatisfactory to use Sharples supercentrifugation because of the considerable losses involved. Aeration at room temperature to exhaust metabolites was found to be unnecessary. Thorough washing was usually sufficient to eliminate any metabolites adhering to the cells. The organisms collected from 16 flasks and suspended in 6 ml. of M/15 phosphate buffer of pH 7.0 were considered a stock suspension; this was adjusted by dilution in order to obtain the desired activity in the subsequent studies. Storage of suspensions at temperatures from 5–25°C. for 12 hours resulted in loss of activity ranging from 50 to 100 per cent. For this reason all suspensions were used within several hours after preparation.

(*c*) *Measurement of Dehydrogenase Activity.*—By replacing the cytochrome system by a suitable hydrogen acceptor, methylene blue, which functions also as an indicator, and removing oxygen from the system, the intermediate reactions effected by the dehydrogenases were studied. In Thunberg tubes of 15 ml. capacity were placed 0.5 ml. of 0.05 M substrate, 0.5 ml. of M/15 phosphate buffer of pH 7.0, 0.25 ml. of distilled water, and 0.5 ml. of the resting cell preparation; in the side arm of the stopper, 0.25 ml. of 1:5,000 methylene blue. The tubes, held in an almost horizontal position, were evacuated by means of a water aspirator for a minimum of 5 minutes with constant shaking. Constant temperature air baths were used instead of water baths

to insure temperature equilibration of the material contained in the side arm, as well as in the main chamber. The time required for equilibration was determined directly by observing a thermometer inserted in a control tube. The dehydrogenation was initiated by tipping the methylene blue into the main chamber, and the time required to bring about 70 per cent reduction measured by comparison with an aerobic control consisting of the same volume of substrate, cells, buffer, distilled water, and the appropriate dilution of methylene blue (3:50,000). Recorded values for reduction time were the average of at least triplicate determinations. No effort was made to standardize resting cell suspensions beyond dilution to a reduction time of 4 to 5 minutes at 45°C. for each substrate studied. Visual measurement of reduction through the glass door of the air bath was found to be more satisfactory for this investigation than photocolorimetric measurements. Determinations were carried out at temperatures within the range of 5–55°C. The temperature of the air baths was held constant in the lower ranges to ±0.1°C., and in the higher ranges to ±0.2°C.

(*d*) *Measurement of the Activity of the Cytochrome System.*—The cytochrome system, consisting of the very labile cytochromes a and b, stable cytochrome c, cytochrome reductase, and cytochrome oxidase, functions as a chain of electron carriers in transferring hydrogen from metabolites and its final combination with oxygen to form water. Cytochrome oxidase is believed to be the terminal oxidizing enzyme of the respiratory chain of aerobic forms and to effect *in vivo* the oxidation by oxygen of the cytochromes, cytochrome c in particular. To test the activity of this system, a reduced material may be introduced into the system; thus, cytochrome oxidase oxidizes cytochrome c and this in turn oxidized a suitable phenol or amine, hydroquinone or *p*-phenylenediamine. Cytochrome b, on the other hand, in the presence of the oxidase, oxidizes *p*-phenylenediamine but does not attack hydroquinone. By using these substrates, the presence and activity of the various components of the cytochrome system can be determined by manometric measurement of the oxygen consumed by the reactions.

Oxygen consumption was measured by means of Barcroft manometers, calibrated with mercury and checked by the bicarbonate method. In the main vessel of the reaction flask were placed 1 ml. of the appropriate dilution of the resting cell suspension, 1 ml. of M/15 phosphate buffer of pH 7.0, and 0.5 ml. of distilled water; in the side arm, 0.5 ml. of M/10 substrate (hydroquinone or *p*-phenylenediamine, Eastman grade) in solution of appropriate pH. The control flask contained an equal volume of liquid, but without the bacterial cells and the reduced chemical. Varying degrees of autoxidation of the two chemicals in question made it necessary to run controls in separate manometers and reduce the values of oxygen consumption obtained in the presence of the enzymes by the value of these blanks. Sodium hydroxide solution was not added to the central well of the flasks, since no carbon dioxide was given off. Determinations were made over the temperature range of 20–55°C. for both substrates. The temperature of the insulated and refrigerated water bath was held constant in the higher ranges to ±0.1°C., and in the lower ranges to ±0.05°C. Temperature equilibration was followed directly in a control flask introduced into the bath at the same time as the test manometer. All gas volumes were corrected for any endogenous metabolism and converted to the standard temperature of 0°C. to make results comparable.

(*e*) *Measurement of the Activity of Catalase.*—Catalase, an enzyme which disposes of the hydrogen peroxide from a number of biological oxidations, was studied by measuring manometrically the oxygen evolved from a standard solution of hydrogen peroxide by a suspension of washed bacterial cells. In the main vessel of the reaction flask of a Barcroft manometer were placed 1.0 ml. of M/15 phosphate buffer, pH 7.0, 0.5 ml. of distilled water, and 1.0 ml. of the resting cell preparation; and in the side arm, 0.5 ml. of 0.3 M hydrogen peroxide (prepared from Merck "superoxol"). In the compensating flask, 1.0 ml. of distilled water was substituted for the bacterial suspension. A carbon dioxide absorbent was unnecessary. Equilibration at each temperature over the range from 15–55°C. was followed directly in a control flask immersed in a constant temperature water bath. The reaction was followed by frequent readings of oxygen evolution over a period of several minutes of uninterrupted shaking. All gas volumes were corrected to 0°C.

(*f*) *Measurement of the Activity of the Complete Respiratory System.*—Aerobic respiration was measured by means of the Barcroft apparatus. Glucose was used as a representative substrate for this study. In the reaction vessel were placed 1.0 ml. of an appropriately diluted resting cell preparation, 1.0 ml. of M/15 phosphate buffer of pH 7.0, and 0.5 ml. of distilled water; and in the side arm, 0.5 ml. of M/10 glucose (Eastman grade). The compensating vessel contained distilled water in place of the bacterial cells. No carbon dioxide absorbent was employed here because the organisms under investigation did not produce any gas in the fermentation of carbohydrates. By following the usual respirometric procedure, the rate of oxygen uptake was determined over the temperature range from 20–55°C. by correcting the manometer readings for any endogenous metabolism, determined in separate manometers as the oxygen-uptake of the resting cells in the absence of added substrate. All gas volumes were converted to 0°C.

III. RESULTS

(*a*) *Activity of the Dehydrogenases.*—The log of the rate of reduction of methylene blue was plotted against the reciprocal of the absolute temperature (for organism 3, see Figs. 1 *a* and *b*), and the linear portion of the curve determined. The slope of the line which fitted the plotted points best was found by a statistical analysis of the data for regression coefficients and standard deviation. The energy of activation, μ, was obtained by applying to the data over the linear range the Arrhenius equation:

$$\mu = 4.58 \left(\frac{\log k_2 - \log k_1}{1/T_1 - 1/T_2} \right),$$

where k is the rate, T the absolute temperature, and

$$\left(\frac{\log k_2 - \log k_1}{1/T_1 - 1/T_2} \right)$$

the slope of the curve in the log plot. The activation energies presented in Table I for the five organisms and in Fig. 1 for organism 3 do not include a notation of standard deviation, but are the "best" values to the nearest 500.

For all substrates the rate of dehydrogenation increased exponentially with the temperature up to 45°C., in accordance with the Arrhenius equation. It is considered most significant that at temperatures far below the minimum temperature for growth of these organisms the activation energies for dehydro-

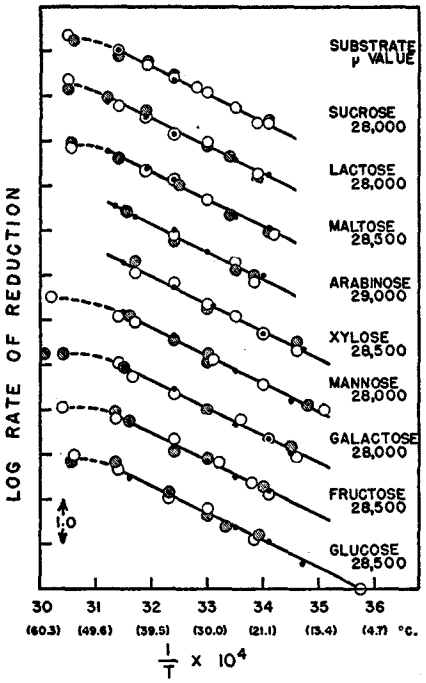

FIG. 1 *a*

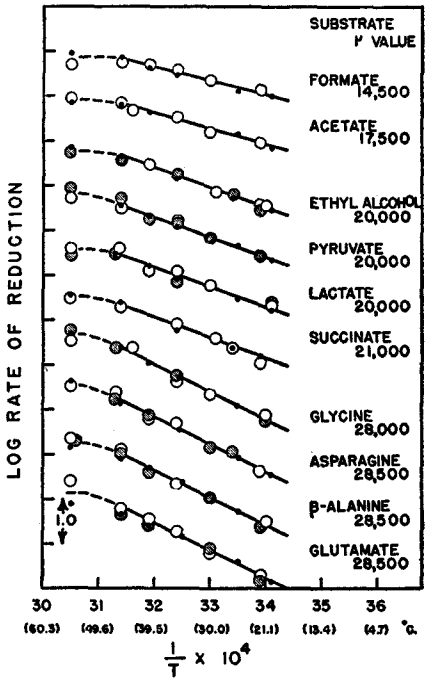

FIG. 1 *b*

FIG. 1 *a* and *b*. Log rate of reduction of methylene blue (to 70 per cent reduction) by resting cell suspensions of organism 3 in the presence of various substrates, plotted aginst $1/T$.

The three symbols used in designating the points refer to three series of determinations for each substrate made with different bacterial preparations.

genation are identical with those for temperatures at which the organisms grow rapidly. In all experiments, however, the reaction ceases to follow the Arrhenius equation above 45°C. Although no attempt was made to determine the optimum and maximum temperatures, the data presented in Fig. 1 show that they exceed 45°C.

An analysis of the data obtained for dehydrogenation of the various substrates by washed suspensions of the stenothermophiles, over a temperature range extending far below the minimum temperature for growth, indicates

essentially the same mean activation energy for the following substrates:

28,000 to 28,500 cal. per gm. molecule: glucose, fructose, galactose, mannose, xylose, arabinose, maltose, lactose, sucrose, glycine, β-alanine, monosodium glutamate, asparagine.[1]

19,500 to 20,500 cal. per gm. molecule: ethyl alcohol, sodium succinate, sodium pyruvate, sodium lactate, sodium acetate.

TABLE I

Summary of the Dehydrogenase Activity of the Stenothermophilic Bacteria as a Function of Temperature

Substrate 0.0125 M	μ Value					Mean μ value
	Organism No.					
	1	2	3	4	5	
	cal./gm. molecule	*cal./gm. molecule*	*cal./gm. molecule*	*cal./gm. molecule*	*cal./gm. molecule*	*cal./gm. molecule*
Glucose (E.K.)*	27,500	29,000	28,500	28,500	28,000	28,300
Fructose (E.K.)	28,500	29,000	28,500	28,000	29,000	28,600
d-Galactose (E.K.)	28,500	27,500	28,000	28,500	28,000	28,100
d-Mannose (E.K.)	28,000	28,500	28,000	29,000	28,000	28,300
l-Xylose (E.K.)	28,000	28,000	28,500	28,000	28,500	28,300
l-Arabinose (E.K.)	27,500	29,000	29,000	29,000	28,500	28,600
Maltose (E.K.)	28,000	28,500	28,500	28,000	29,000	28,400
Lactose (E.K.)	28,000	28,500	28,000	28,500	28,500	28,300
Sucrose (Merck)	28,500	28,000	28,000	28,000	27,000	27,900
Ethyl Alcohol (U.S.P. absolute)	(29,000)	21,000	20,000	20,000	19,500	20,100
Sodium succinate (from E.K. succinic acid)	19,500	20,000	21,000	21,500	20,000	20,400
Sodium pyruvate (from E.K. pyruvic acid)	19,500	19,000	20,000	22,000	21,000	20,300
Sodium lactate (Mallinckrodt 85 per cent reagent lactic acid)	19,000	20,000	20,000	21,000	20,000	20,000
Sodium formate (Baker C.P.)	14,000	15,000	14,500	15,500	17,000	15,200
Sodium acetate (Mallinckrodt U.S.P.)	21,500	19,000	17,500	20,000	20,500	19,700
Glycine (E.K.)	28,500	28,500	28,000	27,500	28,000	28,100
1-Asparagine (E.K.)	28,000	28,500	28,500	30,000	29,500	28,900
β-Alanine (E.K.)	28,000	27,500	28,500	28,500	28,500	28,200
Monosodium glutamate (Amino Product Co.)	28,000	29,500	28,500	28,000	29,000	28,600

* Eastman Kodak Co.

A characteristically different activation energy of 15,000 was obtained for the dehydrogenation of sodium formate.

[1] The temperature characteristic for the dehydrogenation of asparagine has a mean value of approximately 29,000, but the μ values for the individual organisms suggest that this substrate also belongs to the 28,000 to 28,500 category.

(*b*) *Activity of the Cytochrome System.*—Rates were determined from the slopes of the straight lines in plots of oxygen consumption as a function of time. Typical results are presented in Figs. 2 and 4 for culture 3. By plotting log rate against the reciprocal of the absolute temperature (Figs. 3 and 5), an analysis of the curve for the fit of the points to a straight line indicated the accordance of the data with the Arrhenius equation. The energy of activation

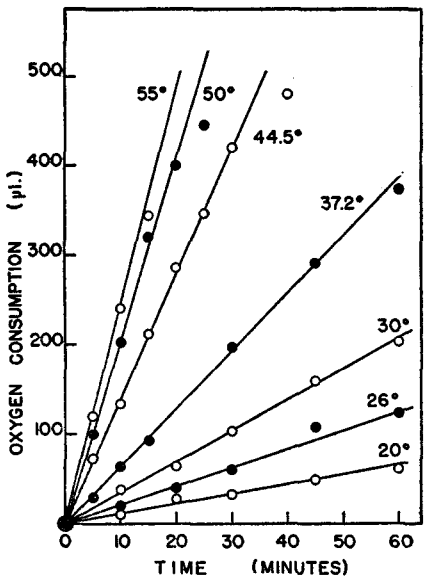

FIG. 2

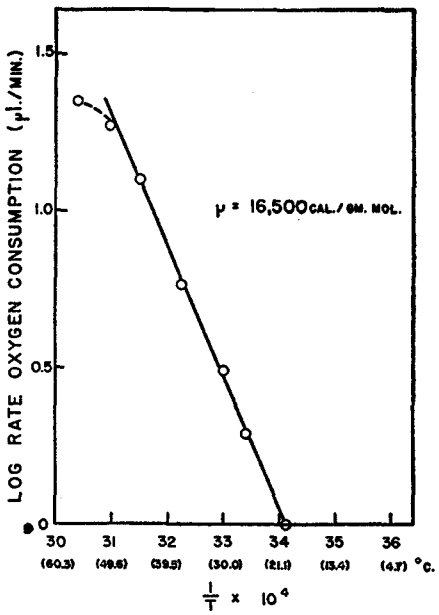

FIG. 3

FIG. 2. Oxygen consumption by resting cell preparation of organism 3 in presence of *p*-phenylenediamine *vs.* time.

$$K_{O_2}^{0°C.} = 2.68$$

FIG. 3. Log rate of oxygen consumption by resting cell preparation of organism 3 in presence of *p*-phenylenediamine as a function of $1/T$.

was calculated by substituting the "best" value for the slope of the line in the equation. A summary of results is given in Table II.

The mean activation energy over the temperature range from 20° to at least 45°C. for the cytochrome–*p*-phenylenediamine system, using washed bacterial suspensions of the stenothermophiles was found to be 16,800 cal. per gm. molecule. The mean value for the rate of oxidation of hydroquinone by cytochrome oxidase–cytochrome c was somewhat higher, 20,200 calories, and inactivation became apparent at temperatures above 50°C. The data show that the optimum and maximum temperatures for the cytochrome system of these organisms lie above 50°C. The identity of the μ value at temperatures at

which these bacterial cells actively grow and multiply and the μ value at temperatures far below the minimum temperature for growth was noted.

(*c*) *Activity of Catalase.*—The rate curves are given in Figs. 6 and 7 for dilute and concentrated suspensions of organism 3. The initial straight line portions of the rate curves were used to determine the energy of activation by a plot of

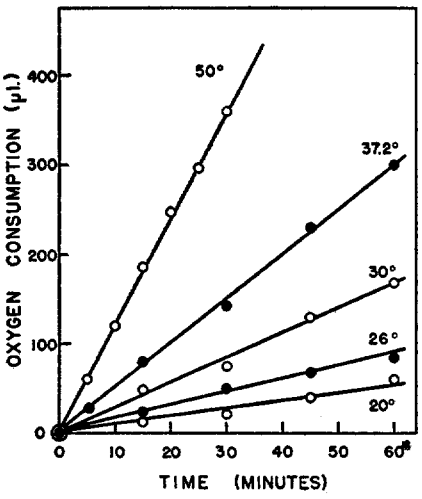

FIG. 4

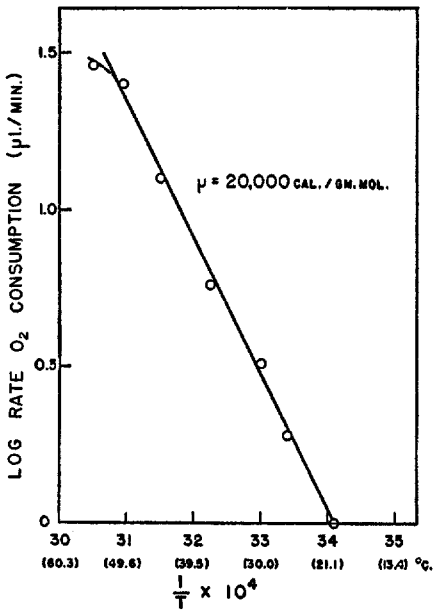

FIG. 5

FIG. 4. Oxygen consumption by resting cell preparation of organism 3 in presence of hydroquinone *vs.* time.

$$K_{O_2}^{0°C.} = 2.68$$

FIG. 5. Log rate of oxygen consumption by resting cell preparation of organism 3 in presence of hydroquinone as a function of $1/T$.

log rate against the reciprocal of the absolute temperature (see Fig. 8). The μ values calculated from the slopes of these curves for the different cultures are given in Table III.

Below the optimum temperature of the enzyme and far below the minimum temperature for growth of the organisms, the rate of the catalase-catalyzed reaction increases with temperature in accordance with the Arrhenius equation up to about 55°C. The mean μ value for the five stenothermophiles studied was 4,100 calories.

(*d*) *Activity of the Complete Respiratory System.*—All gas volumes were converted to 0°C., and oxygen-uptake plotted as a function of time (see Fig. 9).

TABLE II

The Activation Energy of the Cytochrome System of Stenothermophilic Bacteria

Organism No.	Cytochrome oxidase—cytochromes B and C Substrate: *p*-phenylenediamine		Cytochrome oxidase–cytochrome C Substrate: hydroquinone	
	Range	Activation energy	Range	Activation energy
	°C.	*cal./gm. molecule*	°C.	*cal./gm. molecule*
1	22–46	17,100	20–45	19,000
2	22–46	16,600	20–45	20,000
3	20–55	16,500	20–50	20,000
4	20–46	16,800	20–46	19,900
5	20–46	16,800	20–46	22,000
Mean μ value.....		16,800		20,200

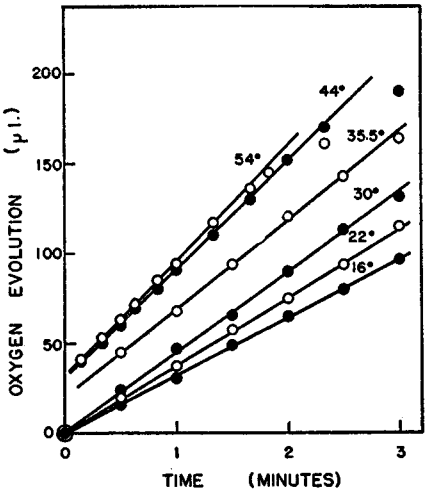

FIG. 6

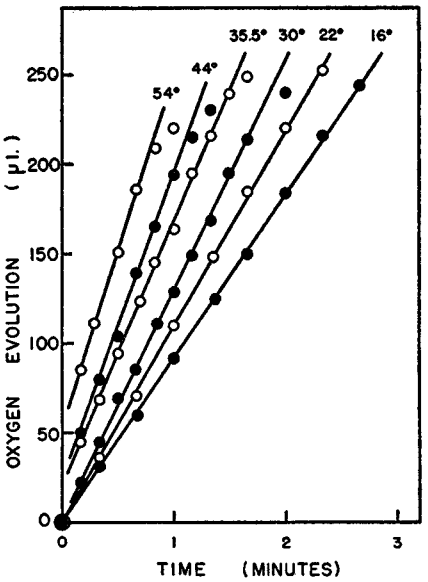

FIG. 7

FIG. 6. Oxygen evolution from 0.05 M H_2O_2 at pH 7.0 by dilute resting cell preparation of organism 3 *vs.* time.

$$K_{O_2}^{0°C.} = 2.68$$

FIG. 7. Oxygen evolution from 0.05 M H_2O_2 at pH 7.0 by concentrated resting cell suspension of organism 3 *vs.* time.

$$K_{O_2}^{0°C.} = 2.68$$

When the log of these rates is plotted against the reciprocal of the absolute temperature (see Fig. 10), the data are seen to be in accordance with the Arrhenius equation. Calculated temperature characteristics for the complete

aerobic respiratory system in the presence of added glucose of the stenothermophilic bacteria are given in Table IV.

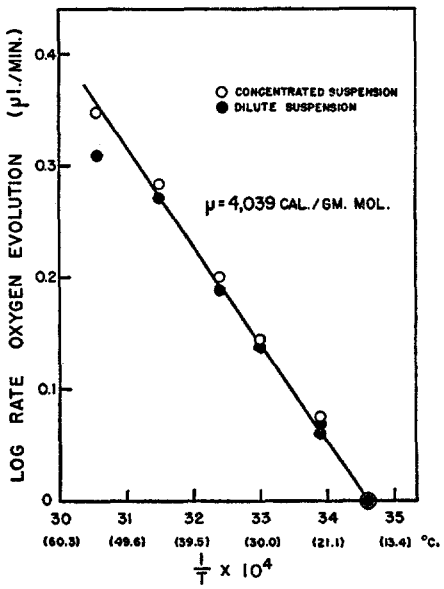

FIG. 8. Log rate of H_2O_2 decomposition by dilute and concentrated resting ce preparations of organism 3 as a function of $1/T$.

TABLE III

The Activation Energy for Catalase of Stenothermophilic Bacteria

Organism No.	Range	Activation energy
	°C.	*cal./gm. molecule*
1	15–55	4,300
2	20–55	3,900
3	15–55	4,000
4	20–55	4,000
5	15–55	4,200
Mean μ value		4,100

At temperatures below the optimum temperature of the component enzymes of the respiratory system studied earlier, and far below the minimum growth temperature of these organisms, aerobic respiration of the stenothermophilic bacteria, with glucose as a substrate, was found to increase exponentially with temperature in accordance with the Arrhenius equation. The mean activation energy for this group of organisms was 29,500 cal. per gm. molecule.

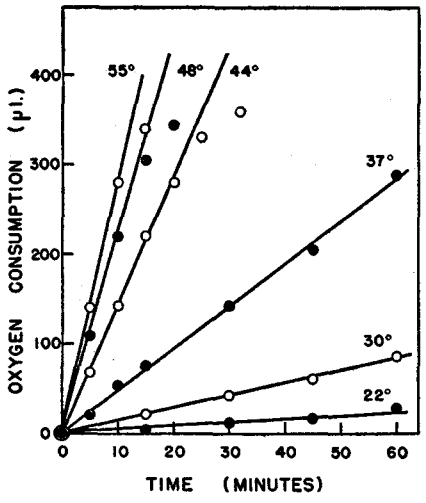

FIG. 9

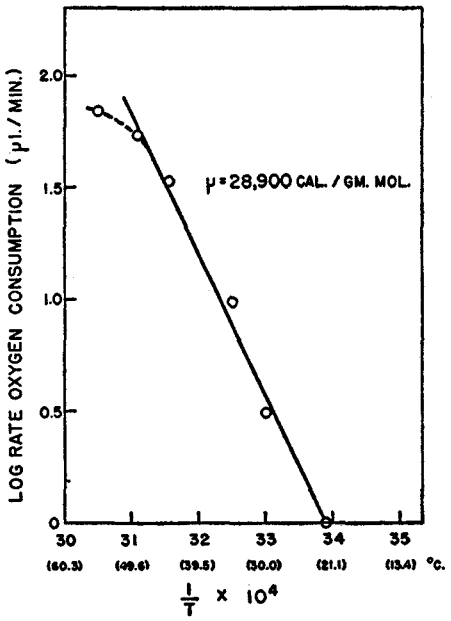

FIG. 10

FIG. 9. Oxygen consumption by resting cell preparation of organism 3 in the presence of glucose *vs.* time.

$$K_{O_2}^{0°C.} = 2.68$$

FIG. 10. Log rate of oxygen consumption of resting cell preparation of organism 3 in the presence of glucose as a function of $1/T$.

TABLE IV

The Activation Energy of the Complete Respiratory System of Stenothermophilic Bacteria

Organism No.	Range	Activation energy
	°C.	*cal./gm. molecule*
1	20–50	28,500
2	20–48	29,500
3	22–55	28,900
4	22–55	29,800
5	22–55	30,600
Mean μ value		29,500

IV. DISCUSSION

The energies of activation for dehydrogenation of most substrates are considerably higher than values recorded in the literature, but apparently not high

enough to prevent dehydrogenation reactions in the lower temperature range. The values of 19,500 to 20,500 for the dehydrogenation of ethyl alcohol, sodium succinate, sodium pyruvate, sodium lactate, and sodium acetate compare favorably with the μ value of 19,400 cal. per gm. molecule reported by Gould and Sizer (6) for the dehydrogenation of several of these substrates by *Escherichia coli*. Crozier (2), however, calculated a lower value, 16,700, from Quastel and Whetham's data (15) for the bacterial dehydrogenation of succinate. A distinctly different temperature characteristic of 15,000 for sodium formate was also obtained by Gould and Sizer (6); the stenothermophilic bacteria gave a mean value of 15,000. Carbohydrates (aldopentoses, aldo- and ketohexoses, and disaccharides) which yielded μ values between 28,000 and 29,000 in the present study of stenothermophiles, gave μ values ranging from 10,000 to 12,600 for *Rhizobium trifolii* (20), and from 19,400 to 25,000 for *Escherichia coli* (6). An activation energy of 25,000 has also been calculated by Crozier for the dehydrogenation of luciferin by *Cypridina* luciferase (2).

An attempt was made to group the activation energies on the basis of different activating mechanisms, as reviewed by Sizer (17). In a variety of instances the same energy of activation has been found for an enzyme acting on several different substrates. A definite relationship of activation energy to the nature of the substrate has been demonstrated by Sizer (16) for the hydrolysis of sucrose and raffinose by invertase and by Gould and Sizer (6) for the dehydrogenation of acetate, glycine, glutamate, lactate, succinate, glucose, mannitol, galactose, xylose, and sucrose by *Escherichia coli*. This identity of μ values obtained for an enzyme acting on different substrates suggests the possibility that this energy of activation characterizes a particular activating mechanism. A relationship of activation energy to the nature of the substrate was apparent in our study. For the stenothermophilic bacteria, the dehydrogenation of formate gave results consistent with the results of Gould and Sizer (6) and Quastel (14); the dehydrogenation mechanism for glucose, galactose, mannose, xylose, sucrose, glycine, and glutamate gave a common μ value, consistent with Gould and Sizer's analysis. However, the substrates characterized by a μ value of 19,500 to 20,500, although rather sharply defined, had no common chemical basis upon which their association in this group could be explained. Tam and Wilson (20), in a similar temperature-activity study of the dehydrogenase system of *Rhizobia*, obtained values for the various substrates which fell into a high, a medium, and a low group, but they did not feel justified in postulating an activating mechanism for each group.

Temperature activation studies of the cytochrome system of bacteria have not been presented in the literature. Resting cell preparations of the stenothermophilic bacteria in the presence of *p*-phenylenediamine, where the dehydrogenases were not involved in the reaction, gave a mean μ value of 16,800

cal. per gm. molecule at temperatures below the growth minimum. Hadidian and Hoagland (7) using heart extract and the same substrate obtained a value of 9,500. In the presence of hydroquinone, where cytochrome oxidase and cytochrome c alone function, a μ of 20,200 was obtained for the stenothermophiles. Corresponding μ values in the region of 20,000, it may be noted, were obtained earlier in the present study for the oxidation of ethyl alcohol, succinate, pyruvate, lactate, and acetate. An analysis of the data indicates that although both cytochrome b and c are present, cytochrome b contributed only a small fraction to the total activity of these mesocatalysts and is apparently more thermostable than generally accepted (19). Thus, the energy of activation of the dehydrogenase system is much higher than that for the cytochrome system, and indicates that the rate of dehydrogenation in the case of these organisms is the limiting rate of the over-all respiratory process (*cf.* reference 8).

The mean activation energy of 4,100 for the catalase-catalyzed reaction, using resting cells of stenothermophilic bacteria, agrees very well with the value of 4,200 reported for beef liver catalase (18), but does not agree with the 1320–1880 cal. per gm. molecule more recently reported by Bonnichsen, Chance, and Theorell (1) for crystalline catalase of horse blood and liver. It has been suggested that the higher values may be attributed to the presence of partly denatured enzyme. However, the magnitude of the values obtained in our present study of resting cells, in which the enzyme was still in the bacterial cells, is most likely not related to the presence of partly inactivated catalase. Data on the catalase of *Escherichia coli*, from an orientation study in the present investigation, gave a μ value in the region of 6,000 cal. per gm. molecule. Catalase apparently also occurs in higher concentration in the thermophiles than in the mesophiles. Peroxidase has not been demonstrated in any of the five strains of stenothermophilic bacilli (4).

As suggested earlier, a temperature-activity study of the complete aerobic respiratory system in the presence of added glucose of the stenothermophiles should give a μ value representative of the slowest reaction in the respiratory chain. Over the temperature range extending from 20°C. to the point at which inactivation became apparent, the rate of oxygen uptake by a resting cell preparation, in the presence of glucose, increased exponentially with temperature. The mean μ value for the five strains studied was 29,500 cal. per gm. molecule. This value is consistent with the value obtained for the dehydrogenation of glucose, 28,500, and suggests that the dehydrogenation reaction is the rate-controlling reaction in the aerobic respiratory system of these bacteria. The reactions involving the cytochrome system, with a μ value of 16,800, will usually proceed at a greater rate than those catalyzed by the dehydrogenases.

The energy of activation of the respiratory system of other organisms is consistently lower than 29,500 cal. per gm. molecule. Crozier (2), from an ex-

haustive analysis of published data, has found the critical thermal increments of respiratory processes in various plants and animals to be of two statistically significant types: μ = 11,500 and 16,100 (perhaps also 16,700). Recent work has substantiated the existence of these modes (*cf.* reference 17). These values have also been found in studies of bacterial respiration: *Rhizobium trifolii*, 10,850 (21); *Bacillus cereus*, 13,000–18,500 (10). Lineweaver *et al.* (12) found a slightly higher μ value for *Azotobacter*, μ = 19,300.

The older literature does provide some respiratory data which, when analyzed by the Arrhenius equation, give values for the energy of activation approximating the values found in this study of the stenothermophiles. Crozier's calculations (2) based on the data of Krogh and of von Buddenbrock and von Rohr on winter frogs indicated 28,000 and 29,500, respectively, in the lower temperature range. Morales (13), however, has demonstrated that the μ value of the over-all respiratory process of the higher plants and animals is not consistent with the μ value for one of its isolated tissues because the limiting process, or "master reaction," is actually the diffusion of oxygen to the respiring tissue. Thus, there appear to be no values in the literature comparable with the mean value obtained for the respiratory process of the stenothermophilic bacteria.

The most significant result of the present study is the identity of the energies of activation of the respiratory system and its enzymic components obtained at temperatures above and below the minimum temperature for growth of the stenothermophilic bacteria. This observation, therefore, indicates that there is no fundamental difference in the effect of temperature on the respiratory systems of stenothermophilic and mesophilic bacteria. In addition, this may suggest a similarity in the nature of the enzymes functioning in the respiratory process of mesophiles and thermophiles.

V. SUMMARY

The results of this study of the effect of temperature on the respiratory mechanism of five stenothermophilic bacteria may be summarized as follows:—

1. The respiratory mechanism and its various components of the stenothermophilic bacteria were found to function at temperatures below the minimum temperature for growth of these organisms. In every case the rates of the individual reactions involved in the respiratory chain increased exponentially with temperature until the temperature at which inactivation became apparent was reached.

2. The mean activation energies, calculated from the "best" value for the slope of the straight lines resulting from a plot of log rate against the reciprocal of the absolute temperature were:

Dehydrogenases: 28,000 to 28,500 calories per gram molecule. Glucose, fructose, galactose, mannose, xylose, arabinose, maltose, lactose, sucrose, glycine, β-alanine, monosodium glutamate, (asparagine).

19,500 to 20,500 calories per gram molecule. Ethyl alcohol, succinate, pyruvate, lactate, acetate.

19,500 to 20,500 calories per gram molecule. Ethyl alcohol, succinate, pyruvate, lactate, acetate.

15,000 calories per gram molecule. Formate.

Cytochrome oxidase and cytochrome b and c (substrate: *p*-phenylenediamine): 16,800 calories per gram molecule.

Cytochrome oxidase and cytochrome c (substrate: hydroquinone): 20,200 calories per gram molecule.

Catalase: 4,100 calories per gram molecule.

Complete aerobic respiratory system (plus added glucose): 29,500 calories per gram molecule.

3. The identity of the energies of activation of the respiratory system and its enzymic components at temperatures above and below the minimum temperature for growth of the stenothermophilic bacteria was demonstrated.

4. An attempt has been made to indicate a relationship between the nature of the substrate and the activation energy by grouping substrates on the basis of common μ values obtained for their dehydrogenation by resting cell preparations of stenothermophilic bacteria. The dehydrogenation reactions have been found to be the rate-controlling reactions in the aerobic respiratory system of these bacteria.

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