THE RATE OF OXYGEN UTILIZATION BY YEAST AS RELATED TO TEMPERATURE

BY T. J. B. STIER*

(From the Physiological Laboratory, Cambridge, England, and the Laboratory of General Physiology, Harvard University, Cambridge, Massachusetts)

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I

The rate of O_2 consumption of a single strain of yeast, *Saccharomyces cerevisiae*, engaged in the metabolic conversion of dextrose, was studied as a function of temperature in the range 3–35°C. An expression of the relationship was sought in the Arrhenius equation: $k_2/k_1 = e^{\frac{\mu}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$ where k_1 and k_2 are velocity constants (or figures proportional thereto) at the absolute temperatures T_1 and T_2; e is the base of natural logarithms, R is the gas constant, and μ, called by Crozier (1924) for such cases the "temperature characteristic," is a constant with the dimensions of calories per gram molecule.

The constant μ was used throughout the experiments as an index of the reproducibility of the relation: rate of uptake of O_2 to temperature. Data upon the constancy of this relation were desired for comparison with temperature characteristics calculated from rates of reduction of cytochrome in the same strain of yeast.[1]

It is realized that in studying such temperature relationships one is probably dealing with the participation of a large number of separate processes in the consumption of oxygen. The measurement, rate of O_2 uptake per unit number of yeast cells, represents the total O_2 consumption of all these metabolic processes. That the total O_2 consumption of all the processes per unit time, when studied as a function of temperature, should satisfy the Arrhenius equation might perhaps seem improbable. It might seem that at constant tempera-

* National Research Council Fellow in Biological Sciences, at the time that most of this work was done.

[1] To be published.

ture the velocity constants of the reactions using O_2 would differ widely among themselves, and also their critical increments, so that no regular linear agreement in the terms of the Arrhenius equation should obtain. Nevertheless, the organization of the diverse cellular processes is such that in some fourteen kinds of related experiments—ranging in diversity from the O_2 consumption of yeast to the O_2 uptake of goldfish—the data do obey the Arrhenius equation with great precision, and moreover give values of the constant μ which fall into definite classes for which the extreme values do not depart by more than 5 per cent from the modes.

Crozier (1924, 1924–25) has proposed that in biological systems the constant μ may refer to the formation of active molecules or ions of the catalyst controlling the velocity of the slowest process in a catenary or otherwise interrelated series of reactions possessing different critical increments. It is thought that the velocity of the organic activity as a whole is controlled by some substance or by such a dynamically linked system of changes. What actual chemical reactions or units of cellular organization are linked together in this manner, how a control of velocities is established yielding such close agreement of values of μ, and correlations with thermal increments obtained in purely chemical reactions, are still subjects of investigation. Perhaps by aid of biochemical procedures such as those developed by Keilin (1929, 1930), Quastel and Wooldridge (1927*a*, *b*), Harden (1932), and others, it may be possible to gain further information desired for this inquiry.

II

Methods

The rate of oxygen utilization was measured with Barcroft differential manometers, using cups of 35 ml. capacity having a vertical tube 6 mm. in diameter and 20 mm. high sealed in at the bottom to hold 5 per cent KOH for absorption of carbon dioxide. It is absolutely necessary to increase the surface of the KOH solution by using small rolls of filter paper (*cf.* Keilin, 1929; Dixon and Elliott, 1930), to insure complete absorption of CO_2. It was found that shaking at a frequency of 120 complete oscillations per minute with a distance of travel of 3 cm. was necessary to obtain reproducible rates of oxygen utilization. Dixon and Elliott (1930) have thoroughly investigated this point.

The calibration constant for each Barcroft differential manometer was obtained

by the method originally described by Hoffman (1913–14), and subsequently simplified by Münzer and Neumann (1917). At constant temperature (20° ± 0.01°C.) small volumes of air measured with a calibrated 1 ml. pipette graduated in hundredths were added to the right hand vessel of the manometer. After correcting for the "dead space" between the right hand tap of the manometer and the surface of the mercury in the pipette by a modification of the method of Münzer and Neumann (1917) the calibration constant K was calculated by the formula $K = \frac{v}{p}$, where v = volume of air in c.mm., and p = pressure of the manometer fluid in mm. The right hand vessel was always used for measuring the rate of O_2 uptake. At the time of calibration each vessel contained 3.0 ml. of phosphate buffer at pH 7.3, 0.3 ml. 5 per cent KOH, and a small roll of filter paper (the volume and area were kept constant for all calibrations and subsequent experiments).

The set-up of apparatus and the procedure of calibrating were essentially the same as those employed by Münzer and Neumann (see their Fig. 3; also, Stephenson, 1930, p. 271).

Münzer and Neumann (1917) showed that K is independent of the temperature. I tested this point for the same manometer at constant barometric pressure. The values of K were as follows: at 20°, $K = 3.94$; at 40°, $K = 4.00$. The deviation is within the experimental error of determining the values of the constant.

The value of K varies reciprocally with the barometric pressure (*cf.* Münzer and Neumann, 1917). A correction for this variation was made for each determination. The example given below shows the steps in the calculations.

A correction for the absorption of oxygen at different temperatures was not made since it was found by calculation that such a correction made a difference of only 0.4 per cent in the ratios of rates of O_2 uptake at temperatures 10° apart in the range from 15–30°C. and 0.9 per cent in the range 4–14°, and so would not appreciably change the significance of the values of the temperature characteristics obtained.

Sample Calculation

Manometer No. 74. $K = 3.26$ at **743** mm. Hg barometric pressure at time of calibration.

Experiment.—March 11.

Right vessel contained 3 ml. of yeast suspension; 0.3 ml. KOH; roll filter paper.

Left vessel contained 3 ml. phosphate buffer; 0.3 ml. KOH; roll filter paper of exactly the same size and weight as in right vessel.

Temperature was 35.5°C.

Barometric pressure was **746** mm. Hg at time of determining O_2 consumption.

∴ Vessel constant at **746** mm. = 3.257 (corrected for barometric pressure).

Average rate O_2 uptake = 51.35 mm. per 10 minutes (*cf.* Fig. 1 for method of calculation).

∴ Average rate O_2 uptake = 166.73 c.mm. per 10 minutes at **746** mm. Hg by the formula: $v = Kp$.

This procedure was followed in calculating the rate of O_2 consumption at each temperature in a series of determinations. Where measurements were made at different barometric pressures, the rates were adjusted to some one barometric

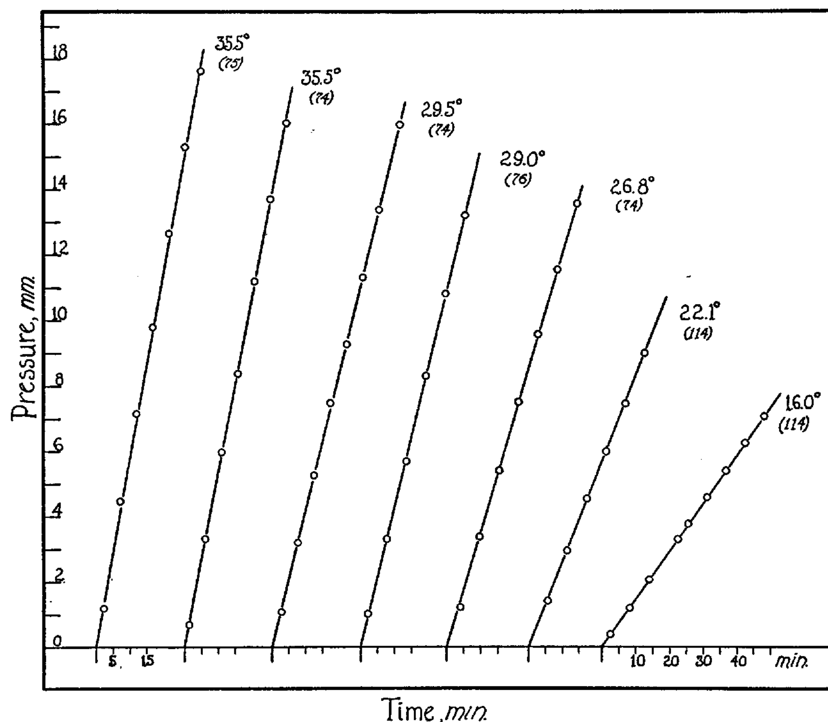

FIG. 1. Oxygen consumption by suspensions of yeast. Ordinate, volume of O_2 taken up, measured as total change in pressure; abscissa, time. Rates of consumption of O_2 were calculated from such graphs. The rate of uptake of O_2 is constant during the period of observation and throughout the range of temperatures employed (3–35°C.). The number placed under each temperature refers to the particular manometer used.

pressure over the whole range of temperatures. In this final form the data were then plotted according to the Arrhenius equation.

A procedure eliminating the use of a calibration constant was also employed for a series of determinations of the temperature characteristics (*cf.* p. 825, Method C). The values of the temperature characteristics as calculated from these relative rates of respiration agree to within 4 per cent with the averages obtained by

the use of absolute rates of O_2 consumption (*cf.* Table I). It is concluded that the simplified procedures used in calibrating the vessels and in calculating the volumes of O_2 consumed per unit of time were sufficiently accurate for the purposes of this investigation.

A method of calculating calibration constants suggested by Dixon (private communication) was also applied to the data obtained in these experiments. The calibration constant K obtained by the Münzer and Neumann method was first corrected to N.T.P. and dryness at 20°C. (the temperature at which all calibrations were made), yielding a new constant K_1. Since K_1 varies as $1/T$ when the volume of the gas dissolved in the liquid in the vessels is negligible compared with the volume in the gas phase (the conditions in these experiments), another constant, K_2, was calculated for each temperature at which the consumption of O_2 was measured. The rate of O_2 consumption was then obtained by the equation $v = K_2 p$.

The average temperature characteristics obtained by these two methods differ only by 1.4 per cent in the range from 4–15°C. and by 1.1 per cent in the range from 15–30°C. The difference between the average rates of respiration calculated by these two methods is also small:—at 22.8°C., 116.4 c.mm. per 10 minutes by the Münzer and Neumann procedure and 113.3 c.mm. per 10 minutes by the Dixon procedure; at 7.8°C., 27.8 c.mm. per 10 minutes by the Münzer and Neumann method and 28.33 c.mm. per 10 minutes by Dixon's method. For convenience the Münzer and Neumann method has been used throughout this paper in calculating the rates of consumption of oxygen. The small differences between the values of the temperature characteristics as obtained by the two different methods of calculation do not change the interpretation of the results. For a more critical determination of the magnitudes of μ, greater refinement in culturing yeast and greater precision in measuring its rate of respiration are required.

The yeast used was obtained through a local dealer from N. V. Nederlandsche Gist-en Spiritusfabriek, Delft, Holland, and was stated by the company to be "nearly a pure culture as far as this can be obtained by production on a large scale." Indeed, these experiments contain additional proof that, over a period of 6 months, yeast from this source behaved physiologically as a pure culture.

Whenever it was necessary to keep the number of cells constant for experiments made at a series of temperatures on different days, the following procedure was employed: 0.2 to 0.8 gm. of fresh yeast was weighed out and suspended in 100 ml. of M/15 phosphate buffer mixture of pH 7.3. By centrifuging samples of this suspension in graduated Hopkins vaccine tubes it was possible to estimate the number of cells per ml., and then by dilution to keep the relative number of cells per ml. equal throughout all the experiments. This method, however, gives no means of estimating or of adjusting the number of active, respiring cells.

A method of arriving at an index of the volume of "live cells," or better, the level of the rate of oxygen consumption for each suspension, was tried in one series of experiments (*cf.* p. 825). The rate of oxygen uptake of each fresh suspension was determined at the same temperature (5°C.); the percentage difference from a

selected rate (20 c.mm. per 10 minutes) was then used in correcting the rate obtained at higher temperatures, in the manner illustrated by this example:

Suspension April 12.	Rate at 5.0°C............	21.5	c.mm. per 10 min.
	Standard rate............	20.0	" " 10 "
	Difference................	1.5	
	Per cent difference........	7.5	
	Rate at 18°C............	62.0	c.mm. per 10 min.
	Corrected rate at 18°C.....	57.35	" " 10 "

In this manner the rates were adjusted for all the temperatures in the series (*cf.* line *B* of Fig. 3).

This method has limited applicability since it can be used only when the supply of yeast is produced under identical conditions of culture throughout the entire period of experimentation. Warburg (1927) found that the same strain of yeast cultured in different media or under different conditions yielded values of Q_{O_2} (c.mm. of O_2 per hour per mg. dry weight) which differed widely. The yeast used in these experiments was produced under identical conditions throughout the entire period of the investigation.

III

Determination of Temperature Characteristics

Method A

0.8 gm. of yeast was suspended in 100 ml. of buffer solution at pH 7.3. The suspension was kept in an ice bath at 1–2°C. Sufficient dextrose to make a 1 per cent solution was added to 25 ml. of the suspension 1 hour before beginning the determination of its rate of oxygen uptake; during this period oxygen was constantly bubbled through the suspension, which was kept in the ice bath. The cups of the manometers were rapidly prepared, with 3 ml. of the suspension in each right cup and 3 ml. of buffer mixture in each left cup. The manometers were immediately placed in the thermostat and shaken at the rate of 120 oscillations per minute. After 15 minutes had elapsed for temperature equilibrium, the stop-cocks were closed and readings taken every 8 or 10 minutes.

The constancy of pH of the yeast suspensions was ascertained at different temperatures with Clark-Lubs indicators. This method is sufficiently accurate for the purpose. (Data of Hastings and Sendroy

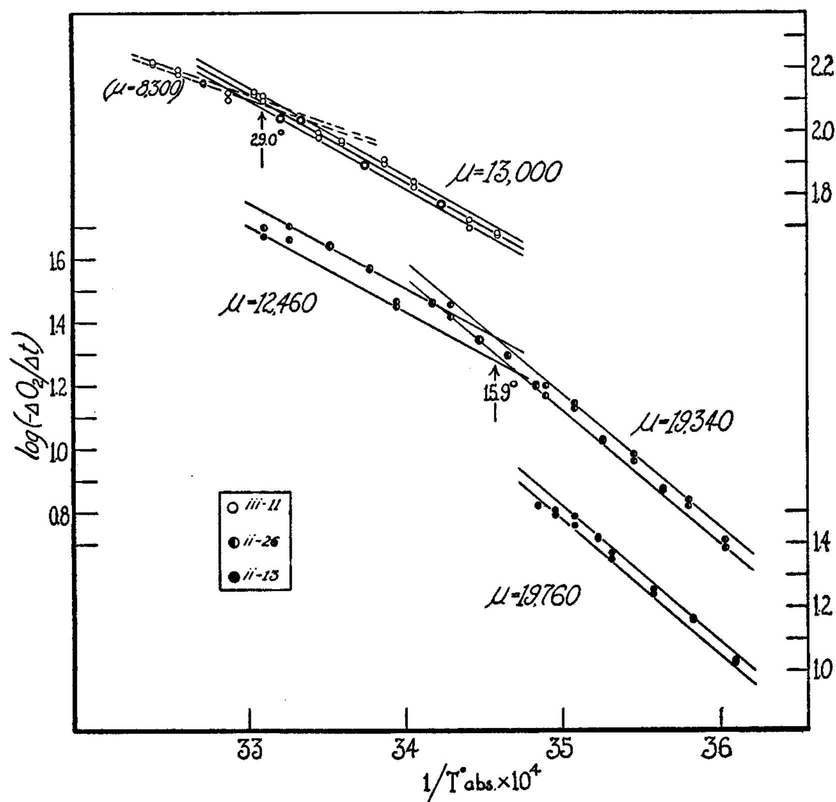

FIG. 2. Data on the relation of rate of O_2 consumption to temperature are plotted according to the Arrhenius equation; μ, called the *temperature characteristic*, computed from the slope of the graph, is a constant having the dimensions calories per gram molecule.

These three sets of data resulted from experiments made on three suspensions of yeast. Each run, lasting not more than 9 hours, was made on the same day. In each experiment the thermometric scale was first ascended by intervals of 2–3°. Observations at alternating temperatures were made by descending the temperature scale in similar steps. Thus any change in the rate of respiration of the stock suspension (which was kept at 1–2°C.) could be detected in the scatter of the points on the arith-log plot. (See also text for further comments on the constancy of the rate of uptake of O_2 by the stock suspension.)

Each point is an average of at least four rates, obtained as shown in Fig. 1, for separate samples of a stock suspension.

Above 30° the data are too few to give great weight to the value of μ; the determinations are chiefly useful in locating the critical temperature at 29°.

The suspension dated ii–13 contained 0.007 gm. of yeast (wet weight) per ml.; ii–26, 0.007 gm. per ml.; iii–11, 0.008 gm. per ml.

Calibration constants obtained by the Münzer and Neumann procedure.

(*cf.* Clark, 1928) show that a M/15 phosphate mixture of pH 7.3 changes only 0.02 pH when the temperature is raised from 20° to 38°C.)

For suspensions containing 0.002 gm. of yeast per ml. and 1 per cent dextrose, it was found that the pH remained constant at 7.3 for 3 hours at 16.4° (the rate of O_2 uptake remaining constant for the same period of time), and for 2 hours at 35°. A suspension containing only 0.004 gm. per ml. remained constant for 1 hour at 35°; and for 30 hours at 1–2°C. Since the determinations of rate of O_2 consumption were made in 30 minutes at 35°, in 50 minutes at 16°, it can be assumed that the data were all obtained at constant H ion concentration.

The temperature of the thermostats was held constant, varying, on the average, by less than ±0.1°C. Above room temperature the cooling of the thermostat was exactly balanced by adding heat from a long carbon filament lamp whose depth of immersion was adjustable and whose heat could be regulated by a hand-operated rheostat. Below room temperature a special cooling unit was devised (Stier, 1931) as a substitute for the more expensive SO_2 compression circuit (Crozier and Stier, 1926–27*b*) which can be used for low temperature thermostats. This device consisted of a copper funnel whose spout was closed by a rubber stopper and had a hopper of heavy linoleum attached to its top. The hopper and funnel were filled with pieces of cracked ice about the size of a walnut. Water from the melting ice was removed by a suction line attached to a water aspirator. The unit was attached to a ring-stand by a clamp which allowed the conical portion of the funnel to be lowered into the water bath to various levels. In operating the device, a depth of immersion is found, by trial, where the heat removed from the bath is about equal to the heat added from the surroundings.

In the absence of a system of automatic thermoregulation a position of balance was maintained by frequently reading the temperature on a calibrated thermometer (about once every 4 minutes) and then adjusting the depth of immersion of the cooling unit in the thermostat. Temperatures as low as 3° when the room temperature was 16° were maintained to within ±0.05°C. With automatic thermoregulation, this cooling unit (with certain modifications) has given a constancy of temperature regulation within ±0.007°C. (Stier, 1931).

By using 4 thermostats it was possible in one series of determinations to make 32 measurements of rates of O_2 uptake at 16 temperatures in 9½ hours over the range 16–35°; in another series of experiments 34 determinations were made in 9 hours at 17 different temperatures over the range 4–30°C.[2] In these two sets of experiments I was assisted

[2] See legend of Fig. 2 for comments on the constancy of the rate of oxygen uptake of the suspension during the 9 hour period of experimentation.

in making observations by Professor Barcroft, Dr. G. Enders, and Dr. P. Rothschild, to whom I am deeply indebted for this help.

Experiments on single suspensions of yeast are recorded in Fig. 2. The average values of the temperature characteristics (μ) for two

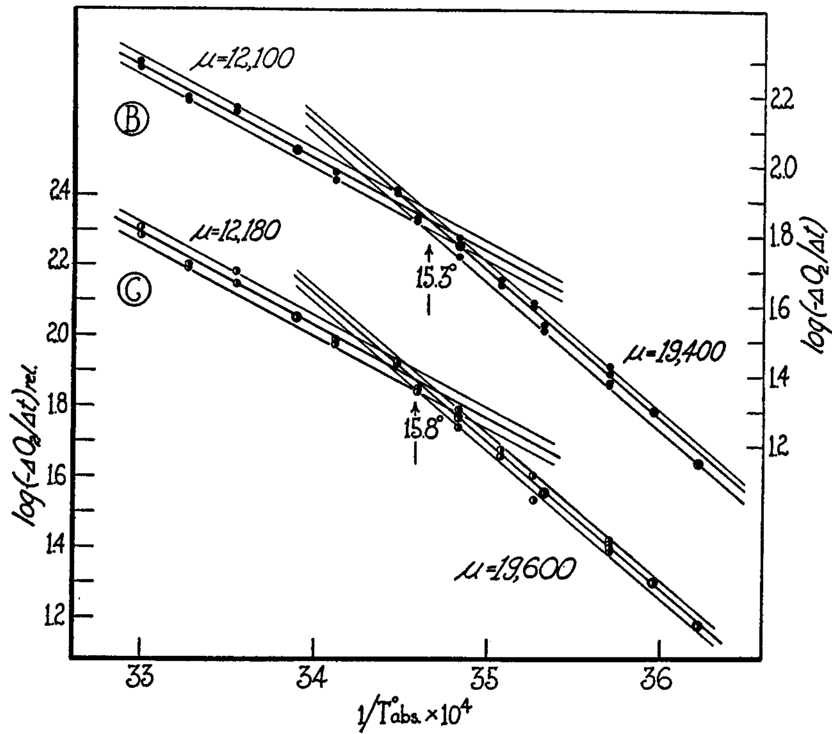

FIG. 3. The data plotted on lines *B* and *C* were obtained on different days, a new suspension of yeast being made up for each determination. The methods of correcting the rates of O_2 uptake for variation in the number of cells in different suspensions are explained in the text.

Each suspension contained 0.016 gm. (wet weight) of yeast per ml. of buffer mixture.

Calibration constants calculated by the Münzer and Neumann procedure.

series of experiments (February 26 and March 11) were found to be: *35–30°*, μ = 8,290 calories; *30–15°*, μ = 12,730 ± 270; *15–3°*, μ = 19,340. In a shorter series covering the range 15–3°, the value of μ was found to be 19,760 calories.

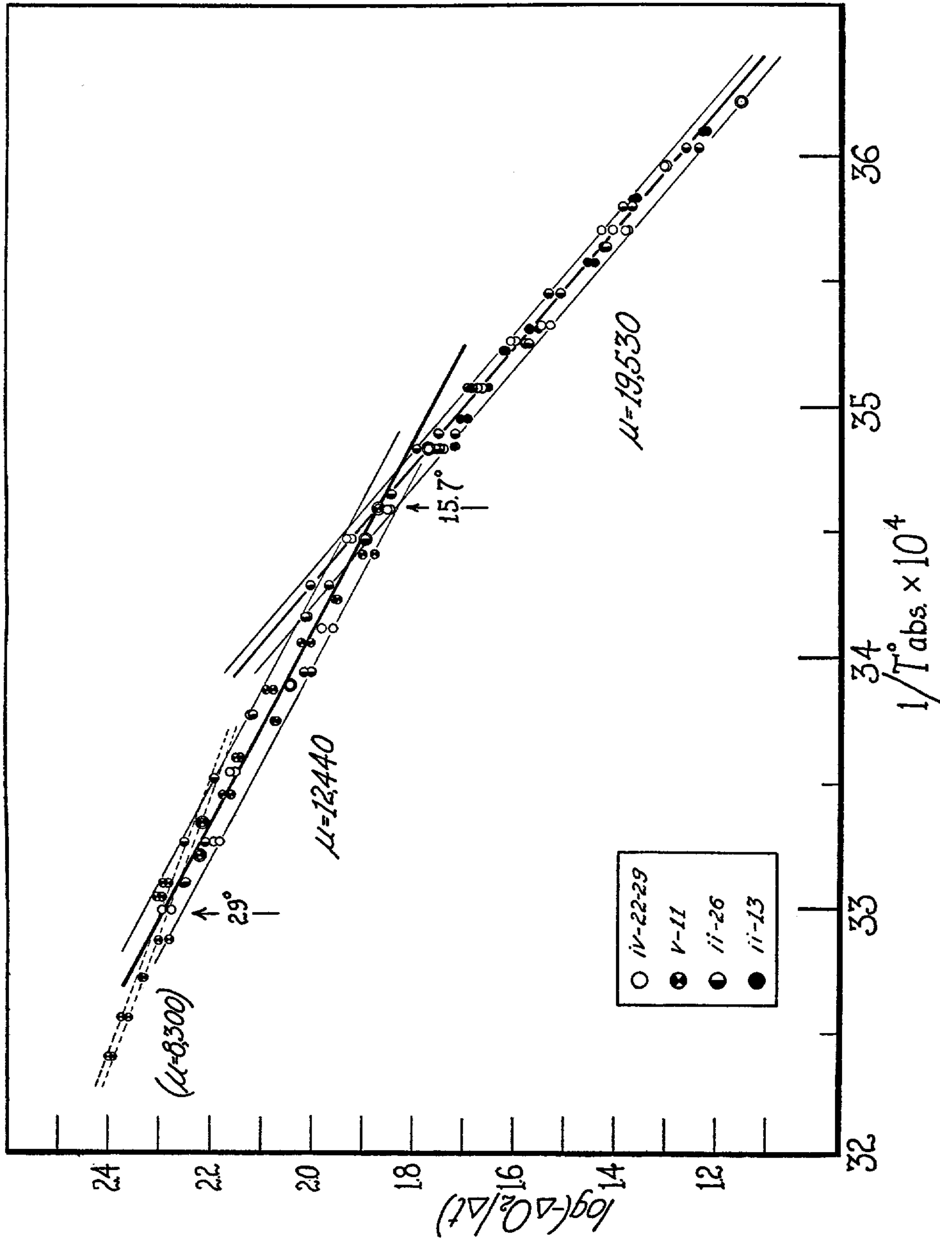

FIG. 4

Method B

The values of the temperature characteristics secured by Method A were compared with values obtained by a procedure in which a fresh suspension was employed at each temperature. To correct for any variation in the total number of "live cells" in new suspensions made up on different days the method of adjustment described on p. 820 was used.

Line *B* of Fig. 3 gives the data obtained by this procedure, the logarithms of the rates being plotted against the reciprocals of the absolute temperatures according to the Arrhenius equation. The graph is similar to that obtained by the previous method of experimentation. The points lie on two intersecting straight lines, the values of the temperature characteristic being: *30–15°*, μ = 12,100; and *15–3°*, μ = 19,400.

Method C

Method C consisted in calculating ratios of O_2 uptake for the temperature range 3–30°, thereby endeavoring to eliminate such errors as might have entered the data during their conversion from readings of pressure in mm. of manometer fluid to rates of O_2 uptake in c.mm. at normal pressure (or at some one pressure for the whole range of temperatures). These ratios were obtained for the same manometer by dividing the pressure difference between the two arms of the manometer, per unit time at 5°, into the mm. difference in pressure appearing per unit of time at each of thirteen different temperatures. When these data were plotted according to the Arrhenius equation (line *C* of Fig. 3) the points were fitted by two straight lines intersecting at 15.8°C.: *30–15°*, μ = 12,180; and *15–3°*, μ = 19,600.

FIG. 4. Mass plot of the data presented in Figs. 2 and 3. The rates of O_2 consumption from the different experiments are brought together with data in the series marked "iv–22–29" by multiplying each average rate of O_2 uptake in a series of determinations by a factor; for series ii–13, factor taken at 8.6° = 1.677; ii–26, factor taken at 11° = 1.774; iii–11, factor taken at 22.8° = 1.503.

O_2 consumption, c.mm. per 10 minutes, is given in Fig. 6.

By Dixon's method of calculating calibration constants (*cf.* p. 819) the average values of the temperature characteristics for the data presented in Figs. 2 and 3 were found to be: *35–30°*, μ = 8,150 calories; *30–15°*, μ = 12,300; *15–3°*, μ = 19,250.

In these experiments 5° was used as the reference temperature. Crozier (1925–26) pointed out that this temperature occurs in a critical zone where irregularities often appear in the relations between rates of vital processes and temperature. The procedure used in Methods B and C, may therefore be open to the criticism that the observed rate of respiration at the reference temperature 5° may be "abnormally" high or low, or irregular. All the facts in the experiments, however, are in accord with the data obtained by Method A: (1) *cf.* especially Fig. 4, where the data obtained by Methods A and B are brought together; (2) *cf.* Fig. 2, where determinations of rate of O_2 uptake for temperatures below 5° fall well on the line; (3) note that

TABLE I

Temperature Characteristics (μ) *Obtained by Different Methods*

Method	μ	Critical temperature	μ	Critical temperature	μ
A (Feb. 13)					19,760
(Feb. 26)			12,460	15.90°	19,340
(Mar. 11)	8,290	29.0°	13,000		
B			12,100	15.25°	19,400
C			12,180	15.84°	19,600
Average......	**8,290**	**29.0°**	**12,440**	**15.66°**	**19,530**

the average temperature characteristics obtained by Methods B and C agree to within 5 per cent with values obtained by Method A in the range 30–15°; to within 0.25 per cent in the range 15–3°. It appears, therefore, that the use of 5° as a reference temperature has not introduced any large error into these particular determinations. This matter will be thoroughly tested by future experiments.

IV

DISCUSSION

a

In the published accounts of respiratory processes of yeast and bacteria there are few data on rates of reactions studied carefully enough

at an adequate number of temperatures so that the measurements can be used for the kind of analysis desired. Slator's data (1906, 1908) on CO_2 production by yeast during fermentation are the only measurements made over a wide enough range of temperatures to be of any use for a temperature analysis.[3] Crozier (1924–25) calculated temperature characteristics for these data and found that the values of μ were within the range of critical increments obtained for other sets of data on rates of respiration. The temperature characteristics are: for brewery, distillery, and wine yeasts, *40–22.5°*, μ = 12,250; and *22.5–5°*, μ = 22,200. These are the same data used by Fulmer and Buchanan (1928–29) as the basis of their criticism of the curve fitting employed in this method of temperature analysis (*cf.* p. 832). I have recalculated values of μ for these data and have also calculated temperature characteristics for measurements of the CO_2 production by brewer's yeast fermenting various sugars (Slator, 1908). These values have been recorded in Table II. The wide scatter of the observations on the Arrhenius plot makes the significance of the calculated values of μ very doubtful (*cf.* footnote 3); the values are given only because of the historical interest of the original measurements. CO_2 production by brewery, distillery, and wine yeasts yields values of μ varying from 12,200 to 13,600 for the range *35–20°*; and 22,200 to 25,200 from *20–5°*. The "break" occurs at about 20.2°C. For CO_2 production by brewery yeast plus dextrose, or with levulose: μ = 12,200 to 12,900, *35–20°*; and μ = 25,300, *20–5°*; "break" at 20.2°. For brewery yeast plus maltose: μ = 12,100, *40–23°*; and 32,200, *23–10°*; "break" at 22.8°. (*Cf.* Table II for further comments on these values.)

The temperature coefficient (Q_{10}) has been employed extensively in the past to describe the "effects of temperature" on various activities of organisms. Although devoid of any theoretical significance, it has been used also in attempts to interpret the underlying chemical

[3] Unfortunately the data are given only in the form of ratios calculated for adjoining temperatures 5° apart, from rates of CO_2 production which did not remain constant during each experiment (see Slator's sample calculation, 1906, p. 136). No mention was made of the constancy of the pH, or of the constancy of the temperature. Consequently calculations of temperature characteristics, or estimations of critical temperatures are without much significance.

mechanisms of vital processes. The value of Q_{10} is not constant over a given temperature range; its magnitude varies as a function of temperature. As a means of classifying vital processes by their controlling reactions, basing the analysis upon correlations with physicochemical data, the "temperature coefficient" is valueless. A consideration of the disposition of the data in the Arrhenius plot on two or three intersecting straight lines makes clear the fact that the value of Q_{10} depends upon the 10° span on the temperature scale selected for the ratio.

Crozier (1924–25, 1925–26*b*) has calculated from published accounts the value of μ for the relation of temperature to the velocities of various vital processes. The frequency distribution of these temperature characteristics as calculated from practically all known series of observations exhibits a number of discrete modes at: 8, 11, 12, 16, 18, 20, 22, 24, 32 thousand calories. There is evidence that the values 11,000 and 12,000 are organically distinct; similar considerations hold for the values 16,000 and 18,000. For living processes in which rates of CO_2 production and O_2 consumption have been measured, the most frequently occurring values of μ have been 11,500 and 16,000. In this connection it is striking that data in the literature on reactions whose rates are controlled by the oxidation Fe^{++} to Fe^{+++}; respiration of echinoderm eggs with which the theory of catalysis by Fe is intimately connected (Warburg and Meyerhof, 1913; Warburg, 1914); and the deoxygenation of hemoglobin (by CO) (Hartridge and Roughton, 1923*a*, *b*)—in all these cases the temperature characteristic was found to be $\mu = 16{,}300 \pm 200$ calories. The value $\mu = 11{,}500$ has been obtained by Rice (1923) for cases where the free hydroxyl ion seems to be the controlling catalyst, and the value $\mu = 19{,}000$ to 20,000 for reactions where the unhydrated H ion is the governing catalyst. Rice predicted that chemical reactions should fall into comparatively few classes, each class having its characteristic thermal increment, where μ refers to the controlling catalyst of that group. Crozier (1924, 1925–26*b*) found this to be suggestively valid for vital processes, since the values of μ could be placed in a small number of classes (*cf.* also Table II).

In Table II I have summarized temperature characteristics for O_2 consumption and for CO_2 production calculated by Crozier and others from measurements already published. From a review of this table

it appears that there is a system of interrelated chemical reactions involved in respiration in living matter of diverse kinds (*cf.* Crozier, 1925–26). Processes as different as the frequency of "activity periods" of organisms (Stier, 1930) and the frequency of heart beat (Crozier and Stier, 1926–27*b*) are under the control of this nexus of processes. There are a number of additional unpublished cases which substantiate this view. Another line of evidence also points to a uniform organization of respiratory systems in diverse kinds of living matter. Investigations by Keilin (1925), Shibata and Tamiya (1930), and others show that the respiratory pigments, especially cytochrome, are widely distributed among organisms (from yeast to mammals), participating as key units in processes of cellular respiration.

Table II shows that the temperature characteristics for O_2 uptake of yeast—8,290; 12,440; 19,530—as reported in this paper, have also been found for respiratory processes in other organisms. This series of temperature characteristics is obtained for (respiratory) pulsations of the cloaca in certain holothurians: in *Holothuria captiva*, μ = 20,500 (Crozier, 1915, 1916); in *Stichopus*, μ = 12,200 (Crozier, 1916); in *Thyone briareus*, aboral end intact, μ = 12,300, and for the isolated aboral end, μ = 8,500 (Crozier and Stier, unpublished).

The "biological reality" of temperature characteristics is best illustrated by a series of careful experiments on the relation of the frequency of breathing movements to temperature recently reported by Pincus (1930–31). Young mice of a selected line of a *dilute brown* strain of mice and mice of the same age of an inbred *albino* strain gave certain definite values of μ and certain definite critical temperatures.

"The F_1 hybrids of these 2 strains, and the backcross generations to either parent strain, exhibit only those 4 values of the temperature characteristic observed in the parent strains and none other. One may therefore speak of the inheritance of the value of the constant μ. Furthermore, there appears to be inherited the occurrence (or absence) of a critical temperature at 20°C. The results of these experiments indicate that the particular values here observed have reality in a biological sense. For one can conceive of no more rigid test of the biological integrity of a process than that its descriptive constants are recoverable in successive generations of crossbreeding (*cf.* Pincus and Crozier, 1929; Crozier, Stier, and Pincus, 1929)" [Pincus, 1930–31, p. 442].

TABLE II

Temperature Characteristics for Consumption of O_2 and Production of CO_2

Object	Observer	Classes of μ, *in thousands*					
		8	11	12	16	20	30
Yeast							
O_2 consumption, baker's yeast	Kubowitz, 1929 (*cf.* p. 477)			(14,700?)		(21,970?)	
CO_2 production {brewery yeast, distillery yeast, wine yeast}	Slator, 1906			(12,200 to 13,600)		(22,200 to 25,200)	
CO_2 production {brewery yeast + levulose, brewery yeast + dextrose}	Slator, 1908			(12,200 to 12,900)		(25,300)	
CO_2 production {brewery yeast + maltose}	Slator, 1908			(12,100)			32,200
Aerobic glycolysis, baker's yeast	Kubowitz, 1929				(16,360?)	(25,420?)	
Anaerobic glycolysis, baker's yeast	Kubowitz, 1929			(13,420?)		(25,086?)	
Velocity of oxidation of glucose	Aberson, 1903				16,000		
Growth: rate multiplication. "Time to produce a standard crop"	Richards, 1928	8,600					
	Richards, 1928					20,200	

The following distribution of temperature characteristics has been made from Crozier's tabulation (1924–25) of values of μ calculated by him for the majority of published experiments of O_2 consumption and CO_2 production. The original references can be found in Crozier's paper (1924–25, p. 200). New values entered by the writer have been noted.

Object	8	11	12	16	20	30
Oxygen consumption		11,800		16,140		
		11,500		16,700		
				16,800	22,000	
				16,850		28,000
				16,200		

Oxygen consumption		11,500			29,500
		11,000	16,300		
			16,000		
		11,500			
			16,500		
			16,100		
			16,800		
				21,000	
		11,700	16,600*		
		13,100		21,050†	
				19,300‡	
Carbon dioxide production			16,700		
	8,100§		16,200		
		12,400			
			16,250‖		
			16,200		
		11,500			29,500
			16,800	22,000	
			16,500¶		
			16,100	24,000**	
				20,750††	
Average temperature characteristics for O_2 uptake of yeast as calculated from data by Methods A and B	**8,290±**	**12,440±**		**19,530±**	

* O_2 consumption of *Lupinus albus* (Tang, 1930–31). † O_2 consumption of *Zea mays* (Tang, 1930–31).

‡ O_2 consumption of *Azotobacter vinelandii* (Lineweaver, Burk, and Horner, 1931–32). § Added from data of Kuyper, calculated by Navez (1928–29); its companion value, 16,200, has already been reported by Crozier (1924–25, p. 200).

‖ CO_2 production of seedlings of *Vicia faba* (Navez, 1928–29). ¶ CO_2 production of seedlings of *Phaseolus aureus* Roxb. (Crozier and Navez, 1930–31).

** CO_2 production of *Lupinus albus* (Tang, 1931–32). †† CO_2 production of *Zea mays* (Tang, 1931–32).

b

Recently, Bělehrádek (1930), and Fulmer and Buchanan (1928–29), have criticized the method employed by Crozier and others of fitting two or three intersecting straight lines to data plotted according to the Arrhenius equation. It has been objected by these authors that the graph should be constructed as a smooth curve. (In making these criticisms they have omitted a large number of pertinent observations supporting the method of treating temperature data.) A method of testing the validity of fitting intersecting straight lines to the data is illustrated by Figs. 5 and 6, where the rates of O_2 uptake are plotted against °C. The plotted points are the actual observations; the outer edges of the cusps were carried over from the extreme edges of the

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Footnote to Table II.—A dotted line connecting two increments means that a "break" has occurred, giving two temperature characteristics, one for the lower range of temperatures, the other for the upper range.

The temperature characteristics for CO_2 production also appear in Table II under the heading "O_2 consumption." The same increments are obtained for both processes (*cf.* Crozier, 1924–25, experiments of Brunow, and von Buddenbrock and von Rohr).

Recent experiments by Cook (1930) on "a comparison of the dehydrogenation produced by *B. coli* in the presence of O_2 and methylene blue" when the organism has been treated with toluol according to the procedure of Quastel and Wooldridge (1927*a* and *b*) give points on the Arrhenius plot which are not rectilinear when replotted upon a fair scale. The best straight line is obtained when lactate is dehydrogenated in the presence of methylene blue. These observations give μ = 15,300 according to my calculations. I have also calculated rates of O_2 uptake from his data—c.mm. O_2 uptake after 15, 30, 45, and 60 minutes—and find that these rates are not constant over the whole temperature range, 40–15°C. The use of inconstant rates is sufficient to account for the curvilinear μ plots obtained for O_2 uptake under his experimental conditions; in any case, no analysis can be attempted when the basic velocity is a function of *time* as well as of temperature (*cf.* Tang, 1930–31; Crozier and Navez, 1930–31). It has been pointed out by Tamiya and Tanaka (1930), and von Euler and Hellström (1930) that upon the addition of toluol to an organism there is a gradual denaturing of the cytochrome system and so a gradually increasing inability on the part of the organism to utilize molecular oxygen. It is obvious that the temperature analysis can be used only when the rate of performance is constant, unless—with a system in which the measured rate is changing in a simple manner—it becomes possible to compute an initial rate undistorted by the progress of changes which are a function of time.

parallel bands enclosing the observations in the mass plot (Fig. 4) in the ranges of temperature: 35–30°; 30–15°; 15–3°. It is preferable to enclose these determinations within three such fan-shaped areas cutting across each other at 15° and 30°—"one" curve cannot fit the data. A full discussion of these matters can be found in: Crozier and Fede-

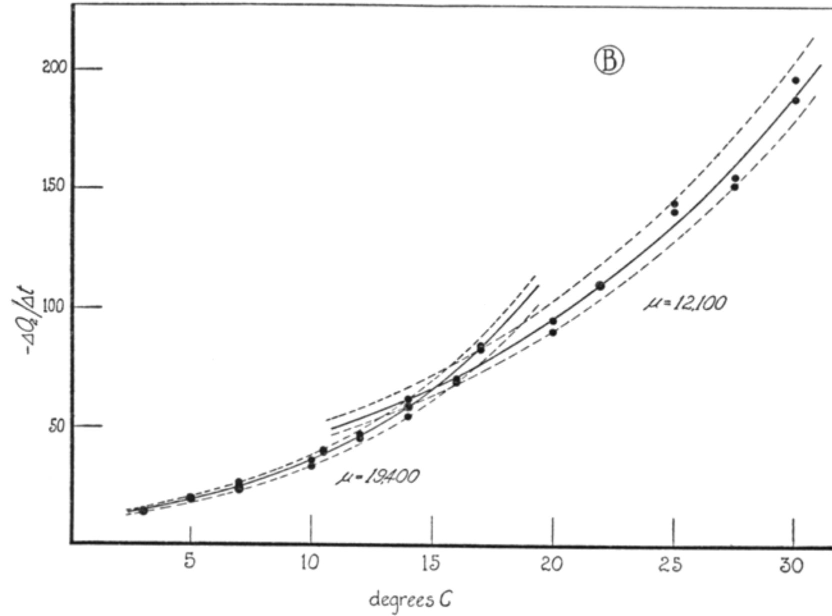

FIG. 5. The rates of uptake of O_2 are plotted in Figs. 5 and 6 as ordinates, and °C. as abscissae. The boundary lines marking the latitude of variation in the Arrhenius plot have been directly carried over from Figs. 3 and 4 to Figs. 5 and 6. Figs. 5 and 6 make it obvious that *one* smooth curve cannot be drawn through the data over the entire temperature range. In Fig. 5 the points are the actual values of rates of O_2 uptake already plotted (in Fig. 3, line *B*) according to the Arrhenius equation. Fig. 6 is a mass plot of all the data used in Fig. 4.

righi (1924–25); Crozier and Stier (1926–27*a*); Brown (1926–27); Navez (1930).

Attention is called to the significance of the fan-shaped enclosures seen in Figs. 5 and 6. Such a distribution of points means that the extreme limits in each fan increase with temperature; *i.e.*, for each range 3–15°, 15–30°, and 30–35°. This kind of spread of the observa-

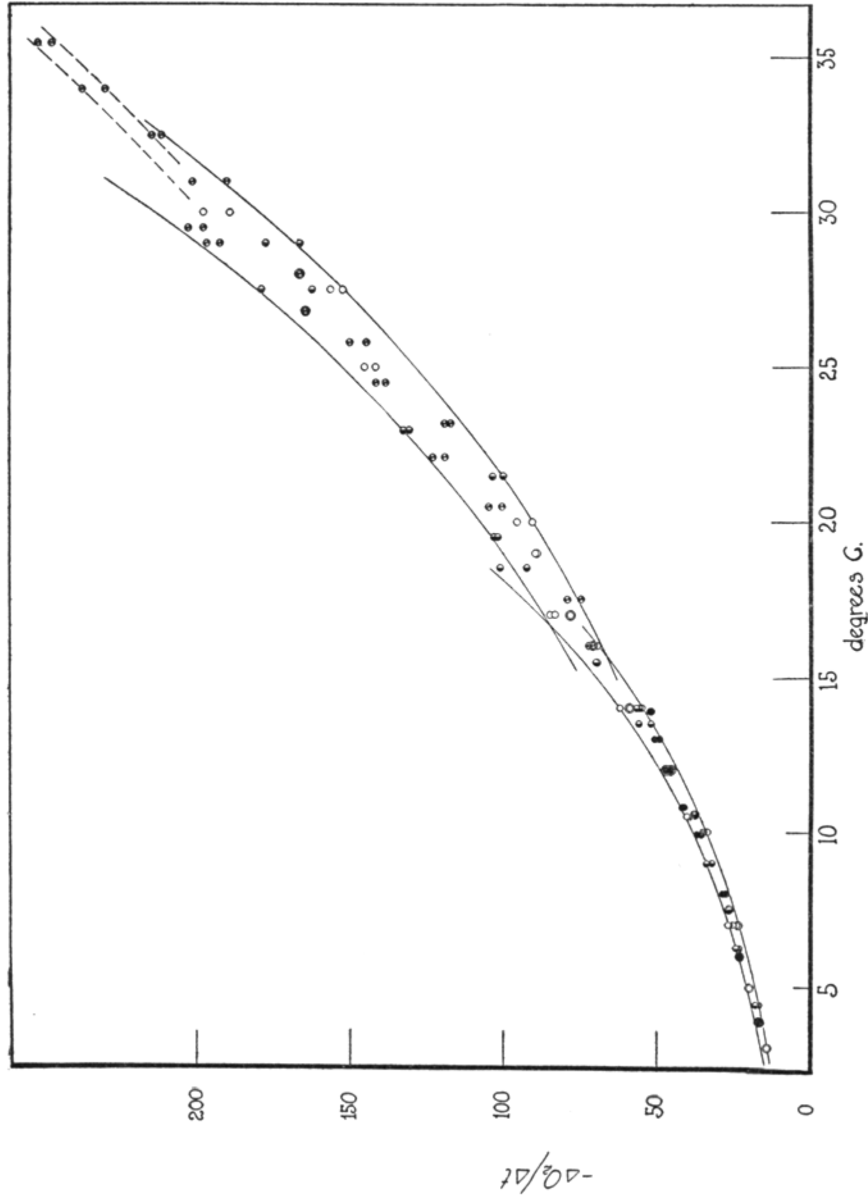

FIG. 6. See legend of Fig. 5.
The symbols used in Fig. 6 refer to the same series of experiments as the symbols in Fig. 4.
Calibration constants calculated by the Münzer and Neumann procedure.

tions cannot be due entirely to experimental errors, since such errors would certainly be a constant amount for each temperature and the percentage differences from the average would then alter with temperature. A constant percentage difference gives parallel sets of lines in the Arrhenius plot. This result has been obtained typically where rates of vital phenomena have been studied as a function of temperature (*cf.* papers by Crozier and associates, *J. Gen. Physiol.*, 1924, to date).

The actual extreme percentage differences at each temperature as calculated from the mass plot (Fig. 4) are found to be constant within each range of temperature: *3–15*°, ±6.1 per cent of the mean; *15–30*°, ±8.5 per cent; *30–35*°, ±1.8 per cent. The latitude of variation changes at a critical temperature, being greatest in the range *15–30*° and least in the range *30–35*°. A part of this latitude of variation is of course spurious, being due to the fact that the variation in different series of measurements is not quite the same; thus, to give one illustration, data which appear in Fig. 2 (iii–11) exhibit in fact a maximum latitude of variation of ±5 per cent over the range *15–30*°.

A similar case in which an increase in the latitude of variation accompanies a change in the value of the temperature characteristic is found in the experiments on speed of gliding motion of *Beggiatoa* (Crozier and Stier, 1926–27*a*).

When the logarithm of the probable error of the mean rate of such a vital process at each temperature is plotted according to the Arrhenius equation the same μ is obtained as was found for the mean rates of the process as a whole (Crozier, 1929; Navez, 1930; Pincus, 1930–31), when n is constant, *i.e.*, the latitude of variation of the observations is a constant percentage of the mean, and both mean and variation of the mean hold the same relationship to temperature. This has been interpreted to mean that the utilization of O_2 at constant temperature, let us say, occurs at a rate which varies not only within the error, but goes through a cyclical variation such that the extreme limits fall on lines which are a constant percentage of the mean throughout the specific range of temperatures. A cyclical fluctuation in frequency of respiratory movements of grasshoppers has already been found (Crozier and Stier, 1925–26). Whether a similar minor cyclical variation in the respiratory activity of suspensions of yeast cells exists will be tested by subsequent experiments.

c

Crozier (1925–26) has designated by the name "critical temperature" a temperature in the Arrhenius plot at which two lines intersect, or a temperature above or below which "destructive" changes occur in the organism, such that the rates measured become a function also of *time*. Critical temperatures were obtained from numerous published accounts of experiments on the relation of temperature to rates of vital processes, and were made into a frequency polygon. The distribution was found to be multimodal, with the greatest number of cases falling in the 15° class. Other modes were at 4.5°, 9°, 20°, 25°, 27°, 30°. Subsequent data (*cf.* papers by Crozier and associates, *J. Gen. Physiol.*, 1925, to date) have confirmed this series of critical temperatures.

The following classes of critical temperatures have been obtained from the published accounts of experiments on O_2 uptake and CO_2 production previously quoted in Table II.

Classes of critical temperatures..........	9°C.	15°C.	17°C.	20°C.	25°C.	27°C.	30°C.
Instances	9.0	14.0	17.0	20.2	25.0	27.0*	30.0
		15.5	17.0	22.7			30.0
		15.0	17.0	19.5			30.0†
		15.0	(18.2)	19.5			30.0
		15.2		21.0			
		15.2		21.0			
				20.0			
				20.0			

The average critical temperatures here found for O_2 uptake of yeast are:

15.7° ±0.27° **29.0° ±**

* Minimum time of sporulation of *Saccharomyces pastorianus* (Hansen, 1883).

† Minimum time of sporulation of *Saccharomyces cerevisiae* (Hansen, 1883).

A critical temperature at 15° has not been obtained previously for respiration of yeast, so far as I have been able to find from published experiments. Slator's data (1906, 1908) give critical temperatures at 20.2°, 22.7°, and 35–40°(?). These values cannot be taken as especially significant, because the original data, as has been noted already, are not really accurate enough for use in a temperature analysis. The data of Kubowitz (1929) for O_2 consumption of baker's

yeast over the range -3.5 to $+37.5$°C. when plotted according to the Arrhenius equation show evidence of critical temperatures at 11.4° and above 30°C. Unfortunately the determinations were made at so few temperatures (six in all) that any evaluation of μ (or of critical temperatures) must be based on two points in the upper temperature range and three in the lower range and consequently is of doubtful significance.

19.5–20°C. was found by Tang (1930–31) to be a critical region for the O_2 consumption of germinating seeds of *Lupinus albus* and of *Zea mays*. Navez (1928–29) found 21.0° to be a critical temperature for CO_2 production by seedlings of *Vicia faba*. Recently Crozier and Navez (1930–31) assigned 20.0° as the critical temperature for the CO_2 production by seedlings of *Phaseolus aureus* Roxb. The CO_2 production by seedlings of *Pisum* yields a critical temperature at 21.0°C. (data of Kuyper, analyzed by Crozier, 1924–25; and Navez, 1928–29). Tang (1931–32) found 20°C. to be a critical temperature for the CO_2 production by *Lupinus albus*, but he found no evidence of a critical temperature for the CO_2 production by *Zea mays* in the range of temperatures studied.

30° is a definite critical zone for yeast. My experiments on O_2 uptake show that this is a critical temperature. Richards (1928) showed that the rate of growth of yeast decreases very rapidly above this temperature and that definite morphological changes occur in the budded cells growing at 30° –. The usually roundish cells found below 30° now grow as "irregular elongate cells" above 30°. Just how the mechanism producing such cells may be related to the value $\mu = 8{,}000$ found for O_2 consumption above 30° has not been ascertained as yet. Estimations by Hansen (1883, data taken from a paper by Herzog (1902–03)) of the time of production of the first ascospore in suspensions of yeast give an apparent critical temperature in the region of 30° ± for *Saccharomyces cerevisiae* and in the zone of 27° ± for *Saccharomyces pastorianus*. However, these values are of doubtful significance since they are obtained from an Arrhenius plot in which the data gave a curvilinear relationship.

In view of these facts one should be cautious when choosing a temperature for comparing effects of substances on rates of respiration. The region 30–37° has been used most frequently for such determinations. Temperatures below 30° would be nearer the range of "normal" physiological activity for yeast and similar organisms.

d

The interpretation of the temperature characteristics obtained for O_2 uptake of yeast in the presence of dextrose is briefly as follows: it

is thought that a value of μ refers to, and characterizes, the slowest or the governing process, under the conditions, in the chain of respiratory processes (*cf.* Crozier, 1924, 1924–25). At a critical temperature a new reaction becomes the "master reaction" (*cf.* Blackman, 1905; Crozier, 1924–25), and so limits the over-all rate of the processes concerned with the consumption of O_2.[4]

Difficulties confronting this interpretation are realized, and have already been stated (p. 815). However, by studying the kinetics of metabolic processes as a function of independent variables such as temperature, oxygen pressure, etc., and making use of methods already indicated, one may expect to establish the identity of these "master reactions" and their controlling catalysts, and then to formulate a more complete picture of the organization of the processes resulting in the uptake of oxygen.

I wish to thank Professor Barcroft, and other members of the Physiological Laboratory, for their kindness and helpfulness to me during my sojourn at Cambridge University as a National Research Fellow.

SUMMARY

Suspensions of the yeast *Saccharomyces cerevisiae* gave reproducible rates of O_2 uptake over a period of 6 months. The relation of rate of consumption of O_2 to temperature was tested over a wide range of temperatures, and the constant in the formulation of the relationship is found to be reproducible. The values of this constant (μ) have been obtained for five separate series of experiments by three methods of estimation. The variability of μ has the following magnitudes: the average deviation of a single determination expressed as per cent of the mean is ± 2 per cent in the range *30–15°*, and ± 0.8 per cent in the range *15–3°C.* This constancy of metabolic activity measured as a function of temperature can then be utilized for more precise investigations of processes controlling the velocity of oxidations of substrates, and of respiratory systems controlled by intracellular respiratory pigments.

[4] Experiments by the author (to be published shortly) on the relation of temperature to the time for reduction of cytochrome give additional evidence supporting this interpretation.

The data plotted according to the Arrhenius equation give average values of the constant μ as follows: for the range *35–30°*, μ = 8,290; *30–15°*, μ = 12,440 ±290; *15–3°*, μ = 19,530 ±154. The critical temperatures are at 29.0° and 15.7°C.

A close similarity exists between these temperature characteristics (μ) and values in the series usually obtained for respiratory activities in other organisms. This fact supports the view that a common system of processes controls the velocities of physiological activities in yeast and in other organisms.

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