Iowa Branch.

University of Iowa, February 7, 1929.

4339

The Thermal Increments and Critical Temperatures of Biological Reactions.

ELLIS I. FULMER AND R. E. BUCHANAN.

From the Departments of Chemistry and Bacteriology, Iowa State College.

It has become common practice to express temperature effects on rates of biological reactions in terms of the Van't Hoff-Arrhenius relationship by the use of one of the following equations:

$$1.\quad \text{Log k} = -\frac{\mu}{4.6T} + \text{constant.}$$

$$2.\quad \mu = \frac{4.6\,(\log k_2 - \log k_1)\,T_2T_1}{T_2 - T_1}$$

in which k_2 and k_1 are the velocity coefficients at absolute temperatures T_2 and T_1. The symbol μ has been called the thermal increment or thermal constant. The latter term is misleading since μ cannot be assumed to be a constant but its constancy must be proved for the temperature range under consideration. It is the purpose in this paper to discuss the value of μ as a function of temperature as calculated by means of Equation 2 for several biological processes and to point out instances where the use of Equation 1 has given misleading results.

In Table I are given values for μ calculated from data given by Sherwood and Fulmer[1] for the growth of *Saccharomyces cerevisiae* in various media. It is evident that μ decreases regularly with increase in temperature. In 3 of the media the value of μ is a linear function of the temperature. Moreover the medium used influences markedly the values of μ. It is of particular interest to note the influence of ammonium chloride both in the synthetic medium and

[1] Sherwood, F. F., and Fulmer, E. I., *J. Phys. Chem.*, 1926, xxx, 738.

in the beer wort. The notations μ_{10} and μ_5 have been used; since μ decreased regularly with increase in temperature its value will necessarily vary with the temperature range employed in its calculation.

TABLE I.

Values of μ for yeast in various media. Sherwood and Fulmer.[1]

Medium	W_0		W_1		E_0		E_1		
T°	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	$k \times 10^2$	μ_{10}	μ_5
0	1.22		4.53				1.13		
5	(2.6)		(7.4)				(2.55)		24,440
10	4.30	19,430	10.92	12,500	3.24		4.05	19,430	16,550
15	(7.1)	16,060	(14.0)	10,200	(5.2)		(6.20)	16,470	13,850
20	10.35	14,150	17.80	8,087	7.77	14,500	8.83	12,580	11,950
25	(13.5)	11,000	(19.4)	5,606	(10.4)	11,890	(11.20)	10,080	8,274
30	16.86	9,210	20.51	2,531	13.61	9,920	13.61	7,658	7,060
35	(19.0)	6,240	(20.4)	990	(13.4)	4,645	(14.2)	4,808	1,545
40	19.36	2,617	20.05	–1,403	13.16	–1,403	14.52	1,281	848

W_0 = beer wort without ammonium chloride. $\mu_{10} = 25{,}000–570t°$.

W_1 = beer wort with optimum concentration of ammonium chloride for each temperature. $\mu_{10} = 17{,}500–480t°$.

E = Synthetic medium optimum for 30°. μ_{10} is not a linear function of t°.

E_1 = Synthetic medium with optimum ammonium chloride for each temperature. $\mu_{10} = 26{,}000–620t°$.

An analysis of data by Slator[2] on the fermentation of different sugars by several yeasts shows that μ decreased regularly with increase in temperature. The value of μ for sporulation rate of *Saccharomyces pastorianus I* as calculated from the data of Herzog[3] is a linear function of the temperature, *i. e.*, $\mu = 44{,}100 - 1300\ t°$.

The above findings are not in harmony with the conclusions of Crozier[4] and Richards[5] for yeast. These authors calculated the value of μ by use of equation 1. They plotted log k against the reciprocal of the absolute temperature. Through these points straight lines were drawn, the slopes of which are $-\mu/4.6$. In Table II, I, are given values of μ calculated by means of equation 2 and compared to the values obtained by Crozier for the data of Slator[2] on the production of CO_2 by yeast at various temperatures. The data from the graph showed a "critical temperature" at about 21° while our figures show no critical temperature. This discrepancy is explained in the following manner. An examination of Eq. 2 shows that the value of μ, a large number, is determined by the accuracy of the

[2] Slator, A., *J. Chem. Soc.*, 1906, lxxxix, 128; *J. Chem. Soc.*, 1908, xciii, 217; *J. Inst. of Br.*, 1911, xvii, 147.

[3] Herzog, R. O., *Z. physiol. Chem.*, 1902, xxxvii, 149.

[4] Crozier, W. J., *J. Gen. Physiol.*, 1924, vii, 189.

[5] Richards, O. W., *J. Phys. Chem.*, 1928, xxxii, 1865.

difference between the logarithms of the velocity coefficients. In drawing a graph of log k against 1/T if the ordinate is not taken sufficiently large the differences in log k which are significant in showing the steady decrease in μ are masked. This is in effect decreasing the accuracy of the experimental values of the function. In the case in hand, the graphing of the values of log k with large enough ordinates shows plainly that log k is not through any temperature range a linear function of the reciprocal of the absolute temperature, but on the contrary decreases more rapidly than the variable.

TABLE II.

Values of μ for several instances calculated by Eq. 2 and by Crozier[4] by means of Eq. 1.

Function	I			II			III		
t°	log k	μ(2)	μ(1)	log k	μ(2)	μ(1)	log k	μ(2)	μ(1)
4							0.718		16,700
5	0.1461		22,200						”
10	0.5378	28,320	”						”
14			”				1.263	19,940	”
15	0.8325	22,100	”						”
20	1.1139	21,840	”						”
24			12,250				1.744	18,870	”
25	1.1318	16,390	”	1.3665		16,700			”
30	1.4771	13,160	”	1.4949	10,660	”			”
34			”			”	1.959	9,020	”
35	1.6075	11,180	”			”			
40	1.7033	7,480	”	1.8416	15,120	”			
45				2.0177	16,150	”			
50				2.2518	22,120	”			

I — CO_2 production by yeast, Slator.[2]

II — Reduction of methylene blue by "resting" *B. coli*, Questel and Whetham.[6]

III — CO_2 production by heart ganglia of *Limulus polyphemus*, Garrey.[7]

In order to find whether a similar discrepancy occurred in other instances, values for μ were calculated by Eq. 2 and compared with those obtained by Crozier by the graphical method. Such values for the reduction of methylene blue by "resting" *B. coli* and for CO_2 production by heart ganglia of *Limulus polyphemus* show in the first case an increase of μ with rise in temperature and in the latter a decrease, as compared to a constant value of $\mu = 16{,}700$ for both cases as inferred from the graphical method.

It is of interest to note that the value of μ for malt amylase as calculated from data by Ernström[8] decreases regularly with rise in

[6] Quastel, J. H., and Whetham, M. P., *Biochem. J.*, 1924, xviii, 519.

[7] Garrey, W. E., *Biochem. J.*, 1920, iii, 49.

[8] Ernström, E., *Z. physiol. Chem.*, 1922, cxx, 190.

temperature, and for invectase according to Euler and Laurin[9] is a linear function of temperature, *i. e.*, $\mu = 22{,}800–205.2$ t°. Spohr[10] found the value of μ for the inversion of sucrose by HCl in homogeneous system to be constant at 12,800 from 25° to 55°. This is in contrast to the reaction in heterogenous system. The fact that μ for single enzymes varies continuously with temperature would lend improbability to a constancy for a complex of enzymes such as are present in the living cell.

It is concluded that μ varies continuously with the temperature in the several biological reactions examined. There is no evidence of a critical temperature nor of master or key reactions in a catenary series, as has been inferred by others from a supposed constant value of μ through a given temperature range. This discrepancy between the results and those of authors cited probably is due to the fact that they have calculated the value of μ on a graph with too small scale ordinates to account in full for the experimental accuracy of the velocity coefficients. It is suggested that a careful examination be made of all instances in which critical temperatures have been claimed in the literature.

4340

Further Observations on Latent Tolerance in Diabetics.

R. B. GIBSON.

From the Pathological Chemistry Laboratory, University Hospital, Iowa City.

A recent report on the stimulating effects on carbohydrate tolerance of successive short periods of a dietary high in sugar and low in protein and salt, with added insulin dosage, included data obtained on 2 mild cases in which improvement was maintained without interruption of the high sugar regime after withdrawal of insulin. Substitutions and additions were made to the diet of one of the patients, a young man 26 years old, until it was essentially normal in character . His condition was followed for 6 months without recurrence of the diabetic status. The present study is an attempt to determine how patients who were formerly insulin cases would react to an essentially similar procedure.

H., a student, age 20 years, was discharged from the hospital

[9] Euler, H. V., and Laurin, I., *Z. physiol. Chem.*, 1919, cviii, 64.

[10] Spohr, J., *Z. physik. Chem.*, 1888, ii, 194.