STIMULATION OF FUNDULUS BY OXALIC AND MALONIC ACIDS AND BREATHING RHYTHM AS FUNCTIONS OF TEMPERATURE*

By IRWIN W. SIZER

(From the Department of Physiology and Biochemistry, Rutgers University, New Brunswick†)

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A study of the quantitative relationship between a biological phenomenon and temperature has in many cases given information concerning the underlying mechanism controlling that phenomenon (Crozier, 1924–25). An analysis is made in terms of the Arrhenius equation:

$$K_2 = K_1 e^{\frac{\mu}{2}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)}$$

where μ represents the slope of the line drawn through the experimental points when log rate of the biological process is plotted against the reciprocal of the absolute temperature. The temperature characteristic μ may characterize the chemical system controlling the rate of a measured process, and is thought to represent the "energy of activation" in calories (Crozier, 1925–26) of the catalyst for the slowest or controlling reaction in the catenary series of processes which determine the rate of a biological phenomenon. Different processes in various organisms frequently give the same thermal increments, and thus the μ values obtained may be used to identify the controlling reactions.

It seemed possible that the response of *Fundulus heteroclitus* as measured by the reaction time to stimulation by the dicarboxylic acids might vary in a measurable manner with temperature. It was hoped

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that the relations of temperature to the quantitative response to chemical stimulation might be used to analyze the connection between properties of the stimulant and the amplitude of reaction as measured by rate of response. Several questions of theoretical significance regarding chemical stimulation might be answered by such an attack. In certain studies on chemical stimulation, factors related to the surface tension of the stimulating agent have been found to be of importance (Cole, 1931–32). A temperature study might reveal whether these are controlling agents in chemical stimulation. It has been shown elsewhere (Sizer, 1934, also Allison and Cole, 1933–34) that a parabolic relationship exists between rate of response and (H^+) for stimulation of *Fundulus*. Adsorption also varies parabolically with concentration. Is the adsorption of acid molecules at the receptor interface a necessary preliminary to stimulation, and if so is this the controlling process determining rate of response? Cole and Allison have stated (1932–33) that since the coefficient of variability (*i.e.* probable error expressed as per cent of the mean) does not vary with reaction time, the mechanism of the reaction to a given acid is the same regardless of the concentration, within the experimental limits. Experiments designed to furnish thermal increments for stimulation by different concentrations of the same acid should show definitely whether the mechanism of reaction changes with concentration. Oxalic, malonic, and succinic acids give different constants in the parabolic equation relating rate of response to (H^+). Does this mean that the fundamental architecture of the stimulation mechanism is the same for the three acids, and that the chemical reaction controlling the rate of the reaction processes is different in the three cases? Stimulation by the dicarboxylic acids in fresh water has been found to be different from stimulation in salt water, although in both cases parabolic relationships were found. Is this evidence of a difference in the chemical processes controlling rate of stimulation in the two environments? An answer to these questions might be indicated by an investigation of the relationship between acid stimulation and temperature.

Method

The experimental procedure was the same as that for stimulation of *Fundulus* by the dicarboxylic acids and their derivatives (Sizer, 1933; 1935). The fish

was placed in a small celluloid reaction chamber through which solutions were passed at the rate of 100 ± 5 cc. per minute. To make a test the salt or fresh water was turned off, and the acid solution turned on at the same rate of flow and at the same temperature. The response was measured with a stop-watch. The temperature was held constant to ±0.1°C. Ice was used as the cooling agent for tests made in salt water below 12°C. When ice was used the temperature sometimes varied by as much as ±0.2°C. Ample time was allowed the fish for adaptation at a given temperature. This time varied from a few minutes at room temperature to several hours at low temperatures. The aquaria containing the individual fish were immersed in the water bath so that the fish might be kept at the desired temperature for an ample period. When the fish was removed to the reaction chamber for stimulation it was already adapted to the experimental temperature. Each fish was stimulated at 2 minute intervals at all temperatures. This recovery time was ample since there was no progressive change in reaction time due to adaptation to the stimulus. For the tests made in fresh water ten reactions were taken on each of six fish at a given temperature, while for salt water work twenty reactions were taken on each of three fish. In both environments sixty readings were taken for each temperature. Above about 15°C. the fish were quite active and moved about unless held in position with a wire screen. Below this temperature the fish were very quiescent, scarcely moving even a fin over long periods of time. At high temperatures cessation or change in rate of opercular movements was the criterion of response, but at low temperatures the first visible unusual movement, whether opercular or not, was considered the response to stimulation. To minimize the personal element as much as possible the range of temperature was studied at 2° intervals; the range of temperature was then covered again, this time response to stimulation being determined at the intervening temperatures. The results were not analyzed until the end of the experiment so that the observer would not be prejudiced in taking readings. Fish occasionally died over a period of a few weeks and were replaced by new ones. Since the variation in individual reaction times is not great this procedure did not noticeably affect the average reaction time. The pH of the solutions both in fresh and salt water was measured daily by the quinhydrone electrode.

It was necessary to take special precautions to make sure that the stimulating solution entered the reaction chamber at exactly the same temperature as the salt or fresh water which it displaced. Otherwise a distinct response of the fish was noticed, but here the stimulating agent was the temperature change, not the acid solution. This response to temperature change suggests a new series of experiments where the stimulating agent is salt or fresh water adjusted to various temperatures and passed in to the reaction chamber which is held at a constant temperature. A modification of this experiment would be to vary the temperature of the reaction chamber as well.

Experiments in Fresh Water

Acid stimulation of *Fundulus* as related to temperature was studied by using as the stimulating agent two widely different concentrations of oxalic acid. 0.002N oxalic has a pH of 3.14 and gives a reaction time of the fish of 5.5 seconds at 18°C. 0.0008N oxalic has a pH of 3.82 and gives a reaction time of 11.8 seconds at 18°C. Stimulation by these acids was tested at temperatures ranging from 1–30°C. The reaction time was corrected as before (Sizer, 1934, also Allison and Cole, 1933–34) by a subtraction of 4 seconds, and then log rate of response was plotted against the reciprocal of the absolute temperature. Each point represents the average of sixty readings, ten taken on each of six fish. This averaging is justified due to the small variability of reaction time among the several fish. An analysis of the graphs made with data from individual fish, as well as the graph made from the mass plot of the data from individual fish showed good agreement with the analysis made on the basis of the averages.

Two straight lines intersecting at the critical temperature, 6.5°C., may be drawn through the points plotted for 0.002N oxalic (Fig. 1). The line drawn for the lower temperatures has a slope represented by the μ value, 33,000, the line for the higher temperatures gives a μ = 15,800. At 24°C. and above the reaction time reaches a minimum and constant value. Below 6.5°C. there is an increased scatter of the points indicative of the fact that a different chemical reaction is in control. An increased scatter of points is also noticed at temperatures above 20°C.

A similar relationship is obtained for stimulation by 0.0008N oxalic as related to temperature (Fig. 1). The curves are displaced along the y axis, but the lines drawn through the experimental points are parallel to those for 0.002N oxalic. However, these lines intersect at 10.5°, instead of 6.5°. This indicates that the shift in the controlling chemical reactions which regulate the rate of response occurs at a significantly higher temperature for 0.0008N oxalic as compared with 0.002N oxalic. A possible explanation for this shift in critical temperature with change in concentration is suggested. If a definite energy level must be reached in order to bring about a change in the slowest or controlling reaction regulating rate of response, then this

certain energy level would be reached at a lower temperature for 0.002N oxalic than for 0.0008N oxalic. Thus 0.002N oxalic would have the lower critical temperature. The reaction time for 0.0008N oxalic does not reach a minimum value at 24°C., but continues to decrease

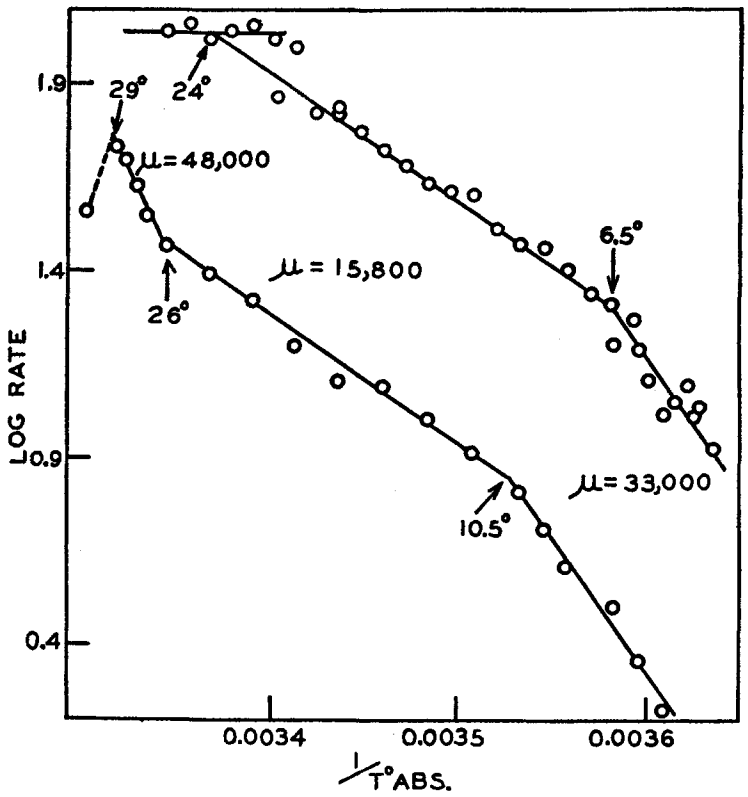

FIG. 1. Log rate of response $\left(\log \frac{100}{R.T.-4}\right)$ of *Fundulus* in fresh water to stimulation by 0.002N oxalic (upper curve) and 0.0008N oxalic (lower curve) plotted against the reciprocal of the absolute temperature. The curves are parallel except at high temperatures but the critical temperatures are different.

with increase in temperature. At 26°C. a new critical temperature is encountered. A line drawn through the points between 26°C. and 29°C. gives a μ = 48,000. Beyond 29°C. toxic effects set in and the reaction time becomes longer again. A similar temperature char-

acteristic for 0.002N oxalic would be expected above 26°C. were it not for the fact that the reaction time has already reached its minimum value at 24°C. Since the thermal increments are the same, it may be stated that the mechanism of reaction does not change when the concentration of oxalic is changed from 0.002 to 0.0008N. Differences existing between stimulation by these two concentrations of oxalic as a function of temperature may be explained on theoretical grounds.

Experiments in Salt Water

Stimulation was measured in salt water at temperatures ranging from 0 to 30°C. At each temperature sixty reactions were taken, twenty on each of three fish and the results were averaged. An analysis of the data for individual fish showed good agreement with the analysis made on the averaged data. Thermal increments determined for the separate fish varied somewhat, but were not significantly different from those determined for the average values.

(*a*) *0.002N Oxalic.*—0.002N oxalic in salt water has a pH of 5.40 and gives a reaction time of 8.7 seconds at 18°C. Stimulation by this acid as a function of temperature is characterized by three different thermal increments (Fig. 2, lower curve, upper curve refers to variability which will be discussed in another section). Over the temperature range of 1–3.3°C. μ = 56,500, from 3.3–15°C. μ = 19,400, and from 15–29°C. μ = 24,100. At 15°C. there is not only a change in increment but a change in rate of response as well. It will be noticed that the points for 20, 22, and 24°C., do not lie along the line as drawn. However, a line drawn through these three points would be parallel to the curve as drawn. It happened that these three points were determined consecutively; the reaction time of the fish had decreased but there was no change in temperature characteristic. This is an example of what Crozier and Stier (1926–27) have called a change in frequency without change in increment. It may be noticed that the μ value is higher for the range from 15–29°C. than it is from 3.3–15°C. This is the reverse of the usual situation for the temperature characteristics at high and low temperatures. Both thermal increments and critical temperatures are different for stimulation by 0.002N oxalic in salt water and 0.002N and 0.0008N oxalic in fresh water. While the mechanisms for stimulation are similar in both environments, as evi-

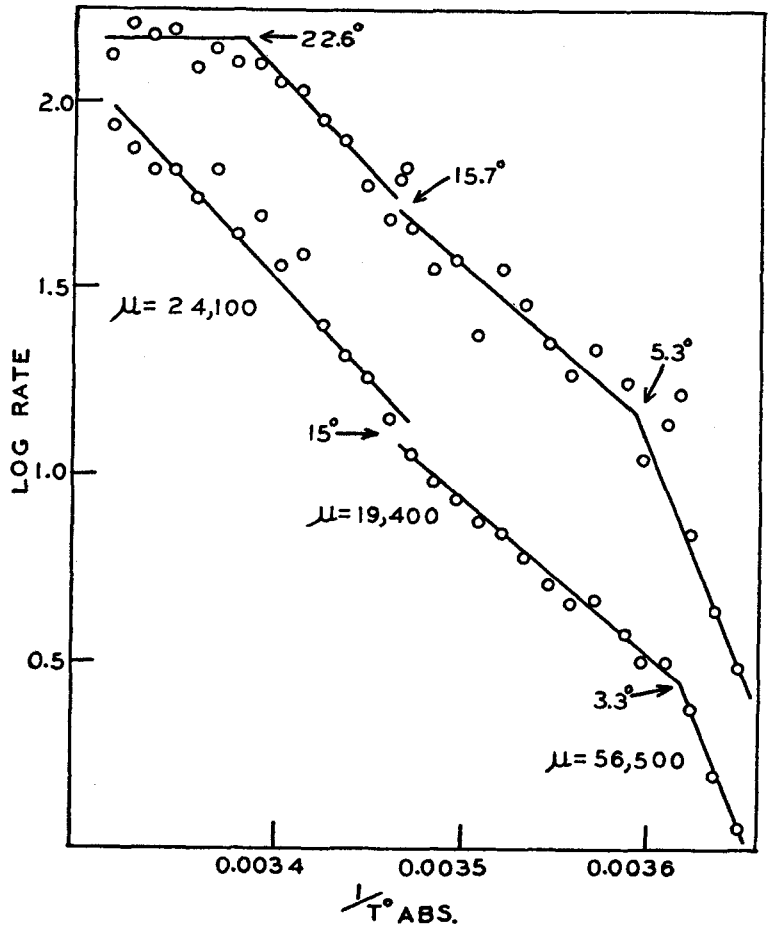

FIG. 2. Upper curve: Ten times the reciprocal of the probable error of the response of *Fundulus* in salt water to stimulation by 0.002N oxalic plotted against the reciprocal of the absolute temperature.

Lower curve: Log rate of response $\left(\log \frac{100}{R.T.-4}\right)$ to the same acid plotted against $\frac{1}{T}$. The two curves are parallel but the critical temperatures differ.

denced by the fact that parabolic equations for stimulation are obtained in both cases, the chemical reactions governing the rate of response are different in the two environments. This difference in

stimulation in salt and fresh water is doubtless correlated with the difference in ionic constitution of the two environments (Allison and Cole, 1933–34). The complex ionic equilibrium at the receptor interface would be altered in quite a different manner by the addition of oxalic to a salt water environment, than it would by adding oxalic to fresh water. Addition of 0.002N oxalic to salt water causes a small amount of calcium oxalate to be precipitated, CO_2 is liberated, but since salt water is highly buffered the (H^+) does not change very greatly. The same acid concentration in fresh water brings about a much greater percentage increase in the ionic concentration of the environment, a much greater increase in (H^+), and a greater acid anion concentration, than it would in salt water.

(*b*) *0.002 and 0.004N Malonic.*—Stimulation by malonic acid in salt water as related to temperature was tested by using 0.004 and 0.002N solutions. 0.004N malonic has a pH of 4.11 and gives a reaction time at 18° of 6.0 seconds. 0.002N malonic has a pH of 5.64 and gives a reaction time of 6.9 seconds at 18°C. It would have been better if a solution more dilute than 0.002N had been used, but practically it is very difficult to measure response to such a weak solution. An analysis of the data shows similar results for the two different concentrations of malonic acid. (See Fig. 3.) In both cases the μ values are 65,000, for the lower range, and 20,600 for the upper range of temperatures. For 0.004N malonic critical temperatures exist at 6.3°C. and 23°C. The reaction time is constant above 23°C. Critical temperatures exist at 6.4°C., and at 25°C. for 0.002N malonic. The reaction time becomes constant above 25°C. For oxalic acid in fresh water and malonic acid in salt water the statement may be made that over the concentration range studied the mechanism of reaction is independent of the concentration for a given acid and a given environment. However, the master chemical reaction, the slowest process in the catenary series of events controlling rate of response, is different for stimulation by oxalic acid in the two different environments. A comparison of the temperature characteristics and critical temperatures for stimulation by oxalic and malonic in salt water shows a distinct difference. This can only indicate that the mechanism of reaction is different for these two members of the dicarboxylic acid series. However, since both acids yield parabolic equations for stimulation it must be assumed

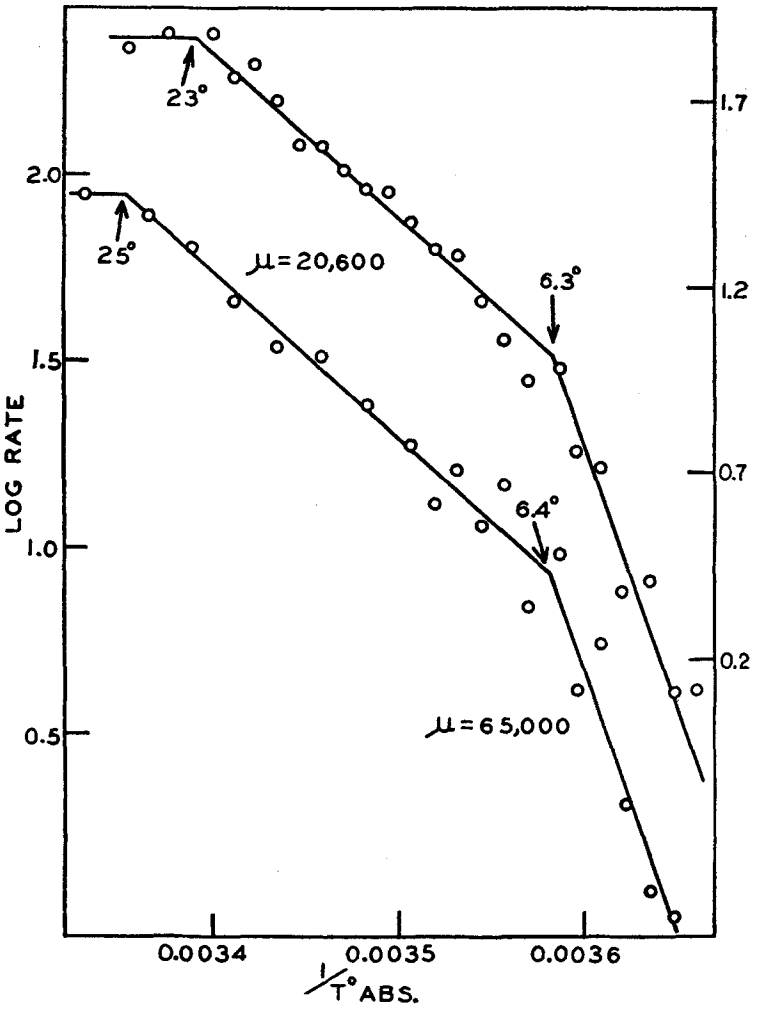

FIG. 3. Log rate of response $\left(\log \frac{100}{R.\ T.-4}\right)$ of *Fundulus* plotted against reciprocal of absolute temperature. Upper curve: Stimulation in salt water by 0.004N malonic. Lower curve: Stimulation in salt water by 0.002N malonic. The two curves are parallel but the critical temperatures differ.

that the fundamental stimulation system is the same for the two acids, and that the difference between the two systems is related to the nature of the chemical reactions which determine the rate of response to acid stimulation.

The critical temperatures for stimulation by 0.004 and 0.002N malonic are practically the same. Moreover their stimulating efficiencies as measured by reaction time are not widely different. If there were a greater difference in the (H^+) of the two concentrations of malonic, a greater difference in critical temperatures might be expected corresponding with the situation for stimulation by two different concentrations of oxalic in fresh water.

DISCUSSION

It was hoped that a study of stimulation as a function of temperature would indicate the nature of the events occurring between the orientation of the acid molecules at the receptor-environment interface and the response of the fish expressed as a change in opercular rate. It has already been shown that stimulation by the dicarboxylic acids and their derivatives is not directly correlated with the surface tension. The fact that it is not a primary factor in stimulation is shown as well by the thermal increments for stimulation by oxalic and malonic acids; they are much higher than those typical of surface tension phenomena. Diffusion of the acid molecules to or through the receptor surface may also be ruled out for the same reason as a primary factor determining rate of response. The adsorption of the acid molecule as a possible preliminary to stimulation was suggested because both rate of response and degree of adsorption are parabolic functions of concentration. While adsorption may be involved, it is not a controlling factor determining rate of response. The μ values are all above 15,000 and clearly show that the factors controlling the rate of response to acid stimulation are not physical but rather chemical in nature.

Several different temperature characteristics have been obtained for stimulation by oxalic in fresh and salt water, and for malonic in salt water. This indicates that stimulation does not depend upon a single sort of process, but rather upon a series of interrelated chemical reactions, each with its own velocity constant. Under varying conditions different chemical reactions may become the slowest or controlling process which determines the rate of response. It might be expected that new μ values would be revealed by testing stimulation by other acids in fresh and salt water as a function of temperature. Higher members of the dicarboxylic acid series give the same parabolic

equation for stimulation (Sizer, 1934). It would be of great interest to know whether or not stimulation by these acids yields identical relationships to temperature.

Little can be said about the actual values of the thermal increments for stimulation by oxalic and malonic acids (see Table I). There are two cases where the μ values are greater for a high range of temperature than they are for a lower range. This is the reverse of the customary situation. The actual temperature characteristics which are found for stimulation by oxalic and malonic acid are not uncommon and are the same as those found for certain other biological processes, indicating similar chemical systems for many biological reactions. It is interesting to observe that all the μ values under 40,000 which are

TABLE I

Temperature Characteristics for Stimulation of Fundulus

μ Value	Acid	Environment	Temperature range
15,800	0.002N; 0.0008N oxalic	Fresh water	6.5–24°; 10.5–26°
19,400	0.002N oxalic	Salt water	3.3–15°
20,600	0.004N; 0.002N malonic	Salt water	6.3–23°, 6.4–25°
24,100	0.002N oxalic	Salt water	15–29°
33,000	0.002N; 0.0008N oxalic	Fresh water	1–6.5°; 1–10.5°
48,000	0.0008N oxalic	Fresh water	26–29°
56,500	0.002N oxalic	Salt water	1–3.3°
65,000	0.004N; 0.002N malonic	Salt water	0–6.3°; 0–6.5°

reported here for stimulation have also been observed for respiratory and oxidative phenomena (Crozier, 1924–25). Thermal increments above 45,000 such as those found for acid stimulation are very rare for completely reversible biological reactions.

The critical temperatures for stimulation by oxalic and malonic are: 3.3, 6.3, 6.4, 6.5, 10.5, 15, 24, 26, and 29°C. These values are not uncommon for biological phenomena.

It has been observed many times that the variability as measured by the relative scatter of plotted points frequently changes at critical temperatures. Little has been done, however, to relate quantitatively such variability of a biological process to the temperature (Crozier, Stier, and Pincus, 1929). It has been shown (Stier, 1932–33; also

Navez, 1930) that the variability of the rate of a biological process bears a constant proportion to that rate. If this is true, then the variability, as measured by the probable error, of the rate of response to acid stimulation should be the same function of temperature as the rate of response itself. An analysis, then, of variability as related to temperature should be an excellent check upon the temperature characteristics and critical temperatures obtained by relating rate of response to temperature. It also follows that the coefficient of variability of the mean, or probable error expressed as per cent of the mean, should not vary with the reaction time; and when plotted against the reciprocal of the absolute temperature a straight line should be obtained having zero slope (Navez, 1930).

Probable errors were calculated for all the data obtained for fresh and salt water stimulation by oxalic and malonic acids.[1] The actual magnitudes of the probable errors cannot be compared for the two environments, since six fish were used in fresh water tests and three in salt water tests. However, we are interested here only in relative values. Instead of plotting probable error against $1/T$, its reciprocal was used, so that the curves obtained might be more easily compared with those for rate of response. A comparison of the probable error curves (Figs. 2, 4, and 5) and those for rate of response (Figs. 1, 2, and 3) reveals an excellent agreement. The points for probable error of 0.002N oxalic in fresh water, however, are scattered, but tend to lie along the line which has the same slope as the curve for rate of response. The critical temperatures are within one or two degrees of those found for rate of response. It becomes apparent that variability of response time is not a haphazard affair but varies with temperature in a manner similar to rate of response itself.

The one striking difference between the series of curves for probable error and those for rate of response is that the variability becomes constant at lower temperatures than does the rate of response. This difference is not apparent for 0.0008N oxalic, for over the temperature range studied neither the rate of response nor probable error reach a constant and limiting value. For the other four acid concentrations the probable error reaches a constant value at a temperature five or

[1] Probable error $= \pm 0.8453 \frac{\Sigma(+V)}{n\sqrt{n-1}}$.

six degrees lower on the average than does rate of response. A comparison of the rate of response and probable error curves for 0.002N oxalic stimulation in fresh water shows that above 20°C. there is an

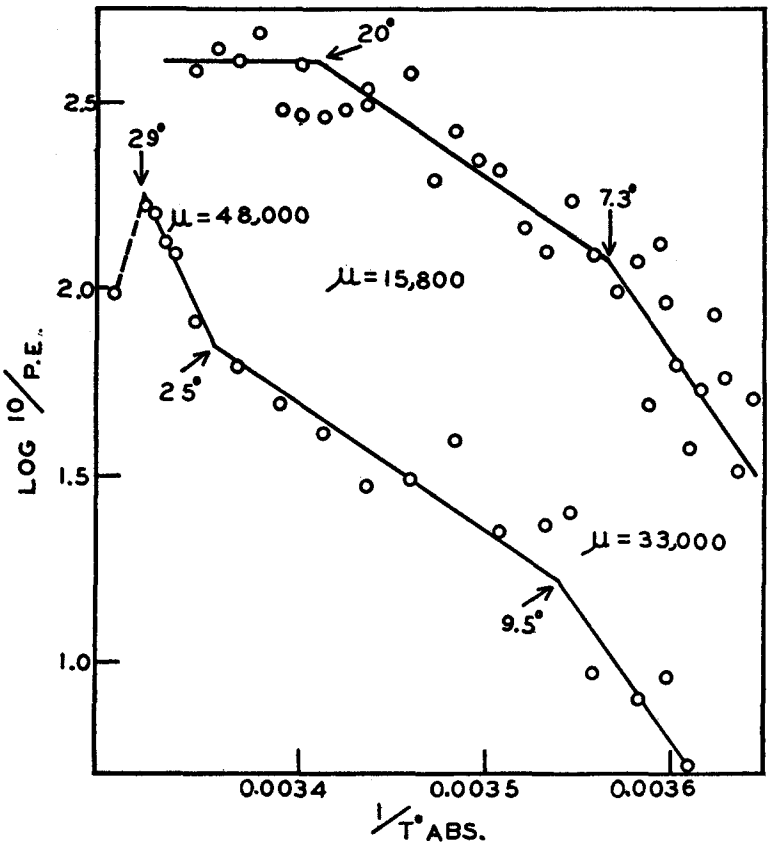

FIG. 4. Log ten times the reciprocal of the probable error of the response of *Fundulus* in fresh water to 0.002N oxalic (upper curve) and 0.0008N oxalic (lower curve) plotted against $\frac{1}{T}$. The curves are parallel except for high temperatures, and have the same slopes as the curves for stimulation by these acids. The critical temperatures are different, however.

increased scatter of points for rate of response. At this same temperature and above the probable error becomes a constant. The reaction time itself, however, does not become a constant until the temperature has reached 24°C.

Crozier has suggested that the variation in a biological process may be due to changes in the effective amounts of catalyst involved in the

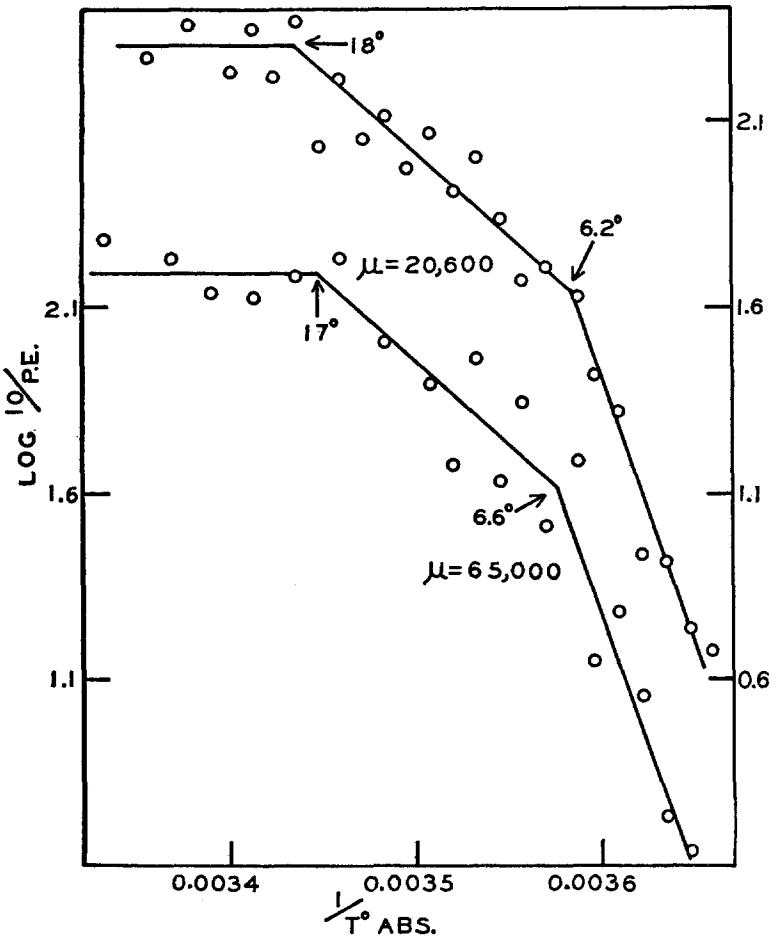

FIG. 5. Log ten times the reciprocal of the probable error of the response of *Fundulus* in sea water to stimulation by 0.004N malonic (upper curve) and 0.002N malonic (lower curve). The curves are parallel and have the same μ values as those for rate of response to these acids. The critical temperatures are different, however.

chemical system determining the rate of that process. If the catalyst concentration concerned in the stimulation mechanism becomes con-

stant at high temperatures, then variability in rate of response will cease to vary as a function of temperature. The rate of response, however, is determined among other things by the magnitude of the initial disturbance in the chemical system as well as by the velocity

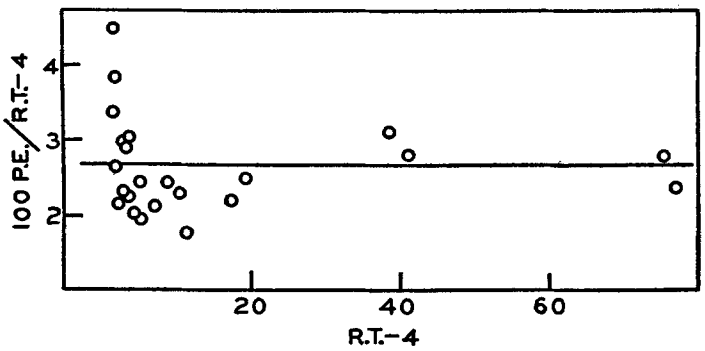

FIG. 6. Per cent probable error of the corrected reaction time for stimulation of *Fundulus* in salt water by 0.004N malonic plotted against the corrected reaction time. The line drawn represents the average probable error of 2.71 per cent.

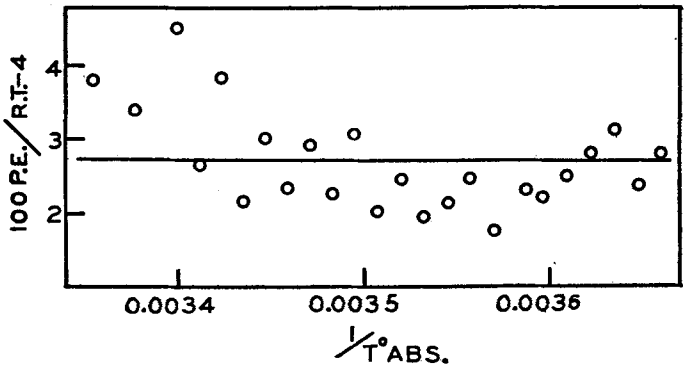

FIG. 7. The same as Fig. 6 plotted against $\frac{1}{T}$. The line drawn represents the average probable error of 2.71 per cent.

of subsequent reactions. This initial disturbance might continue to increase with temperature at temperatures even above those at which catalyst concentration and variability of response have become constant. Due to the physical limitations of the stimulation mechanism

the response time also reaches a limiting value at somewhat higher temperatures.

Since the probable error curve follows that for rate of response the relationship:

$$\frac{100 \text{ P.E.}}{R.T. - 4} = \text{Constant}$$

would be expected to hold. The truth of this statement is clearly shown by plotting coefficient of variation for stimulation by 0.004N malonic in salt water against reaction time (Fig. 6). This coefficient of variability appears to be roughly independent of the temperature as may be seen in Fig. 7. At temperatures above which the probable error has reached a constant and limiting value it ceases to be a constant per cent of the mean reaction time and increases in value. This is indicative of the fact that at these higher temperatures factors are affecting the rate of response of the fish which are not ordinarily related to the stimulation mechanism. This increased variability at high temperatures signifies a change in the mechanism of reaction to stimulation. The average value for the probable error expressed as per cent of the mean reaction time is 2.71 per cent for 0.004N malonic. A similar analysis would yield comparable results for the other acid solutions which have been studied.

The fact that the reciprocal of probable error is the same function of temperature as rate of response, and that the coefficient of variability does not vary either with reaction time or with temperature, should be interpreted as indicating that variation itself is not a variable changing independently with temperature. Variation in response as measured by probable error is a direct function of reaction time and is determined by the same catenary series of events which determine rate of response to stimulation.

Breathing Rhythm of Fundulus As Related to Temperature

In connection with the study of chemical stimulation as related to temperature it was thought necessary to make a simultaneous study of the rate of opercular breathing movements as related to temperature. Cessation or change in rate of opercular movement is taken as

a criterion of response to stimulation by acids. Rate of response, as related to concentration of the acids is thus intimately associated with opercular rate, and the possibility existed that both rates were similarly affected by temperature changes. However, experiments designed to test that possibility have revealed that the temperature characteristics and the critical temperatures for the two processes are distinctly different. It was therefore concluded that the catenary reactions leading to chemical stimulation are independent of those processes governing breathing rhythm.

A study of the opercular movements of *Fundulus* as a function of temperature has brought out some significant facts which deserve consideration. The experimental set-up for studying opercular rate is the same as that for chemical stimulation. At least an hour's adaptation time in the experimental dish passed before the time for ten gill movements of the fish was measured with a stop-watch. At 0° and 1°C. opercular movements practically cease, and no great reliance can be placed on data obtained at these temperatures. For the salt water tests one reading was taken on each of three fish and the average of the three used. Six fish were used in fresh water and an average taken. Such averaging is justified for data from individual fish treated separately gave essentially the same results as the averages from the several fish. An analysis of the data for the salt water tests showed that when log opercular rate was plotted against the reciprocal of the absolute temperature a linear band of points was obtained (Fig. 8). There is no apparent break in this relationship between the limits of temperature used. Although it does not show clearly from the plot, the opercular rate reaches a maximum and constant value at 25°C. and above. The value of μ obtained from the slope of the parallel lines bounding the band of plotted points was found to be 8,400. There is a striking agreement between this value of μ and the value found by other investigators for similar respiratory movements.

The μ value has been thought to represent the "energy of activation" of the catalyst for the slowest process in the series of catenary events controlling the velocity of the phenomenon being studied (Crozier, 1924–25). Of course, with change of temperature or some other factor, a different process in the series may become the slowest, and μ would correspondingly change to a value typical of the catalyst for this new

reaction. Certain processes such as O_2 consumption, and CO_2 production are common to all protoplasm, and it might be expected that such reactions might have similar catalysts, and hence similar values. Such is the case, for certain phenomena dependent on cellular oxidation have typical oxidation temperature characteristics. Perhaps the

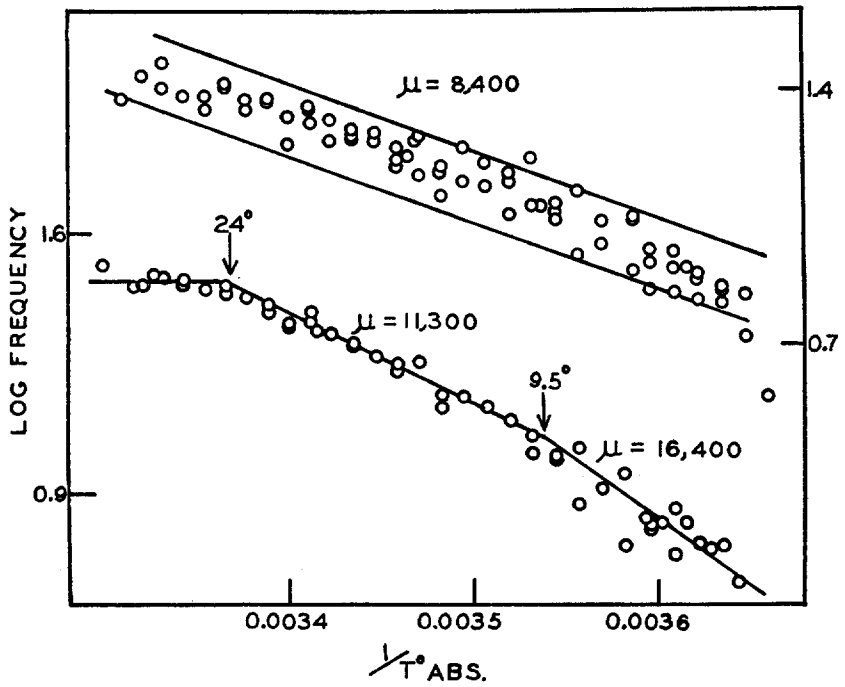

FIG. 8. Log frequency (ten times the reciprocal of the time for ten movements) of rhythmic opercular movements of *Fundulus* plotted against $\frac{1}{T}$. Upper curve: Breathing rhythm in salt water. Lower curve: Breathing rhythm in fresh water.

most common values which have been found for respiratory oxidations are 8,000, 11,000, and 16,000.

Crozier and Stier (1924–25 *b*) suggested that the value 8,000 may reflect the velocity of synaptic processes concerned in respiratory movements of fishes and other vertebrates. A value of around 8,000 has been reported several times for the rate of respiratory movements.

Crozier and Stier (1924–25 *a*) reported the value of 8,600 for pharyngeal breathing in the frog. A few years later Cole and Allison (1929) confirmed that value for the frog. 8,600 was also reported for gill contractions in larval *Amblystoma* (Crozier and Stier, 1926–27). Navez (1930) found the value 8,200 for breathing rhythm of dogfish. By subjecting goldfish to a temperature of 25°C. for 3 hours Crozier and Stier (1925–26) obtain the value 8,300 for gill rate instead of the usual 16,500. Various examples from the invertebrates also might be cited where a μ of about 8,000 is obtained for respiration.

The relationship between *Fundulus* opercular rate and temperature is distinctly different in fresh water from that in salt (Fig. 8). Below 9.5°C. the temperature characteristic is 16,400; above 9.5°C. it is 11,300; at 24°C. the gill rate becomes constant. A further indication that the two μ values, 11,000 and 16,000, are markedly distinct is the fact that the variability of gill rate for fresh water is very much greater below 9.5°C., than it is above this temperature. Apparently a change in environment from salt to fresh water has so altered the catenary series of events that the slowest process in the chain is no longer the reaction whose catalyst has a thermal increment of 8,400. The values obtained for *Fundulus* breathing rhythm in fresh water, 11,300 and 16,400, are both characteristic of respiratory biological oxidations. It is evident that the temperature characteristics for *Fundulus* opercular rhythm may be experimentally altered by changing the environment of the fish from salt to fresh water.

A few examples will be cited to show that the μ values, 11,000 and 16,000, are indicative of oxidative and respiratory phenomena. A value of roughly 11,000 has been found for respiration in *Sepia*, oxygen consumption in *Lupinus* (Tang 1931–32), oxidation in *Arbacia* eggs (Rubenstein and Gerard, 1933–34), and for pulsation frequency in "accessory hearts" of *Notonecta* (Crozier and Stier, 1926–27). The value of 11,500 has been associated with catalysis by the hydroxyl ion (Crozier, 1924–25). The value of roughly 16,000 has been found for CO_2 production by *Lupinus*, *Phaseolus* (Crozier and Navez, 1930–31), *Pisum*, and *Vicia faba* (Tang, 1931–32); for pulsation frequency in accessory hearts of *Notonecta;* for CO_2 production by nerve ganglia; for the reduction of methylene blue by bacteria; for the deoxygenation

of oxyhemoglobin by carbon monoxide; and for respiration in the dogfish (Navez, 1930). The value of roughly 16,000 is often definitely associated with iron catalysis (Crozier, 1924–25).

It is evident, therefore, that the temperature characteristics 8,000, 11,000, and 16,000 are definitely associated with biological oxidations, or with reactions which are limited by the velocity of cellular oxidations. These three values represent distinct reactions in the oxidative metabolism of protoplasm. However, these may be catenary reactions (Crozier and Stier, 1924–25 *b*) and it might be predicted that under altered experimental conditions the organism would exhibit a corresponding change in thermal increment.

Examples of such experimental modification of temperature characteristics have been furnished chiefly by the work of Crozier and Stier (1924–25 *b*). The temperature characteristic for pharyngeal breathing movements of the frog is 8,600. 8 days after decerebration the μ value was definitely altered to 11,000. In grasshoppers decapitation causes μ to change from 7,900 to 16,200 and 11,200. The typical value for goldfish opercular breathing rhythm is 16,500 (Crozier and Stier, 1924–25 *b*). Subjection of the fish to 3 hours at 25°C. causes this value to change to 8,300. The temperature characteristic of the heart rate of *Limax* changes from 16,300 to 11,500 according to the season of the year. A μ value of 11,500 for *Limax* heart rate was changed to 16,200 by feeding the slug sugar. After 4 days the temperature characteristic reverts to its original value; therefore the effect is reversible.

It is not surprising, therefore, that the thermal increment for opercular breathing rhythm of *Fundulus* changes when the environment is altered from salt to fresh water. The μ value changes from 8,400 over the whole temperature range in salt water to 16,400 below 9.5°C., and to 11,300 above this temperature in fresh water.

SUMMARY

1. Chemical stimulation as a function of temperature was studied by using oxalic acid in fresh and salt water and malonic acid in salt water as stimulating agents on *Fundulus*. According to the Arrhenius equation the following μ values were obtained for the various acid solutions between 0 and 29°C.: for 0.002N oxalic in fresh water—

15,800; 33,000; for 0.0008N oxalic in fresh water—15,800; 33,000; 48,000; for 0.002N oxalic in salt water—19,400; 24,100; 56,500; for 0.004N and 0.002N malonic in salt water—20,600; 65,000. At a critical temperature there is a sharp transition from one thermal increment to another.

2. The chemical processes controlling stimulation do not change with concentration, for different normalities of a single acid yield the same μ values. Distinctly different temperature characteristics were obtained for stimulation by oxalic in salt and fresh water. Likewise stimulation by oxalic and malonic in salt water yielded very different increments. This temperature study indicates that the controlling chemical reactions determining rate of response are different for the same acid in two different environments, or for two dibasic acids in the same environment. Other work indicates, however, that the fundamental stimulation system is the same for all the acids in both environments. Chemical rather than physical processes limit the rate of response since all the values are above 15,000. Stimulation depends upon a series of interrelated chemical reactions, each with its own temperature characteristic. Under varying conditions (*e.g.* change of temperature, environment, or acid) different chemical reactions may become the slowest or controlling process which determines the rate of response.

3. The variation of response, as measured by the probable error of the mean response time of the fish, is the same function of temperature as reaction time itself. Hence variability is not independent of reaction time and is controlled by the same catenary series of events which determine rate of response to stimulation.

4. Breathing rhythm of *Fundulus* as related to temperature was studied in both salt and fresh water. In salt water the temperature characteristic is 8,400 while in fresh water it is 16,400 below 9.5°C., and 11,300 above this critical temperature. These μ values are typical of those which have been reported by other workers for respiratory and oxidative biological phenomena. A change in thermal increment with an alteration in environment indicates that different chemical reactions with characteristic velocity constants are controlling the breathing rhythm in salt and fresh water.

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