TEMPERATURE AND FORWARD MOVEMENT OF PARAMECIUM.*

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I.

Paramecium is extremely sensitive to slight changes in its environment and in consequence has been used, since the days of Spallanzani, as a delicate biological indicator. Yet, despite the three thousand or more papers dealing specifically with this organism, quantitative studies of its relations to the environment have been exceedingly rare and very limited in scope. So far as concerns temperature, for example, we find Pütter (1903),¹ in a curious state of confusion, denying that the production of mechanical energy by this animal is in any way proportional to an increase in heat intensity. Since then, Kanitz (1915)² has found in Khainsky's (1911)³ results on *Paramecium caudatum*, support for his own earlier and even more irregular experiments with the contractile vacuoles of other Infusoria, while Woodruff and Baitsell (1911-12) determined $Q_{10} = 2.84$ or 2.70 , for the fission rate over an interval of only 4° . With respect to temperature, certainly, the advantages of sensitivity in Paramecium remain largely unexplored.

The analysis here proposed deals with the effect of temperature on forward motion. This is perhaps the most common act which the animal performs and is, therefore, at all times available for experiment. The purpose of the study does not include an attempt to illuminate the mechanical arrangements that propel the organism;

* Owing to Dr. Glaser's absence in Europe, proof of this paper has been read by the Editors.

¹ Pütter (1903), p. 88.

² Kanitz (1915), p. 62.

³ Khainsky (1911), p. 32.

on the other hand it does involve the employment of temperature effects to detect the basic chemical reactions that supply the energy required for an execution of the ciliary beat.

The movements with which we are concerned result from a rotation dependent on the oblique direction of the ciliary stroke. These cilia are the only machinery of propulsion and a change in activity on their part means a corresponding change in the rate of progress. Under ordinary conditions, therefore, the speed with which the animal spins on its major axis and bores its way through the water may be considered proportional to the rate at which the cilia move. Since, however, we are interested merely in the forward resultant, only the rate of progress of the entire animal was measured; this was done by observing the time required by the organisms to travel a given distance at different temperatures.

II.

In order to minimize the effect of disturbing influences only short distances were considered. Moreover, the organisms were confined in a capillary channel—the readings being made in a locality where the diameter remained constant at 1.5 mm. over a distance of at least 1 cm. The full sized ends of this tube, 10 cm. apart, were bent up at right angles and were clamped in the metal holder provided with two thermometers held parallel with the horizontal runway. If all the adjustments are correct and there are no interfering factors outside, the rate at which the animals move forward in the capillary is entirely consistent and not influenced by direction.

For each set of experiments such a tube was loaded with culture medium—an aerated, filtered extract from 20 gm. of sterile timothy hay which had been boiled for 30 minutes in 500 cc. of water distilled from glass, and to which, after correcting for evaporation, there was added 50 cc. of a Ringer's solution containing, per liter, 6.5 gm. NaCl, 0.14 gm. HCl, and 0.12 gm. CaCl_2 . The *Paramecia*—all from a single pure line—were then introduced together with enough medium to raise the level in the uprights to a fixed mark. Finally the entire system, with its two concordant thermometers pointing in opposite directions, was submerged to the level of the liquid within the speed-trap, in a water bath provided with a stirrer and controlled by means of a micro burner and, on occasion, by ice.

With equilibrium established after a few moments, the readings were taken with the aid of a black staircase ocular micrometer as the organisms passed through the field of a binocular microscope. For each *Paramecium* the time consumed in covering a certain scale distance was determined by means of a stop-watch. The records of all the animals at a given temperature were then reduced to a common basis, namely the time in seconds required to cover one unit of the scale at a temperature constant within 0.1°C . as given by the average reading of the two thermometers. With a horizontal magnification of $\times 20$, the value of one scale unit in absolute terms was very nearly 500 microns. Every record is the average of not less than six such performances, in each of which the average distance moved by the organisms was six scale units.⁴ Perhaps a higher degree of accuracy could have been achieved if the records at all temperatures had been made with a single individual. Certainly the original itemized lists contain variations. At times one finds a "racer" or an individual unusually slow. The effects of deviations of this sort are, however, swamped completely in averaging a large number of observations. This was definitely the outcome of a preliminary statistical analysis yielding the modal time at several temperatures. When it became apparent that such modal points at most differ only in the second decimal from the crude averages, there seemed to be no reason for not employing the latter immediately as data. These are given in Table I and graphically in Fig. 1. They demonstrate not only the ineptitude of Pütter's (1903) remark but also that the process under consideration, like other biological actions, varies as an exponential function of the temperature.

III.

The analysis of this material assumes that forward motion depends on energy derived from a series of chemical reactions, and that to cover a given distance always requires the same amount

⁴ The number depended on the length of time during which the temperature remained constant. Many more observations were made at 30° because the first results at this temperature were quite discordant. The difficulty was traced to two sources—a contaminated pipette and a slight difference in the composition of the medium.

of energy. A series of underlying reactions of this sort necessarily has a definite sequence, and the most elementary consideration suffices to show that the rate at which a system of linked processes

TABLE I.
Average Time Required by Paramecium to Swim a Unit Distance at Different Temperatures.

Temperature. °C.	No. of observations.	Time.		
		Observed, average.	Calculated. $\mu = 16,000$	Calculated. $\mu = 8,000$
		sec.	sec.	sec.
6.6	7	2.64	2.65	
7.0	7	2.58	2.60	
9.9	6	1.98	1.98	
10.7	18	1.89	1.81	
10.8	10	1.80	1.80	
14.8	10	1.22	1.22	1.19
15.0	10	1.17	1.18	1.18
16.0	26	1.10	1.08	1.12
17.5	50	1.04	0.94	1.04
18.0	10	0.91	0.90	1.01
18.5	24	0.89	0.86	0.99
18.7	10	1.09	0.84	0.98
19.0	40	0.95		0.97
19.4	20	0.91		0.95
21.2	10	0.81		0.87
21.5	15	0.71		0.85
22.0	36	0.83		0.83
23.0	32	0.80		0.80
25.0	10	0.74		0.73
25.4	11	0.73		0.72
25.5	15	0.77		0.72
27.0	14	0.65		0.67
27.3	12	0.63		0.65
29.0	40	0.69		0.62
30.0	100	0.56		0.59
39.0	30	0.43		0.41
40.0	7	0.40		0.39

can proceed as a unit depends absolutely on the component with the slowest rate. In its relations to temperature, therefore, forward translation should reflect the corresponding properties characteristic of the controlling reaction. Accordingly, the experimental

points should be calculable by means of the Arrhenius⁵ (1915) formula of 1889 which describes the changes in the rate of acceleration of a chemical process at different temperatures and which has been employed with such success by Hecht (1919, 1918-19) in his studies on the influence of temperature on photic sensitivity in *Mya*.

FIG. 1. The Arrhenius equation applied to the relation between temperature and time in the forward translation of *Paramecium*. For the left-hand curve, $\mu = 16,000$; for the right-hand, $\mu = 8,000$.

In the Arrhenius expression, the ratio between the velocity constants K_1 and K_0 of a chemical reaction taking place first at one, then at another temperature is related logarithmically on the Napierian base e to the gas constant, the absolute temperatures, and to a constant μ which characterizes a particular reaction. As formulated,

$$\frac{K_1}{K_0} = e^{\frac{\mu}{R} \left(\frac{1}{T_1} - \frac{1}{T_0} \right)} \quad (1)$$

⁵ Arrhenius (1915), p. 49.

From the values in Table I velocity constants cannot be calculated. However, for K_1 and K_o respectively, we can substitute the reciprocals of the times, $\frac{1}{t_1}$ and $\frac{1}{t_o}$, required to perform the same amount of work. The temperature characteristic, μ , can then be easily determined by transformation and a convenient change from the Napierian to the Briggsian base.

Calculated in this manner for the entire range from about 6–40°, μ , far from being constant, shows a marked decline. This, however, is not proof of a theoretical misconception at the outset, for as Crozier and Pilz (1923–24)⁶ have pointed out, over a temperature range of this extent the effect is not necessarily described by a single curve, even if smooth. On the contrary two curves, each with its own characteristic value for μ , may be required for adequate description. In the present case, and within the limits of experimental error, a curve with $\mu = 16,000$ fits closely from 6.6° to about 16°, whereas a second, with $\mu = 8,000$ simulates the data remarkably well from about 15–40°. How closely within their respective ranges the values given by the theoretical curves coincide with those experimentally found, is shown strikingly in the plot and also in the last three columns of the table.

IV.

The divergence of the two theoretical curves between 15° and 19° is significant. Within this range of temperature several of the points might be attached perhaps equally well to either curve; nevertheless, in this region originates a change of direction which above 20° is unmistakable. What does this mean? On the principle of a controlling rate which we have assumed as the basis for our analysis, the problem finds a simple solution. The speed of the entire system of underlying reactions is increased by a rise in temperature, yet it does not follow that all components of the system have the same acceleration. A specific reaction—the slowest one prior to a certain change in temperature—may be replaced by another which even if originally faster, nevertheless with rising temperature drops into the last place.

⁶ Crozier and Pilz (1923–24), p. 720.

The idea involved here is not new. As Cohen-Stuart (1912)⁷ found it necessary to emphasize, van't Hoff himself expressly stated that in chemical systems Q_{10} remains constant only at certain temperatures. However, the existence and possible significance of a definite break in biological data, particularly at points where the processes under consideration begin to occur at supernormal temperatures, were clearly brought to light only recently by Crozier and Federighi (1923-24) (see also Crozier and Pilz, 1923-24⁶). In certain arthropods, the process underlying the state of reflex immobility (so-called "death-feigning") at low temperatures has a μ value of 24,000, while from 16-30°, $\mu = 9,200$. For other processes (Crozier, 1924-25, *a*, *b*, and *c*) corresponding extremes are 16,400 and 11,200. Hecht (1918-19) reported for the latent period of *Mya*, in the lower ranges, a value of 19,000, and slightly over 11,000 at 31°. With these instances forward translation in *Paramecium* aligns itself easily, for aside from the fact that in *Mya* μ declines gradually, all these cases have all these points in common: high values of μ go with low temperatures and *vice versa*; the high values fall within one general order of magnitude, and the low ones within another; and when breaks occur, these are found abruptly where the temperatures exceed those at which the animals or tissues normally operate.

V.

For our analysis the physical meaning of μ has important consequences. Historically this constant traces back to 1872 when Guldberg (Arrhenius (1912)⁸) found the rate of change in the logarithm of the dissociation pressure directly proportional to the heat of dissociation, and inversely, to the gas constant and to the square of the absolute temperature. The expression of this was applied by van't Hoff in 1885 to chemical velocities, and in 1889 appeared the Arrhenius simplification. The descent of μ in the theoretical line, then, is perfectly clear.⁹ In its latest guise it bears the original rela-

⁷ Cohen-Stuart (1912), p. 1162.

⁸ Arrhenius (1912), p. 83.

⁹ In his reference to the empirical equation of Arrhenius, Rice (1923, p. 2808) does not distinguish sufficiently between a formula which is truly empirical and one whose use, even if discovered by arbitrary methods, nevertheless depends on constants thoroughly grounded in physical theory.

tions to the absolute temperature and the gas constant. As used by Arrhenius, therefore, μ fundamentally stands for, and in the most favorable data is proportional to, and perhaps identical with, a heat of dissociation or reaction.

Quite recently, F. O. Rice (1923) has brought forward considerations which if fully substantiated must greatly increase the utility of μ . Arrhenius explained the effect of temperature on the hydrolysis of cane-sugar by HCl in watery solution by postulating that the sugar was present in two forms, the active and the inactive, in mass action equilibrium, and that at equilibrium the concentration of the active form was very low. Rice assumes that in acid catalysis the H ion rather than the sugar exists in two forms, the hydrated ion and the unhydrated, and that the latter is the active form and is present in low concentration because of the great affinity between the H ion and water. An exactly comparable assumption is applied also to hydroxyl ion catalysis. In this way it appears possible to explain why μ has practically identical values for so many reactions superficially different. Far from being a meaningless accident, the fact becomes rationally clear if we follow Rice's suggestion and focus our attention on the chemical mechanism by which transformations are brought about rather than as heretofore on the primary substances transformed (Crozier, 1924-25, *a, c*). If this point of view is correct, chemical reactions must group themselves into a relatively small number of classes, and each class must possess a characteristic μ , because in each class of reactions the mechanism involved is the same.

In his analysis of the data on the mammalian auricle, echinoderm eggs, the breathing rhythm of insects, the reduction of hemoglobin, and respiration in nervous tissue and in bacterial processes where the velocities measured are probably those of oxidation, or at least, are functions of respiration, Crozier (1924-25, *a* and *b*; Crozier and Federighi, 1923-24) has found consistently values in the neighborhood of 16,000. The quite independent discovery of $\mu = 16,000$ for forward translation in *Paramecium* suggests, therefore, an oxidation as the controlling reaction for the lower ranges.

In the present state of our knowledge an attempt at the class identification of the reaction whose rate of acceleration is measured by $\mu = 8,000$ is perhaps even more hazardous. In order of magni-

tude, this value might not fall outside the class of hydrolyses. It is true that Rice (1923) finds 10,950 from Reicher's data on the hydrolysis of ethyl acetate by sodium hydroxide and from Wilsdon's studies on the hydration rates of acetic, propionic, and camphoric anhydrides, an average of $\mu = 11,100$. On the other hand, in a list of μ values for a variety of processes, Arrhenius¹⁰ includes 7,540 for the saponification of cottonseed oil by the lipase of castor beans, while for the tryptic cleavage of casein, $\mu = 7,400$. Until we have much more definite information in this field, commitments regarding specific reactions are hardly warranted.

VI.

In conclusion we may ask whether it is possible to conceive a chemical reaction system capable of accounting for the distribution of the data and for the apparent transfer of velocity control from one process to another in different temperature ranges.

The observations themselves and their analysis do not limit us particularly to any specific mechanism. The simplest conception applicable to the controlling reactions is a catenary series $O \rightarrow A \rightarrow E$ in which an original source of supply O is changed into the available form A , whose destruction to the end-products E , yields the energy resulting in translation. If $O \rightarrow A$ has a μ value of 8,000 while for $A \rightarrow E$, $\mu = 16,000$, the depletion of A at higher temperatures would proceed at a faster rate than its replenishment, and hence the increase in the rate of the process as a whole would become less as time went on.

As an explanation, this scheme cannot be supported by the sort of evidence that would rule out every other possibility. We may in fact be dealing, at the higher temperatures, with the increasing effect of some process that interferes with the flow of $A \rightarrow E$ by diverting a fraction of A . Indeed, the data apparently will bear an interpretation similar to the one which led Hecht (1918-19) to postulate a heat inactivation in connection with the photic response of *Mya*. Such a mechanism, however, is not so simple, and the data for *Paramecium* do not enable us to decide between these two possibilities.

¹⁰ Arrhenius (1915), p. 54.

While the less complicated idea accounts for the facts and hence is preferable as a first approximation, the mere dealing with velocity controls only, lends a deceptive simplicity to the suggested mechanism. As a matter of fact, if we consider A in the catenary series a reservoir in the main supplied from O , and consider E as partly oxidized and partly reversible to O , Meyerhof's conception of carbohydrate metabolism in muscle becomes quite applicable (Shaffer, 1923). *Paramecium* undoubtedly also oxidizes carbohydrates, and unless the metabolism is radically different, the process runs in a cycle and is composed of two main steps. In muscle, according to Meyerhof, glycogen is hydrolyzed to glucose and lactic acid during the anoxidative phase, whereas the oxidative results in the oxidation of both these products as well as in the reconversion of three-quarters of the lactic acid to glycogen. If we attach the lower μ value (8,000) to the synthesis of glycogen from lactic acid—an assignment reasonable in view of the large quantities of water produced during this phase—Meyerhof's conception should give a fairly well substantiated reaction system capable of the velocity controls demanded for the present case in *Paramecium*. Moreover, Meyerhof's circular scheme accounts well not only for the transfer of control but allows for the fact that the data can be interpreted as involving at the higher ranges a deficit of A . If we assume for normal temperatures a quantitative adjustment such that the prevailing rate $O \rightarrow A$ keeps the concentration of A constant at the prevailing rate of oxidation and of the reaction $A \rightarrow E$, and then assign $\mu = 16,000$ to the oxidative phase, temperatures lower than normal would result in no change of control, but possibly in an accumulation of A . At higher ranges on the contrary, owing to the depletion of A , the rate of the system as a whole would come to depend more and more on the synthetic transformation of E to O , the forerunner of A , and thus, concretely, if *Paramecium* were a muscle, on the conversion of the lactic acid back to glycogen. Obviously such details must await further investigation. The available data merely bring the analysis of forward translation of *Paramecium* into the sphere of physical-chemical methods and conceptions.

SUMMARY.

1. The rate of forward movement in *Paramecium* as affected by changes in temperature can be described accurately in terms of the Arrhenius equation.

$$\frac{K_1}{K_0} = e^{\frac{\mu}{2} \left(\frac{T_1 - T_0}{T_1 T_0} \right)} \quad (1)$$

2. For the range from 6–15°, $\mu = 16,000$; from 16–40°, $\mu = 8,000$. These values fall within the limits characteristic for chemical processes.

3. On the principle of velocity control by the slowest rate, it is assumed that in *Paramecium* at temperatures above normal, control passes from one underlying reaction to another.

4. The views expressed by Rice, the recent results of Crozier, and certain μ values given by Arrhenius all suggest that $\mu = 16,000$ may represent an oxidation, and $\mu = 8,000$ either a modified oxidation or an hydrolysis.

5. For the system of controls, the catenary series $O \rightarrow A \rightarrow E$ with the lower μ value attached to the precursor reaction is adequate. We may also assume a cyclical system analogous to Meyerhof's conception of carbohydrate metabolism in muscle. In this case it is necessary to assign $\mu = 16,000$ to the oxidation of A and E and $\mu = 8,000$ to the synthesis $E \rightarrow O$. This model also accounts for the fact that the data might be interpreted as involving, apparently, a depletion of A at the higher temperature.

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