TEMPERATURE CHARACTERISTICS FOR SPEED OF MOVEMENT OF THIOBACTERIA.*

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I.

The gliding movements of certain *Cyanophyceæ*, *Thiobacteriales*, and gregarines provide relatively uncomplicated types of comparable activities suitable for quantitative observation. In spite of much discussion, little is known as to the mechanism of this sort of progression. Interpretations have been advanced by a number of writers,¹ but data required for formulation of a theory of this sort of movement have been lacking. For the present, we are not concerned so much with the mechanics of the movements of *Oscillatoria*, *Beggiatoa*, and other forms creeping in ways apparently similar, but in employing the rate of this type of movement as an index of metabolic changes.

For one kind of *Oscillatoria* it has been shown (Crozier and Federighi, 1924-25) that the rate of translatory movement, in this instance uncomplicated by rotation, obeys the Arrhenius equation for change with temperature. The rates of movement at different temperatures permit evaluation of the constant E , or μ , in the equation

$$\text{Velocity} \propto e^{-\frac{\mu}{RT}},$$

where e is the Napierian base, R the gas constant, and T the absolute temperature. The value of μ obtained in the experiments cited was 9240.

In these observations the light intensity was practically constant.

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¹ For recent views consult Fechner (1915); Schmid (1918, 1923); Prell (1923, *a* and *b*); Krenner (1925).

To discover the way in which the magnitude of E might be dependent upon light intensity, it was desired to know, among other things, the relations between speed of movement and temperature in a form free from effects due to photosynthetic activity. For this reason, in part, we turned to the leuco-thiobacteria. It is found that the relation of motility to temperature in *Beggiatoa* and *Thiothrix*, as in *Oscillatoria*, points clearly to the controlling influence of chemical processes. The values of the critical increments (E , or μ) in fact agree sufficiently with those repeatedly obtained for other activities of various organisms, and specifically for catalyzed respiratory oxidations (*cf.* Crozier, 1924-25). The value of E derived for speed of movement in *Beggiatoa* thus emphasizes the fact that the magnitude obtained with *Oscillatoria* (Crozier and Federighi, 1924-25) does not accord with any commonly encountered (*Crozier, 1925-26, b*) in connection with biological processes adequately studied. The investigation of the relation between E and light intensity is therefore expected to yield interesting suggestions as to the significance of the critical increment for movement in the case of *Oscillatoria*. This will be discussed in a subsequent paper. In the meantime, it may be pointed out that the movement of *Beggiatoa* appears to be governed by chemical processes similar to those revealed in a number of other vital activities; the details of the relationship between speed of translatory movement and temperature show certain features of general interest for this method of analysis.

II.

The organisms employed for the measurements were kept in shallow culture dishes containing the usual variety of forms occurring in brackish water putrefactive sulfureta (*cf.* Bavendamm, 1924; Baas-Becking, 1925). Two species, identified as corresponding to *Beggiatoa alba* and to a species of *Thiothrix* (*T. tenuis* ?), were taken for study from particular spots in one culture. Thin smears were mounted between two cover glasses, the lower one small enough to be placed within the glass ring of a van Tieghem cell. The cell was sealed with paraffin or chicle, and had in it a small volume of the culture liquid. The sealing was necessary to prevent dilution when the preparation was submerged in a thermostat; access of tap water

caused cessation of progression movements. The cell was mounted in a mechanical stage on the platform of a microscope so adjusted as to have the preparation submerged to a depth of 10 cm. in a water thermostat. The mechanical stage and the fine adjustment of the microscope were controlled by suitable attachments projecting above the water level. With good stirring no difficulty was experienced in maintaining desired temperatures. Light from a housed tungsten bulb was reflected from a mirror beneath the microscope. Variations in light intensity were apparently without effect on the movements of the sulfur bacteria, but for practically all of the measurements the light was of approximately 30 m. c. intensity. Within periods of 6 hours or longer, even up to 24 hours, no progressive changes in speed of movement were detected. Hence the sealed atmosphere in the observation cell produced no special effect.

The measurements were made of the longitudinal progression of straight filaments, and so far as could be determined in the absence of mechanical impedance. With a 5 mm. objective and $7.5\times$ ocular, ten divisions of the ocular micrometer used corresponded to 0.05 mm. The time required for each filament to traverse this distance was taken with a stop-watch, a number of readings being secured at each temperature. With each preparation used, precautions were taken, through time records and by reversing the sequence of temperature changes, to insure the absence of irreversible thermal effects.

III.

According to the current understanding of the mechanism of movement in *Oscillatoria* the longitudinal membrane (Hinze, 1902) of a filament is pierced by pores, through which a carbohydrate mucus is extruded (Fechner, 1915; Schmid, 1918, 1923; Prell, 1921, *a*; Krenner, 1925; Ruhland and Hoffmann, 1925). This would account for the phenomena which gave rise to the older conception of "extracellular protoplasmic streaming." Another view regards the movement as due to "modifications of surface tension," perhaps caused by osmotic processes (Coupin, 1923); though suggestive, no particularly relevant evidence supports this idea. Krenner (1925) found the speed of translatory movement of *Oscillatoria* to vary inversely with the diameter of the species, and that the osmotic pressure of the proto-

plasts is higher in the narrow forms (measured by plasmolytic shrinkage method). Krenner therefore supposes that the specific speed of motion is determined by the turgor. For *Oscillatoria* and its relatives it is known that in general the stouter forms are the more slowly moving. On this basis, one might rather expect the specific speed to be determined by some relationship of surface to bulk. But among the sulfur bacteria we find that with forms occurring side by side in the same culture, the larger species move more quickly,—for two forms, in about the ratio of 1 to 1.5, at the same temperature, when the filament diameters are in the ratio 3.22:1.

We are by no means clear as to the meaning of the optical evidence for “extracellular protoplasmic streaming” (*cf.* also Crozier and Federighi, 1924–25; and Krenner, 1925), nor as to the homology of the superficial slime-covering in *Beggiatoa mirabilis* (Hinze, 1902; Ruhland and Hoffmann, 1925), which we have also observed, with surface structures in the forms we have employed for measurements of speed of movement.

According to Schmid (1923), who studied fragmented filaments, all parts of a filament of *Oscillatoria* are motile. Prell (1921*a, b*,) found that the cells of a filament “cooperate,” although there seems to be no conduction of stimuli from one part of a filament to another. This agrees with the observation (Crozier and Federighi, 1924–25) that the speed of movement does not vary with the length of the filament. Mr. E. S. Castle has made similar observations on *Anabæna*. It has been noticed, however, that very small groups of cells do not move (Krenner, 1925). In *Beggiatoa* very short fragments, even comprising but three to five cells, do move, but only for very short distances; the frequency of reversal in direction is very high. It is to be noted, as bearing upon unity of action in long filaments, that there is frequently apparent a failure of the parts of a filament to cooperate. With very long filaments (2 mm.), the two terminal regions may be moving in opposite directions; or a hook bend at one end may be moved forward bodily, in such fashion as to indicate that the bent tip region is not at all contributing to the movement. Similar cases occur in which reversal of direction of movement is not synchronous over the whole filament (*cf.* also Keil, 1912). Aside from their bearing upon the mechanism of movement, these points are of practical moment for

the measurement of speeds of progression under comparable conditions.

The speed of movement declines as the culture containing the thio-bacteria ages and the cells of the organisms become vacuolated. During the most active period of growth the speed of translatory movement is quite sufficiently uniform to permit significant measurements. The speed is independent of the length of the filament. Successive estimations with a single filament show satisfactory constancy, as may be illustrated by several sets of readings:

Filament.	Temperature, <i>t</i>	Time to travel 10 micrometer divisions.
	°C.	sec.
<i>A</i>	10.2	34.6
		38.6
		39.8
		39.0
		36.8
<i>B</i>	16.8	23.8
		22.2
		21.8
		22.2
<i>C</i>	19.3	14.4
		17.6
		17.2
		18.0

It could not be shown that these slight variations are related to the incidence of reversals of direction.

The latitude of variation in such series does not significantly differ from that in series obtained from a number of different filaments. This was tested sufficiently to ensure the possibility of employing averages based upon measurements with a number of filaments. It is practically impossible, however desirable, to obtain readings over a range of temperatures from single filaments. There is indication of fluctuating variation in speed of movement, not correlated with time of day, in which the period is rather long. This is in part responsible for the scatter of the plotted means (Fig. 1). The probable error of the plotted means is less than 5 per cent of the corresponding means

(usually less than 4 per cent). For purposes of the present account we have employed data from filaments in one culture, between January 14 and January 28, 1926. Throughout this period no systematic changes in speed of movement were detected. The number of observations was 431.

IV.

The results are plotted in Fig. 1. Contrary to the case of *Oscillatoria* (Crozier and Federighi, 1924-25), the motion of *Beggiatoa* ex-

FIG. 1. Speed of gliding motion of *Beggiatoa* as function of temperature. The circles give positions of average speeds in series of readings; the vertical lines extending from them cover the latitude of variation in each series. The rate is taken as 100/ time to travel 10 micrometer divisions (0.05 mm.).

hibits a sharp change in the relation to temperature at about 16.5°. The lines providing a satisfactory fit to the two portions of the log speed- $1/T^{\circ}$ abs. graph have slopes respectively, yielding $\mu = 8,400$ and $\mu = 16,100$ as temperature characteristics.

It may be suggested that the data are equally well fitted by a single unbroken curve. To this there is definitely opposed the fact that in such a case the slopes of the fitted straight lines could not very well be

expected to agree with those found in numerous cases where a single rectilinear relationship holds over the whole of the temperature scale. The impossibility of describing such series of observations by means of a single smooth curve is adequately shown by plotting *rates* against centigrade temperatures; the points fall upon two sharply intersecting curves. And there is also to be emphasized the fact that the temperature at which intersection of the proposed straight lines is located, as determined solely by the distributions of the relevant points, agrees so closely with one at which such irregularity is commonly or very fre-

FIG. 2. Speed of movement in *Thiothrix* as related to temperature. The points are averages of 6 to 8 measurements each.

quently manifest in other vital processes (Crozier, 1925-26, *b*). Additional considerations justifying this procedure are discussed in another place (Crozier and Stier, 1926-27). Less extensive observations on the movement of *Thiothrix* provide data for the graph in Fig. 2. The temperature characteristic, $\mu = 8,300$, agrees well with that for the corresponding temperature range with *Beggiatoa*.²

² As with *Oscillatoria*, question also arises here as to the character and mechanism of reversal in direction of movement. According to Coupin (1923) *Oscillatoria* filaments, on Knop medium to which gelose had been added, show no regular periodicity in the reversal of movement; but it is necessary to maintain constant conditions of light and temperature before the matter can be tested. It is clear

In addition to the occurrence of a critical "break" at 16°, the temperatures 5.3° and 33° were established at points at which progressive slowing of movement with time becomes evident; at 33° or above "jerky" side to side movement is evident, with little forward motion.

The "break" at 16° is made obvious in another way. The latitude of variation at temperatures below 16° is definitely less than at higher temperatures. For some time it has been desired to find instances in which it might be possible to discover if the latitude of variation is a property of the organism or tissue as a whole, or of the process whose critical increment is being measured. It is clear, we believe, that in general, and depending on the nature of the activity considered, both these types of variation must be recognized as possible. In many instances it has appeared that the latitude of variation may change without affecting the temperature characteristic (*e.g.*, Crozier and Stier, 1925-26, 1926-27); on the other hand, the latitude may be sensibly constant when the increment changes. The present case is one in which there is apparent alteration of the latitude accompanying a change of increment.³

V.

SUMMARY.

The speed of translatory movement of *Beggiatoa alba* is governed by temperature in such a way that between 5° and 33° the temperature characteristics $\mu = 16,100$ and $\mu = 8,400$ respectively obtain for the temperature ranges 5° to 16.5° and 16.5° to 33°. The "break" at 16°-17° is emphasized by the occurrence of a wider latitude of variation in speed above this temperature. Above 16° the progression of *Thiothrix* yields $\mu = 8,300$. The possible relation of these values to that previously obtained for similar movement in (photo-synthetic) *Oscillatoria* is commented upon.

that the frequency of reversal is related to the length of the filament, being greater with short filaments, and certainly it increases with elevation of temperature. Reversal is more frequent in *B. alba* than in *Thiothrix*, under the same conditions. In forms we have observed the frequency of reversal has a higher temperature coefficient than the speed of translation.

³ This may also be the case with the locomotion of *Paramecium* (Glaser, 1925-26).

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