THE RELATION BETWEEN TEMPERATURE AND THE PEDAL RHYTHM OF BALANUS.

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The characteristic contractions and expansions of the pedal cirri,—a respiratory and feeding mechanism,—in the rock barnacle, *Balanus balanoides* L., have proved to be reliable indicators of stimulation. The animal as a whole, and the cirri alone, are very sensitive to chemical, mechanical, photic, and thermal stimuli, the effects of which may be measured by observing the corresponding changes in the character and rate of the pedal rhythm. It is possible therefore to investigate such stimuli qualitatively and in some cases quantitatively, either separately or in appropriate combination, while the others are so far as possible kept constant. A preliminary study of this sort was recently made[1] on one group of *B. balanoides*. The relations between temperature and changes in the pedal rhythm are described in this paper.

Consistent results from changes of temperature are obtained only when the other external conditions are kept as constant as possible. The effects of various external factors, as well as of internal conditions, if not controlled, would lead to an erroneous conclusion concerning the behavior of the barnacle. Here is an animal activity influenced by several factors, but it would be absurd to conclude that their effects cannot be studied separately. If the various factors can be determined and controlled the analysis of any one becomes possible. Merely because an activity is "complex" is not sufficient reason for abandoning efforts to analyze it (*cf.* Herber and Slifer, 1928). In the laboratory regular movements of the cirri demand running water, with a rate of flow within definite limits. This was obtained by means

[1] The experiments were done during the summer of 1928 at the Mount Desert Island Biological Laboratory, where Director Dr. H. V. Neal extended many appreciated courtesies.

of a water gauge which registered the flow in cubic centimeters per minute within an error of 2 per cent. To prevent as much as possible contact stimulation by particles in the water without decreasing the food supply, the water was filtered through cloth with a 1 mm. mesh. Contact stimuli due to feces were rare since the latter sank upon extrusion. Vibrational stimuli were decreased to a minimum by mounting the aquarium on rubber balls set on a layer of moderately soft sheet rubber 15 mm. thick. Illumination was furnished by northern sky light or by Mazda lamps the intensity of which did not change enough during an experiment to elicit the well known shading reflex. To avoid effects due to changes in the food supply or to the physical constants of the water such as pH, specific gravity and salinity, the experiments were done only when any variations in those factors were physiologically insignificant. Interfering stimuli caused by moulting or defecation were eliminated from the results by omitting observations during and immediately following those activities.

A group of 41 animals attached to a stone was placed in a glass aquarium of 650 cc. capacity. The water entered at the bottom at the rate of 250 cc. per minute and overflowed around the top. The resultant immersion of these intertidal animals, continuous for 9 weeks, had no ill effect that could be determined. The temperature of the water was controlled by sending the current through a lead coil (1.5 m. long) immersed in a 12 liter water bath, to which ice, or cold or hot water was added as desired. The temperature of the aquarium water was changed between 0.0° and 28.0°C. either upward or downward by not more than 3° at a time, and not oftener than once in 40 minutes. Allowing 15 minutes for equilibrium the temperature remained constant to within 0.1° for periods of from 15 to 60 minutes, depending upon the ratio of the temperatures of the air and the bath. At each temperature the time required for 10 contractions and expansions was measured by a stop-watch to 0.05 second. The averages of 5 such readings were found to be significant because of the remarkable regularity of the movement under constant conditions. Complete records of 17 individuals, the basal diameters of which varied from 4 to 15 mm., were secured over a period of 9 weeks, involving 965 averages of 6,775 observations at 186 different temperatures between 2.0° and 27.0°C. Twelve hundred observations on 10 other animals showed similar results but are not included in this report because of their incompleteness over an adequate range of temperatures.

In general, the rate of the pedal rhythm of the barnacle increased regularly with temperature from 2.0° to 21.0°C., revealing a critical temperature above which the increment was slightly decreased. Below 2.0° the movements were extremely irregular and often spasmodic. Between 21.0° and 27.0° three different effects appeared in different cases: (1) the rate decreased or became irregular, (2) the rate continued to increase, or (3) the valves closed. Beyond 27.0° closure of the valves regularly occurred. In the case of the few animals whose rates continued to increase beyond 21.0° closure at 27° was sudden and not preceded by irregularity. Alteration of the rate by temperature was due primarily to a change in the duration of the expanded phase, without much change in the contraction period, a fact easily demonstrated at the lower temperatures. It may be said therefore that temperature effects in the barnacle are primarily "diastolic."

In any one animal the frequency of movement at a given temperature on different days varied only about 5 per cent. Since this latitude of variation was constant throughout the temperature range, data obtained over a long period were combined. Having once determined the relation between frequency and temperature it was possible to predict the temperature of the water to within 1.0° from observations on the rate of movement. Although different animals exhibited different absolute values, two intersecting "hyperbolas" were always obtained when time was plotted against temperature. It would be expected therefore that if the logarithm of the rate were plotted against $1/T°$ the points would fall on two intersecting straight lines, indicating the applicability of the so called Arrhenius equation for temperature effects. Separate plots for the 17 animals were so made, and in every case the points could best be represented by two intersecting straight lines. The values of μ (obtained from the slopes of the lines) fell easily into three classes, so that the plots in each class could be combined, it being necessary in some cases to multiply the ordinates by a constant to bring the lines nearer coincidence. These plots, from which a few duplicate points have been omitted for clarity, are reproduced in Figs. 1–3, and show how closely the Arrhenius equation fits the data.

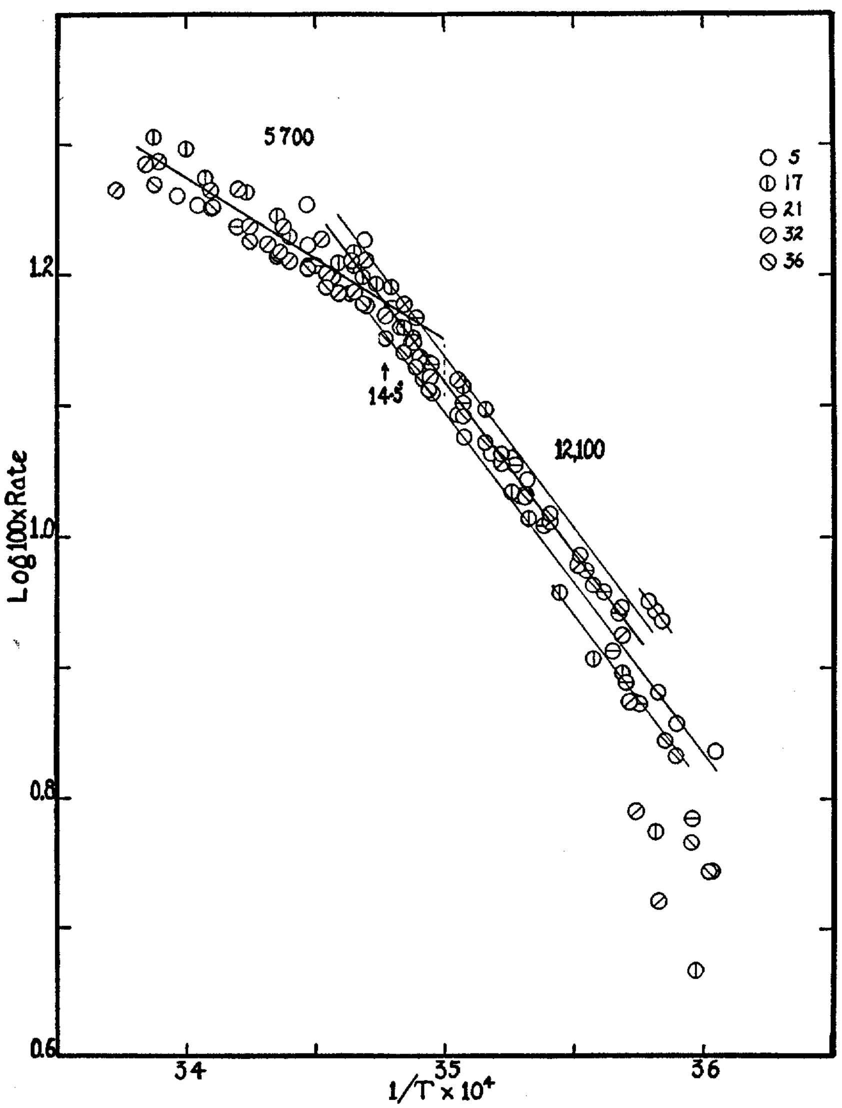

FIG. 1. Data on the rate of the pedal rhythm of *Balanus*, from 5 animals, between 4.4° and 23.4°. Critical temperature is at 14.5° above which μ = 5,700, and below which μ = 12,100. Irregularities appear below 7.0°.

In Fig. 1 are the data from 5 animals, which yielded temperature characteristics of 5,700 from 14.5° to 22.5°, and 12,100 from 7.0° to

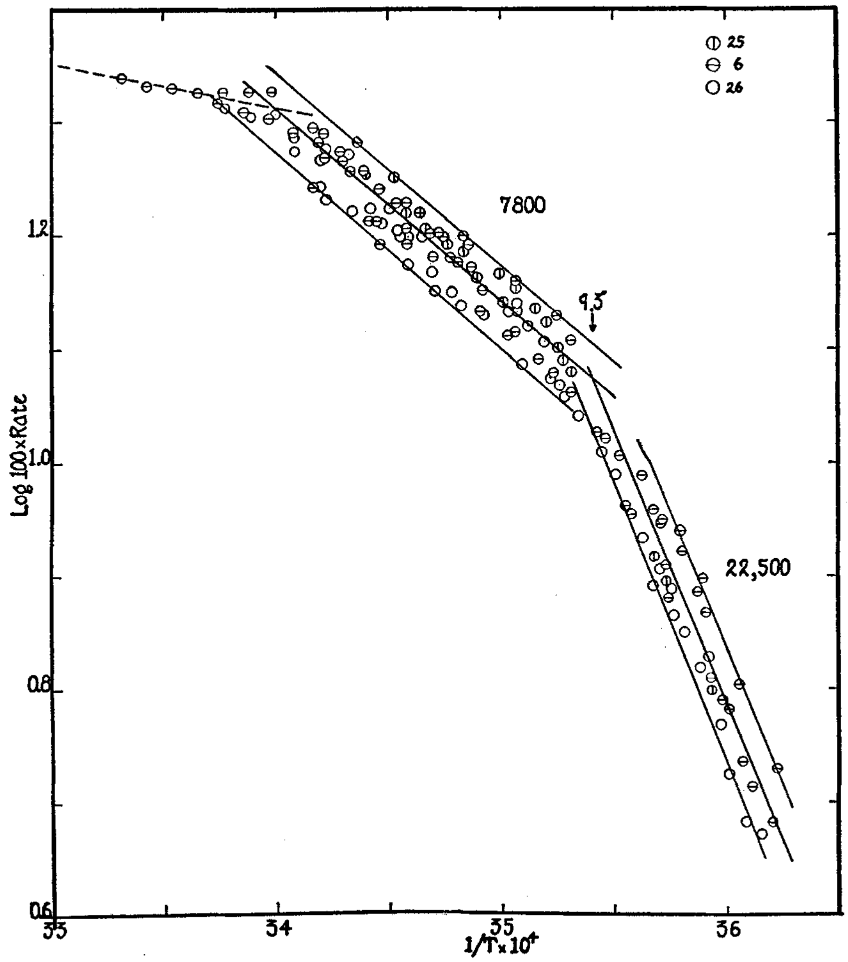

FIG. 2. Data on the rate of the pedal rhythm of *Balanus*, from 3 animals between 2.4° and 27.1°. Critical temperature is at 9.3° above which μ = 7,800 and below which μ = 22,500.

14.5°. Below 7.0° the results were very irregular. In Fig. 2 the values were 7,800 from 9.3° to 21.0°, and 22,500 from 3.1° to 9.3°. Animal 6 in this group continued to show an increasing rate, with a smaller increment, up to 27.0°, at which closure was sudden. In Fig. 3 the data from 9 animals showed temperature characteristics of

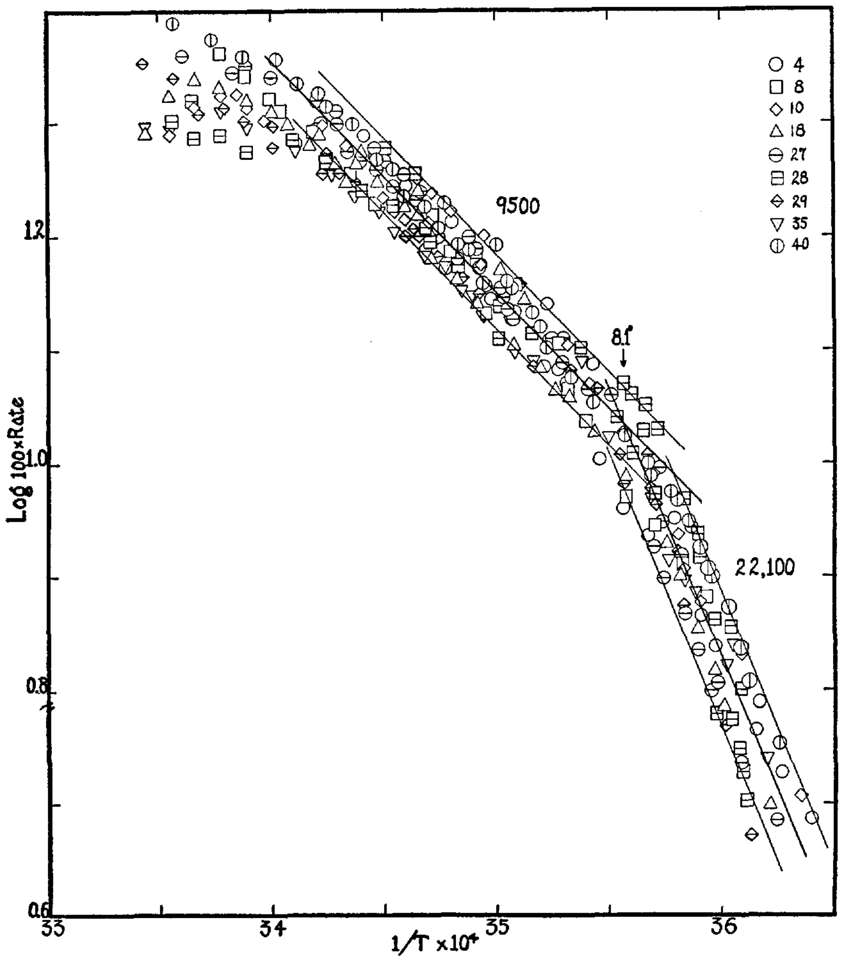

FIG. 3. Data on the rate of the pedal rhythm of *Balanus*, from 9 animals between 1.6° and 25.8°. Critical temperature is at 8.1° above which (to 21.0°) μ = 9,500, and below which μ = 22,100.

9,500 from 8.1° to 21.0° and 22,100 from 2.4° to 8.1°. Above 21.0° the plot showed several rates increasing slowly up to closure. Different temperature characteristics from different animals over the same temperature range may be due to the fact that different members of a catenary series of reactions which determines the pedal rhythm assume control of the series under differing conditions of age, sex, nutrition, and the like.

The values of μ obtained for the barnacle are similar to those reported for various other animal activities. It is not surprising to find values near 8,000 and 22,000 for a mechanism which is at least partly respiratory, the former frequently associated with processes involving gaseous exchange. The characteristic of 12,100, however, has been associated with non-respiratory neuromuscular rhythms in arthropods (*cf.* Crozier, 1924; Crozier and Stier, 1925, *a*; 1925, *b*). Wolf (1928) found the same combination of 8,000 and 22,500 in normal *Gonionemus* for frequency of pulsation, which is also a respiratory and food-getting function. These three increments might be expected for an activity which is concerned partly with respiration but operated by a neuromuscular mechanism, if the general indications of the results from temperature analyses are sound.

Increments of about 9,000 calories have been previously found for several different organic processes, such as movement of *Oscillatoria* (Crozier and Federighi, 1924; Navez, 1928), heart beat in *Tiedemannia* (Glaser, 1925), and in *Notonecta* (Crozier and Stier, 1927), and for growth of sporangiophores (Castle, 1928). In some of these cases, however, the value appeared only infrequently and was not considered typical for the process. That this 9,500 is not due to a mixture of values near 8,000 and 11,000 is shown by the following series of values calculated from the individual plots: 8,900, 9,030, 9,040, 9,470, 9,560, 9,600, 10,200, 10,370, 10,400, the average of which is 9,620. So far it has been impossible to detect its significance. The records of μ = 5,700 from the barnacle and μ = 4,800 for locomotion of *Planaria* (Cole, 1926) stand by themselves and any attempt to correlate them with known chemical or biological processes must await further investigation; particularly since they occur at such relatively high temperatures. The critical temperatures revealed in the three sets of data are very close to those reported in many other activities. Changes in the control of the rate of a series of reactions frequently occur near 9.5° and 15.0°; near 8.0° such changes are not so common (*cf.* Crozier, 1926).

Fig. 4 is a mass plot of the results from all the animals and illustrates very clearly the inability to interpret temperature effects

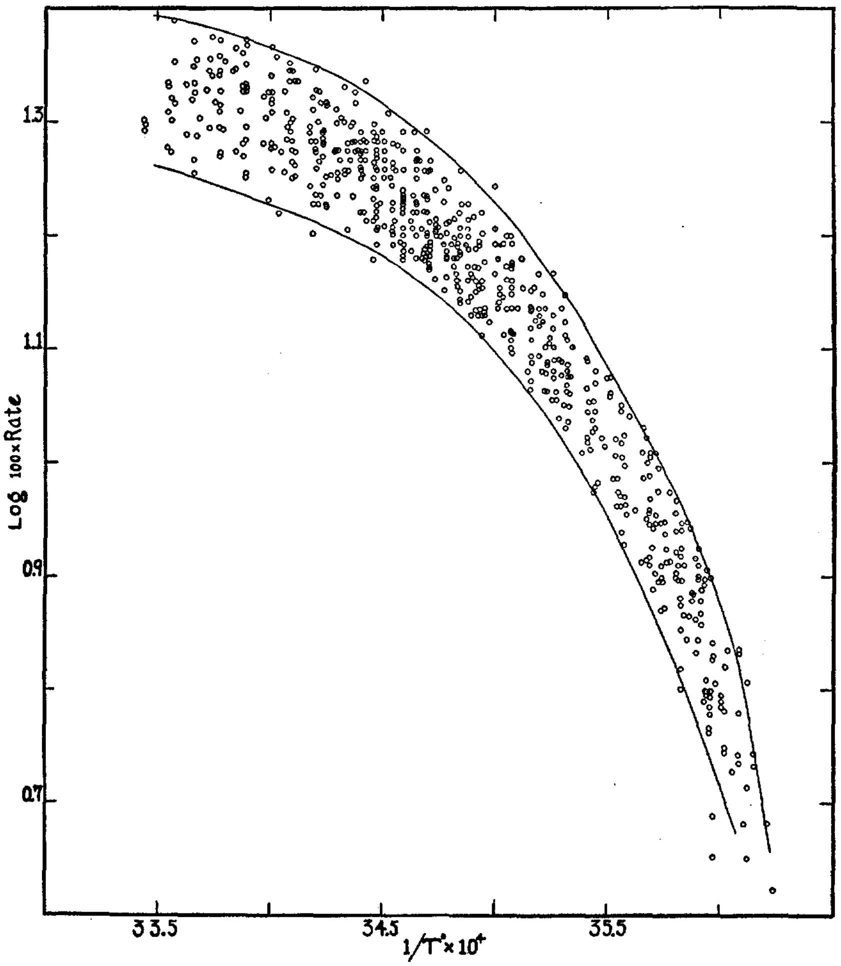

FIG. 4. Data on the rate of the pedal rhythm of *Balanus* from 16 animals between 2.9° and 26.0°, plotted *en masse*. Curvilinear lines indicate different temperature characteristics among the different individuals and the necessity of individual plots.

correctly when the animals show individual differences. The curvilinear shape of the best fitted line is due to the different temperature characteristics over the medium range of temperatures, and to the varying irregularities above 21.0°C. (Crozier, 1924; Crozier and Stier, 1925, *a*; Glaser, 1924.) If the Arrhenius equation is to be applied to such data individual plots must be made (*cf.* Castle, 1928, page 412; Glaser, 1925).

A second series of experiments was performed to detect any effect of diluting the sea water upon the temperature relations, the pH being kept constant. Enough observations were collected to demonstrate that any dilution greater than 4 per cent caused irregularity of movement; that 3 and 4 per cent dilutions progressively decreased the rates, without altering the temperature characteristics; and that dilutions of 2 per cent or less caused no significant change in rate. The results indicate the extreme sensitivity of this barnacle to fresh water as far as regularity of movement is concerned. It is well known however that much larger amounts of fresh water are tolerated by barnacles without permanent injury, although the pedal rhythm may be temporarily affected.

SUMMARY.

1. The relation of temperature to the pedal rhythm of *Balanus balanoides* L. has been studied under otherwise constant conditions.

2. The frequency of movement increases with temperature, showing three groups of thermal increments and three critical temperatures. Five animals yielded μ = 5,700 above 14.5° C. and 12,100 below; 3 gave μ = 7,800 above 9.3° and 22,500 below; while 9 showed μ = 9,500 above 8.1° and 22,100 below.

3. The upper critical temperatures, above which different effects appeared in different animals were 23.4°, 26.0°, and 27.0°. Above 27.0° none of the valves remained open.

4. Excepting the values 5,700 and 9,500, the increments are similar to those previously found to be associated with respiratory and with neuromuscular activities.

5. Dilution of the sea water with from 3 to 4 per cent fresh water decreases the rate without altering the increments. More than 4 per cent dilution causes irregularity.

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