THERMAL INCREMENTS FOR PULSATION-FREQUENCY IN "ACCESSORY HEARTS" OF NOTONECTA.

BY W. J. CROZIER AND T. J. B. STIER.

(From the Laboratory of General Physiology, Harvard University, Cambridge.)

(Accepted for publication, January 20, 1927.)

I.

For development of a theory of the temperature characteristics of vital processes it is necessary to deal with types of material in which it may be expected that experimental treatments can evoke one or another of a series of interrelated critical increments (Crozier, 1924–25, a, b; Crozier and Stier, 1924–25, a; 1925–26, b). In this paper we give an initial account of temperature relations in a kind of rhythmic activity which is in certain respects especially favorable for such tests.

Pulsatile organs, "accessory hearts," were first observed in the legs of juvenile aquatic hemiptera (Notonecta, Ranatra, Corixa, and others) by Behn (1835). These organs, located generally in the tibia just distal to the femoral articulation, or in the tarsus, were conceived by Behn to be a kind of "membranous valve" important in maintaining the peripheral circulation. Their "spontaneous" rhythmic movements were apparently confirmed, although misinterpreted, by Dufour (1835); they were discussed by Dugès (1838), and by Verloren (1847), and a summary of the early observations on the "accessory hearts" in aquatic and other hemiptera was given by Edwards (1858). It was noted (Verloren, 1847) that in Tettigonia the pulsatile activity might be interrupted for more or less lengthy periods, but that when active the frequency of the quick, sharp contractions exceeded that of the heart. The "hearts" were rediscovered by Mitchell (1858). Locy (1884) also described their movements, in Ranatra, Notonecta, and Bdelostoma, subscribing to their rôle in connection with the circulation; he noted that the movements would continue for a time in amputated legs, and even when the "heart" was itself cut into parts. More recent

1 Dugès, 1838, p. 441.
2 Verloren, 1847, p. 82.

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observations, with figures, are given by Bröcher (1909). The existence of these organs is briefly referred to in entomological texts (Houlbert, 1920; Schröder, 1913), but nothing further appears to be known about these curiously inviting instances of perhaps myogenic rhythm. Among a number of possible sources of pulsatile organs in the bodies of insects (cf., e.g., Bröcher, 1916; 1917; 1919) they are by far the most amenable to experimental manipulation. They can be observed in the intact animal, and proper technique provides preparations in which the organs are active for days in detached legs. Six preparations may be gotten from a single individual, permitting for some purposes unusual control material.

In our experience the locomotor muscles of amputated legs of Corixa are likely to exhibit twitching movements, which interfere with the activities of the “accessory hearts,” and in Ranatra the latter are difficult to see. Common species of Notonecta, however, are obtainable in large numbers, live very well in the laboratory, the “hearts” are easily seen, and the limb muscles of the detached legs are absolutely quiescent. The following account is based entirely upon the behavior of “accessory hearts” in the two swimming-legs of adult Notonecta undulata. It will be shown that although two preparations are obtainable from each individual these may nevertheless behave in quantitatively different ways, so that perfect control observations are not possible. But no necessity for such controls arose in the present work, which was designed to obtain primarily the temperature characteristics for pulsation-frequency during the survival of the isolated limb.

Our purpose was to discover if in different freshly isolated legs, and during the course of the irreversible death phenomena, there should not appear, as in the breathing movements of grasshoppers (Crozier and Stier, 1924–25, a), a series of differing critical thermal increments for pulsation-frequency, which might thus reveal certain of the interrelated processes involved in determining the rate of pulsation.

We were especially anxious to do this because of the possibility of obtaining information about typical governing processes in insect muscle, for comparison with central nervous activities (Crozier, 1924–25, a; Crozier and Federighi, 1924–25, a, b; Crozier and Stier, 1924–25, a, b; Fries, 1926–27). Although possibly containing nervous elements, the “hearts” in isolated legs are obviously beyond central nervous influence. It turns out that the typical temperature char-
acteristics obtained for frequency of pulsation agree quantitatively with those known for respiratory phenomena (Crozier, 1924–25, b), and for breathing movements of insects (Crozier and Stier, 1924–25, a), but with the addition of a further member of this “set” of critical increments. They differ sharply from the value (μ = 12,300) which is characteristic for frequency of heart contractions in arthropods (Crozier, 1924–25, a; Crozier and Federighi, 1924–25, a; Crozier and Stier, 1925–26, b; Fries, 1926–27). The associations observed between the four chief increments obtained are of additional interest for the analysis of vital processes from this standpoint.

II.

The beating of the leg-“hearts” is easily observed in the intact animal. The back-swimming habit of Notonecta makes it possible to prepare an individual by mounting it, ventral surface upward, upon a small block of hard rubber, to which it is attached by a small amount of vaseline, in such a way as to be at the surface of a vessel of water, with the legs outstretched in the surface film. Occasionally, movements of the appendages are seen; but the intervals between these more or less periodic movements are fairly long. The rate of pulsation in any one leg is very uniform; thus in one case, observed at frequent intervals over 24 hours, at 17°C., the time for ten contractions varied between 9.6 and 1.06 seconds; in another, between 5.2 and 5.6 seconds during 36 hours; frequently there is some decline in rate after 12 hours.

The beats are irregular for brief periods; sometimes a contraction is skipped. There is no evidence of diurnal rhythm. When the two swimming-legs of one individual are compared, there is usually a distinct difference between them:

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Leg</th>
<th>Time for ten pulsations, seconds</th>
</tr>
</thead>
<tbody>
<tr>
<td>N1 19°</td>
<td>Left</td>
<td>5.4 5.6 5.3 5.5 5.5</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>7.1 6.7 7.3 7.3 7.8</td>
</tr>
<tr>
<td>N2 19°</td>
<td>Left</td>
<td>5.0 5.1 5.1 4.7 4.1</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>4.4 4.9 4.7 5.6 5.1</td>
</tr>
<tr>
<td>N3 17°</td>
<td>Left</td>
<td>8.3 7.9 7.9 8.3</td>
</tr>
<tr>
<td></td>
<td>Right</td>
<td>7.4 7.0 7.6 7.0</td>
</tr>
</tbody>
</table>
One leg—"heart"—may abruptly cease operations for a time, the other continuing without pause. When the animal is stimulated, the beat may be hastened or retarded and irregularities appear.

The uncorrelated nature of the rates of contraction in the two legs and their independent variation speak for local rather than central nervous control of the activities of the leg—"hearts." Yet when the intact animal dies the frequency of the pulsations changes and to some extent their character also. When an animal in which the "heart" of one leg has been pulsating for some time at a steady frequency of 10.5 seconds for ten beats is completely covered by a thick layer of vaseline the rate remains constant for a time; but then, after several hours, the rate suddenly decreases to about one-half its former value, in both legs, and at this point the animal as a whole ceases to respond to stimulation. If left attached to the body the leg—"hearts" continue to beat for some 14 hours, but with decreasing although regular frequency. If detached from the body the legs show sustained pulsation for a much longer time. If the attached or isolated limb be swathed in vaseline no change in pulsation rate occurs.

These and other observations show that the rate of pulsation is locally determined, but that a secondary control, of nervous character, possibly, or dependent upon changes in the hemolymph pressure, is also important.

Two general types of result follow amputation of a swimming-leg. The rate of pulsation of the "heart" may remain the same as before amputation, the beat becoming at once more regular, and then, after about an hour (18°), the beat suddenly becomes much slower. In other cases the rate immediately after amputation may be about doubled, for several minutes, followed by sharp cessation of contraction which lasts about an hour; contractions are then resumed at about one-quarter of the original frequency. At the moment of pinching the leg with scissors the beats slow down, then become very rapid, then settle down to the initial rate. We have plugged the ends of amputated legs by exposing them to the air for 5 minutes, then by coating with vaseline; or the leg may be cut while imbedded in vaseline. No effects of these procedures were detectable.

It is important that the nature of the pulsatile movement of the leg—"heart" retains a very uniform character throughout the tempera-
ture range. Contraction is abrupt, relaxation slower, followed by an interval of quiescence. The contraction is maximal and always complete. The sheet of muscle fibers constituting the “heart” (cf. Bröcher, 1909) is cap-shaped at the proximal end, from which a (muscle) band passes to the femorotibial joint; distally, the “cap” spreads out into a flat contractile band. As a rule, contraction first appears at the apex of the “cap,” and travels as a wave to the distal band. Occasionally the contraction wave is reversed. As death approaches the relaxation phase is prolonged, and the quiescent interval almost disappears; this also happens if the leg is sealed in a tube of water previously boiled.

III.

For observation, the isolated legs of Notonecta were attached by vaseline to a glass slide placed in a small glass vessel of water. This vessel, having a flat bottom, was sunk in a chamber filled with water. The chamber was water-tight, and addition of hot or cold water for regulation of temperature was by way of a coil of copper tube, with many small apertures, connected with an external supply. This chamber was immersed in a large stirred thermostat. Through the cover of the vessel containing the preparations the objective of the observing microscope passed, and also a thermometer reading to 0.01°. The objective was used as a water-immersion lens, thus avoiding troublesome fogging, all the metal surfaces being coated with paraffin to obviate oligodynamic effects. The regulation of the temperature of the innermost vessel was secured by maintaining very slight differences between the temperature of the large thermostat and of the chamber immediately surrounding the observing vessel. Two, three, or four legs could be mounted together, and by adjustment of the microscope they could be viewed in succession or as desired. Light was reflected up through the thermostat and through a window in the inner box.

The muscles of an isolated leg, including the contractile fibers of its “heart,” must of course be regarded as a system proceeding toward death, irreversibly. Therefore, if the relationship is to be obtained which exists between temperature and frequency of pulsation it is necessary to work rapidly in order to secure observations at a number
of temperatures before the underlying mechanism shall have changed materially. With these preparations it is possible to do so, largely because the latitude of variation in frequency of pulsations at constant temperature is so very slight.

After removal from the animal about 1 hour (at room temperature) is often necessary before the isolated "accessory heart" settles down to a steady rate of contraction. Not infrequently, however, this steady rate, lower than before amputation of the leg, is attained almost at once. A few successive readings of time for ten pulsations enable one to judge if the preparation is in a state suitable for experimentation.

In order to discover the degree of constancy to be expected under uniform temperature, graphical records were obtained with the aid of a signal key and a chronoscope registering 1.0 second intervals upon a smoked drum. In this way a sufficiently precise record was obtained of continuous series of pulsations over periods of about \( \frac{1}{2} \) hour. The high degree of constancy in "time for ten pulsations" in any one preparation is shown in the following table:

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Preparation</th>
<th>Mean time for ten contractions</th>
</tr>
</thead>
<tbody>
<tr>
<td>°C.</td>
<td></td>
<td>sec.</td>
</tr>
<tr>
<td>16.4°</td>
<td>A¹</td>
<td>19.0 ±0.4</td>
</tr>
<tr>
<td>16.4°</td>
<td>B₂</td>
<td>21.6 ±0.2</td>
</tr>
<tr>
<td>16.4°</td>
<td>E₂</td>
<td>19.2 ±0.3</td>
</tr>
<tr>
<td>16.1°</td>
<td>I</td>
<td>17.95±0.95</td>
</tr>
<tr>
<td>15.2°</td>
<td>K</td>
<td>15.7 ±0.4</td>
</tr>
</tbody>
</table>

In this table the ± limits give the maximum latitudes of variation, which average 4.9 per cent of the means. The same type of variation is evident in observations made at intervals, with a stop-watch, over periods of some hours. The maximum latitude of variation for any one heart is not over 10 per cent, and is as a rule less than 5 per cent of the mean at a given temperature. This variation of course includes errors of observation. The same type of fluctuation is apparent throughout the temperature graphs. In good preparations constancy in pulsation rate is maintained for many hours, at uniform temperature.
IV.

When the temperature is varied it is quite necessary to study each preparation individually. Averaging observations from different "accessory hearts" is not permissible. Even the two swimming-legs from the same individual may differ significantly (Figs. 7, 8), although they not infrequently give parallel results (Figs. 1 to 5).

![Graph](image)

**FIG. 1.** In this and succeeding figures the temperature characteristics for frequency of pulsation in the accessory leg-"hearts" of *Notonecta* are obtained graphically by plotting log frequency (= log [100/seconds for 10 beats]) against reciprocal of absolute temperature.

Preparations Nos. 29 (circles) and 30 (crosses), (the latter having the rates multiplied by 2.0 for comparison) give \( \mu = 8,800 \). It may be noted that the latitude of variation, with the large scale-units employed, corresponds to an extreme difference, at the left end of the graph, of only 1.4 seconds in a mean of 23.4 seconds for ten pulsations.

In this way we have examined a large number of preparations, of which about 30 were studied in detail. The total number of observations was above 3,000. Since we desired especially to know the kinds of critical increments which might appear during the onset of death, it was necessary to avoid so far as possible the production of irreversible effects by exposures to very low or to very high temperatures. It was also necessary to work rapidly in order to make sure of time in
Fig. 2. Legs Nos. 27 (open circles) and 28, from the same individual, give $\mu = 11,400$.

Fig. 3. Legs Nos. 19 and 20 give sensibly identical results; $\mu = 16,000$. 

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which to travel in reverse order the excursions up or down the temperature range before intrinsic change of temperature characteristic should supervene. The absence of great natural variation in rhythm, coupled with the non-interference of spontaneous activities of the leg musculature, greatly facilitated such observations. The agreements in values of \( \mu \) obtained show that confusion from these sources was successfully avoided.

The lower critical temperature for continued pulsation was 5°; in the isolated legs 20° was found a very definite upper limit for regular rhythm. In a number of preparations pulsations ceased at 9.5° ± 1.0°. The intermediate temperatures at which “breaks” appeared, indicating change of increment or of frequency, varied from 10.8° to 17.2°; the reason for this variation, as subsequently discussed, is found in the progressive exhaustion of the pulsating system, with induction of physicochemical changes independently of the thermal ones.

V.

The results of these observations are sufficiently illustrated by the examples given in Figs. 1 to 11. Including those instances in which
"breaks" occurred, as exemplified in Figs. 6, 8, and 11, the following is a summary of the critical increments found to describe the change in frequency of contraction as determined by temperature.

<table>
<thead>
<tr>
<th>Mean μ</th>
<th>Extreme range</th>
<th>Number of instances</th>
</tr>
</thead>
<tbody>
<tr>
<td>8,190</td>
<td>7,900 to 9,150</td>
<td>14</td>
</tr>
<tr>
<td>11,350</td>
<td>11,290 to 11,500</td>
<td>4</td>
</tr>
<tr>
<td>16,200</td>
<td>15,100 to 17,000</td>
<td>16</td>
</tr>
<tr>
<td>19,800</td>
<td>19,600 to 20,000</td>
<td>4</td>
</tr>
<tr>
<td>24,530</td>
<td>23,000 to 25,000</td>
<td>5</td>
</tr>
<tr>
<td>32,200</td>
<td>30,000 to 34,000</td>
<td>12</td>
</tr>
</tbody>
</table>

The most precise determinations of μ are of course only obtainable from "runs" over a good range of temperatures; since in some cases such ranges were impossible to obtain in the present experiments, a certain variation in each value of μ as ascertained is to be expected. Certain details regarding the observations are discussed in the legends of the several figures.

The other magnitudes of μ, save 32,200, have already been recognized as occurring repeatedly in connection with a variety of biological processes (Crozier, 1924-25, b; 1925-26, b). Their reappearance here adds to the conviction that they correspond to chemical realities in living matter, perhaps to the heats of activation of commonly occurring catalysts (Crozier, 1924-25, a).

It will be noticed at once that the most frequently occurring magnitudes are, approximately, 8,000, 16,000, and 32,000 calories. Bliss (1925-26) has described a case in which temperature characteristics of these general magnitudes apply respectively to different parts of the total temperature range as affecting the time required for a particular developmental phase in Drosophila. This kind of progression tempts speculation. The additive nature of heats of activation is recalled. But we believe that for the present the fact may most safely be recorded devoid of speculative fringes. This is especially so because if the individual records are examined it is found that with any one preparation there is no orderly association or succession of values such as 8,000; 16,000; 32,000 in different parts of the temperature range, or at different times. The increment 16,000, for example,
may be associated with 32,000, or with 8,000; or 8,000 with 32,000. This speaks for a certain random association of the processes manifested by the appearance of the respective increments; with this restriction, however, the lower of two increments always pertains to the higher temperature range.

In an isolated and irreversibly deteriorating system such as is presented by the isolated leg-"heart" it is to be assumed that changes in \( \mu \) should also be evident as a function of time.

By "a change which is a function of time" we mean to imply that the processes of exhaustion and death should influence the occurrence of "breaks" in the relation between pulsation-frequency and tempera-
ture. The simplest illustration of such an effect is given by cases such as that shown in Figs. 5 and 8. It is obvious that the differential effects of exhaustion upon each of several processes such as might possibly control the frequency of pulsation may result in these elements of the nexus governing pulsation being successively revealed as determining the relationship to temperature. In numerous cases

![Graph](image)

**Fig. 6.** Two preparations from different individuals, show transitions from $\mu = 9,050$ to $\mu = 30,700$ at lower temperatures.

where this sort of result is evident, reversibly, on passing from one zone of temperatures to another (Crozier, 1924–25, b; 1925–26, b) it has been assumed that it may be due to the fact that a catenary chain of reactions underlies the determination of frequency of pulsation (Crozier, 1924–25, b); the fact that the temperatures at which these shifts occur are not distributed at random (Crozier, 1925–26, a)
makes it necessary to suppose that physical changes, essentially of a grossly discontinuous character, are also implicated. In dealing with the Notonecta leg-"hearts" it was expected that the degradation of the excised system would have similar effects, and that in consequence the occurrence of an intermediate critical temperature should be blurred. This is the fact, but there is ample evidence, nevertheless, that in the neighborhood of 15° (10.8° to 17.2°) abrupt changes most frequently occur in the relationship between rate and temperature.

A further sort of complication in these curves is due to what we have characterized in other instances (Crozier and Stier, 1925–26, a) as a change of frequency or velocity without change of increment, or, what
is probably connected therewith in an intimate way (Crozier and Stier, 1925-26, b), a change in the latitude of variation at constant temperature without change of increment. This type of effect occurs in certain Notonecta preparations (Figs. 9, 10, 11) and might easily confuse interpretation. To what extent these shifts may be due to change of pace-making location in the pulsating organ we cannot decide; in most instances, we feel, this explanation would not be correct.

Fig. 8. Two preparations from one individual give, in one case $\mu = 8,200$, in the other $\mu = 8,200$ above $12^\circ$, $16,100$ below $12^\circ$; on reversing the course of the temperature changes (dashed line), next day, the increment $16,100$ is now found to hold in this case above $12^\circ$. This type of change is one of those presumed to depend upon the irreversible progress of exhaustion in the isolated legs.

VI.

The results of these experiments have been considered in terms of regularities in the nature of the influence of temperature on the frequency of pulsation in the leg-"hearts" and in a wide variety of other
objects. The magnitudes of the Arrhenius constant are not distributed at random. For this the explanation has been proposed that constants $E$ or $\mu$ serve to characterize catalytic reactions governing the frequency of pulsation. It is possible to suggest a number of reasons why this sort of regularity "ought" not to be found. One method of dealing with the direct observations is to invent empirical formulæ for their expression. One such has recently been proposed by Bělehrádek (1926, a, b) in the form $y = \frac{a}{x^b}$, where $x = \text{temperature (Centigrade)}$ and $y = \text{time necessary for a given phenomenon, and } a$ and $b$ are constants. This is obviously the well known empirical formula of Esson (Harcourt and Esson, 1895; Harcourt, 1912) for chemical reactions, with the substitution of the Centigrade temperature for $T^\circ \text{ abs.;}$ it is difficult to conceive cogent reason, theoretically, for the proposed change, which simply requires making one of the constants larger without at all improving the fit. In addition to the

![Graph: A "break" in the curve of temperature relations was found in four cases, one of which is here shown, which is similar to that earlier described by Crozier and Stier (1924-25,b) in connection with the breathing rhythm of Anurans. The course of the observations was exactly reversed on raising the temperature. (The slope of the fitted lines ($\mu = 16,100$) is the average of those fitting the upper and the lower segments.)](image)
fact that this particular formula is of a type which can be made to describe *almost* any sort of curve not possessing too abrupt discontinuities, and that at best it has simply the status of an interpolation formula, there is the insuperable objection that it does *not* fit the facts. To illustrate this we may choose examples in which a large number of observations are available (Fig. 12). The statement (Bělehrádek, 1926, a) that a single simple curve can describe instances of the sort shown in Fig. 6 is obviously futile (*cf.* Brown, 1926–27). The attempt to employ his formula has moreover led Bělehrádek (1926, b) into the assertion that his constant $b$ reflects the primary importance of viscosity in connection with the velocities of vital processes, and that it changes systematically with the age of the organism. The notion that the temperature coefficient of a given activity decreases or increases regularly with the age of the organism is simply untrue, as our
own experience with a considerable number of cases enables us to state quite definitely. We make no apology for choosing to rely upon the one type of equation which actually describes the data and which seems to stand a good chance of physical interpretation; and until some other comprehensive explanation is provided for the regularities which its use discloses we are not impressed by objections grounded, largely, on the argument that protoplasm is “too complex.” The efficient answer to such very general objections is, that the complexity, or rather haziness, frequently resides in the mind of the observer, and can be dissipated by increased refinement of experimental procedures.

A more interesting sort of obstacle has recently been discussed by Murray (1925–26). In his experiments the temperature characteristics deduced for frequency of pulsation in cultured explants of chick myocardium failed to show uniformity, and, in the number of preparations studied, failed to be grouped about modal values. We are not familiar with the performance of such cultures, but it can be suggested

3 It is perhaps of interest, since the present material enables several points of some moment to be illustrated, to comment upon an objection sometimes voiced to the practice of fitting two or even three lines to segments of such data as are plotted, for example, in Fig. 6. It may be said that the implied transitions are too sharp. If one were really dealing with the effect of temperature upon a catenary series of reactions there should be a region of curvature connecting the two sensibly rectilinear zones. The answer is that in suitable instances just this indication of curvature is actually found. Most series of measurements exhibit a latitude of variation which makes it difficult to decide the precise nature of the union between the two straight lines. A number of series in the present experiments (e.g., Fig. 6) do however show this sort of “rounding off” when the latitude of variation is quite small. Objections to considering the whole sweep of such series as a single curve have been mentioned in a preceding paper (Crozier and Stier, 1926–27, a). Sharp transitions of the sort implied in these broken graphs are of course well known in physics; we may refer to transition points, and to curves of magnetic susceptibility. Moreover, as illustrated in a recent paper from this laboratory (Brown, 1926–27), the data so fitted cannot be described by a single smooth curve when plotted directly as velocities against temperature. Again, as has been insisted previously (Crozier, 1925–26, a), there is indication of “physical” alterations occurring at just those temperatures most frequently found to be transition points on the thermal scale. The reason for the occurrence of just these critical temperatures must be sought in the properties of protoplasm as a physicochemical system.
that in such a preparation, essentially an unorganized mass structurally though exhibiting regularity as to pulsation, there must exist a number of possible pace makers. It is to be expected that the net result of their fluctuating dominance might obscure the preponderating in-

Fig. 11. Occasionally, instances were found in which an abrupt change of increment was associated with an abrupt increase of rate.

fluence of any one, since it is fair to assume that their several inner metabolic states might be differently adjusted. As we have pointed out in detail in a following paper, this interpretation permits certain deductions which the figures in Murray's paper seem to us to justify.
Fig. 12. Data from one experiment (in Fries, 1926-27) on the frequency of cardiac contractions in *Blatta* are fitted by the Arrhenius formula with $\mu = 12,600$ between $10^\circ$C and $38^\circ$C. In the original presentation (Fries, 1926-27, Fig. 2) these data appear plotted as log frequency vs. $1/T^\circ$ abs., where the nature of the "break" at $10^\circ$ is more clearly apparent; below $10^\circ$, $\mu = 18,100$. The dashed lines are transposed from the lines defining the extreme latitude of variation in the log plot (Fries, 1926-27, Fig. 2). The superiority of the logarithmic representation (vs. $1/T^\circ$ abs.) consists not only in the clearer appearance of conditions on either side of a critical temperature (e.g., $10^\circ$ in this case), but also in the fact that the departures from the line of best fit are confined within a band the upper and lower edges of which are parallel to the central line (cf. Crozier and Federighi, 1925). The latter fact means that it is necessary to deal, in fitting the Arrhenius equation to such data, not with the absolute departures of the observed average rates or frequencies, but with the relative or proportionate divergences; this is of great importance if it be suggested that curves such as that in the present figure should be fitted by the method of least squares (cf., also, Crozier and Federighi, 1924-25, b, 1925).

To curves obtained by the method just described there has been added (a, b, -- --- --- ---) the result of an attempt to fit the observations by means of the formula proposed by Bělehrádek (1926, a). This formula, $time = A/(^\circ C)^b$, (or rate = $(^\circ C)^b/A$), tested by appropriate plotting of the observations as log rate vs. log $(^\circ C)$; in such a plot the best fitting straight line was adjusted, and has then been transferred to the present figure. It is obvious that the fit is anything but significant; nor can the adjustment of the ends of the curve be made such as to improve the fit; from the nature of Bělehrádek's formula, correction of the lower end of the curve, for example, merely increases the deviation at the upper end.
Extensive data upon the myogenic heart of *Limulus* larvae (Crozier and Stier, 1926–27, b) have convinced us that the relation of pulsation frequency to temperature is of a perfectly regular sort, with definite and recurrent values of \( \mu \).

VII.

This paper is by intention an introductory account of material which it is designed to employ for certain types of experiment. But we may point to several conclusions which the preliminary examinations seem to justify. The rhythmic neuromuscular activities of arthropods, so far as tested, fall into two general categories as regards the critical thermal increments which they reveal. In one class are found movements of heart and of locomotor appendages and the rhythms of stridulation and of luminous display (Crozier, 1924–25, a; Crozier and Federighi, 1924–25, a, b; Crozier and Stier, 1925–26, a; Fries, 1926–27; and some other instances as yet unpublished). These we have reason to regard as controlled by nerve centers, and they show a high degree of consistency in providing \( \mu = 12,200 \) to 12,500. Occasionally this value is associated with a higher one over the lower portion of the temperature range, \( \mu = 18,200 \) or 23,500. On the other hand, movements of respiration (Crozier and Stier, 1924–25, a; and other data unpublished), presumably also determined by nerve center activity, but demonstrably of a different kind, consistently yield other increments,—those, namely, associated with cell respiration itself (Crozier, 1924–25, b).

The isolated leg of *Notonecta* with its “heart” is remote from central nervous control of the type pretty certainly involved in the movements of the dorsal vessel (cf. Alexandrowicz, 1926). We are not yet able to say just what the thermal relations are in the intact animal, but in the isolated leg—“heart” the increment 12,300 ± does not appear among the several values encountered. On the other hand, the values actually found (Table I) are those characteristic of oxygen utilization, heat production, and CO\(_2\) production. We do not conclude from this, of course, that the leg—“hearts” are therefore concerned with respiration, but merely that the metabolic activities underlying and controlling the rates of pulsation are certainly different from that determining the increment 12,300.
It is notable that in material of this sort we should rather expect serious disturbance of rectilinear relationships between \( \log \text{rate} \) and \( 1/T \), such that plainly curvilinear graphs would be gotten, or progressive shifts of \( \mu \). But the fact is that here, as in the case of grasshopper respiratory motions (Crozier and Stier, 1924–25, a) and in that of the heart of *Limax* (Crozier and Stier, 1925–26, b), when change of \( \mu \) occurs it takes place abruptly and by a definite amount. This speaks strongly for the individualized character of the several processes which may control the rate of pulsation.

The presence of increments often associated with respiratory phenomena led us to attempt to alter the increment by controlling the oxygen supply. We were unable to obtain consistent differences by comparing hearts in legs immersed in water through which \( O_2 \) constantly bubbled, legs swathed in vaseline, or legs sealed in tubes with a small volume of water. The differences found are for the greater part attributable rather to the metabolic condition of the whole insect. This we expected to find influenced by laboratory confinement. It is of interest, therefore, to note that the increments 32,000 ± were chiefly, although by no means exclusively, obtained from individuals not more than 2 weeks after collection. The increments 19,800 and 23,800 appeared only in the cases of legs from *Notonecta* kept for some 2 months or more in the laboratory.

**VIII.**

**SUMMARY.**

The frequencies of pulsation of the "accessory hearts" in the isolated swimming-legs of *Notonecta* were studied in relation to temperature, with the idea that in such organs central nervous control is impossible, and that in an isolated system irreversibly proceeding toward death it might be expected that further evidence would be found regarding the supposed specific significance of critical thermal increments. A number of values of \( \mu \) are found, commonly 8,200; 16,200; or 32,200; less frequently 11,400; 19,800; and 24,500. These values are definitely contrasted with that (12,300) typical for heart beat frequencies in arthropods. They exhibit interrelationships of the sorts already found in other cases. There occur also sharp irreversible changes in
frequency of pulsation, which may or may not be accompanied by change of increment. The net result is held to be confirmatory of the interpretation of thermal relations proposed in earlier papers.

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