PULSATION FREQUENCY OF THE ADVISCERAL AND
ABVISCERAL HEART BEATS OF CIONA INTESINALIS
IN RELATION TO TEMPERATURE

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(Accepted for publication, May 23, 1932)

Pulsation frequency of the heart beat in relation to temperature
has been studied in a variety of organisms (cf. Crozier, 1924, 1926,
a, b; Crozier and Federighi, 1925 a, b; Crozier and Stier, 1925, a, b,
1926, 1927, a, b; Fries, 1926; Murray, 1925–26; Glaser, 1929; Parpart
and Glaser, 1930). Typical values for temperature characteristics
(μ) and critical temperatures were repeatedly found, which fall into
definite groups. Of particular interest were cases in which during
embryonic development or by way of operations, changes in thermal
increments could be observed. Studies on the embryonic heart of
Limulus (Crozier and Stier, 1927, b) showed that more, different μ
values could be found in earlier stages of development (myogenic
pulsation) than later on or in the adult (neurogenic control of pulsa-
tion). Similar conditions were met in embryos of Fundulus (Glaser,
1929), and in the chick embryo (Parpart and Glaser, 1930). Cases
where changes in temperature characteristics were produced subse-
quent to operative interference are found in Notonecta (Crozier and
Stier, 1927, a) and in Gonionemus (Wolf, 1928). Findings of this
kind suggest the assumption that during embryonic development or
by way of operation the mechanism which controls pulsation has been
altered. In organisms where we find different centers which initiate
pulsation we might expect to find different controlling mechanisms or
pace makers, and an investigation of such cases might throw some
light upon the question of the number or diversity of different pace
makers in control of pulsation.

In tunicates we meet the fact that the heart beat regularly reverses
after certain intervals, so that the blood is pumped for some time into
the region of the gills and afterwards backward into the body region
(van Hasselt, 1824, Krukenberg, 1880, Knoll, 1893, Loeb, 1899,
Schultze, 1901, Straub, 1901, Hunter, 1902 and 1904, Nicolai, 1908,
Hecht, 1917–18, Brücke, 1925, von Skramlik, 1926. In Ciona intesti-
nalis, for example, the heart beats regularly in advisceral direction
about 140 times, after a few irregular beats the pulsation becomes
reversed for about 70 to 100 beats. The total number of pulsations
in one or in the other direction is not constant; it differs with age and
size of the animal. In general however it can be said that there are
always more advisceral pulsations than abvisceral ones. In Salpa
(von Skramlik, 1926) and other forms the change of direction happens
very much more irregularly than in Ciona. The circumstances we
meet in tunicates suggested a study of the two types of heart beat
in relation to temperature.

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The material used was collected by the fishing crew of Naples Zoological Sta-
tion at Mergellina and Capo Posilipo during September, 1929. Usually the
animals were kept in running sea water overnight before being used for experi-
mentation. Brought in from the Gulf of Naples the tunica is in most cases covered
so heavily with dirt, and the intestinal tract is so little transparent, that it is not
possible to see the heart without cutting the tunica. To avoid any kind of an
operation which might affect internal pressure and thus influence the heart beat,
as described by Hecht (1917–18), it was only necessary to leave the animals for
several hours in clean water, where they became more transparent so that one can
see the heart quite easily through the tunica by placing the animal so that the anal
opening shows to the right hand of the observer. When the heart was easily
visible the animals were taken for observation. Less attention was paid to the
size of individuals, but in general only smaller animals 6 to 12 cm. in length when
fully expanded were used. Stop-watch readings of the time for ten pulsations
of the heart in advisceral direction as well as in abvisceral direction were taken,
and the results thus obtained were treated separately. Altogether twenty-two
animals were used. Over 3000 stop-watch readings were taken for ten pulsations
each; in the graphs there are plotted 300 points representing averages of these
readings.

During experimentation the animals were placed in dishes about 8 cm. in
height, submerged in a large glass aquarium in which the temperature could be
changed by adding ice or hot water. The temperature was kept constant by a
stirring motor during the run of an observation for about 15 to 20 minutes, to
within about 0.3°C. Between the readings at one temperature and another,
when the change of temperature took place, no readings were taken for at least 20 to 25 minutes, to give the animal time enough for thermal adaptation. All the animals were placed in their containers in the same manner. If by contraction of one of the syphons an individual changed his position by rolling over so that the heart was no longer visible, it was brought back into its original position by touching it gently with a thin glass rod. The beating heart was observed by a low power Zeiss binocular microscope.

The successions of pulsation in adviceral and abvisceral directions are somewhat different, the total number of adviceral pulsations being generally greater than the number in abvisceral direction. Similar conditions have been described in earlier papers as cited. In general the sequence of beats is very regular at a given temperature except for the first few and the last few beats before the reversal of direction of the pulsation. The first few beats always follow one another faster and the last beats slower and more irregularly. In the absolute frequency no significant difference could be found between ad- and abvisceral pulsations at any temperature. This fact contradicts somewhat the statement of Roule (1884), who claims to have found a higher pulsation frequency for adviceral beats in younger forms of Ciona intestinalis. The results obtained for adviceral pulsations are plotted with the ones for abvisceral pulsations in the same figures. The open marks give the values for the adviceral, the solid marks for the abvisceral pulsations.

The data obtained from twenty-two animals used in these observations are given in Figs. 1 to 3, where the logarithm of the frequency of the heart beat has been plotted against the reciprocal of the absolute temperature. Each point represents an average value of ten readings taken at the corresponding temperature during a run. The different series describe narrow ribbons through which straight lines can be drawn. The relation of pulsation frequency to temperature adheres to the Arrhenius equation \( \frac{k_2}{k_1} = \exp\left(\frac{\mu}{R} \left(\frac{1}{T_1} - \frac{1}{T_2}\right)\right) \), where \( k_1 \) and \( k_2 \) are proportional to velocity constants at the respective temperatures \( T_1 \) and \( T_2 \), \( R \) is the gas constant, and \( \mu \) is the "critical thermal increment" or temperature characteristic.

The data obtained for each animal were at first plotted separately and the values for the temperature characteristics calculated. According to the values found and to the location of changes in the curve of pulsation frequency, the animals were brought into different groups. The rate of pulsation is not always the same in different animals at the same temperature, so that in bringing the animals of one group
Fig. 1. Observations on pulsation frequency of the heart of *Ciona*. Data from eight animals. $\mu = 7750$ calories above 20°C, and $\mu = 11,820 \pm$ below 20°C. The readings for advisceral beats are indicated by white marks, for abvisceral beats by black marks.
Fig. 2. Observations on pulsation frequency of the heart of *Ciona*. Data from thirteen animals. $\mu = 16,000$ calories below 15°C. Above 15°C, the values are different. One group showing $\mu = 16,000$, the second $\mu = 12,000$, and the third $\mu = 8000$. The readings for advisceral beats are indicated by white marks, for abvisceral beats by black marks.
together the frequency had to be multiplied by a certain factor in each case. It has to be said, however, that in no case where a "break" occurs have the two parts of the plot above and below the critical temperature been multiplied by different factors. Thus the mean values for the temperature characteristics could more easily be determined, and in case a "break" occurred its location was more readily found.

The first group contains observations made on eight animals between 8° and 32°C. At higher temperatures a \( \mu \) value of 7750±

\[ \text{calories was obtained. At a temperature of about 21°C, a break occurs, and below this temperature the } \mu \text{ value is 11,820± calories. These values fit the data both for pulsation in advisceral and in abvisceral direction (Fig. 1).} \]

The results from 13 other animals investigated are represented in Fig. 2. For the temperature range below 15°C., for all these individuals, we find the temperature characteristic 16,000 calories. Above 15°C. the individuals behave differently. In some of the cases the
\( \mu \) value is 16,000 calories just as below 15°C., no change in the curve of pulsation frequency being noticeable. For three animals we find a break at 15°C., with the \( \mu \) value about 12,000 above the critical temperature. In four other animals the break occurs at the same temperature (15°) but the \( \mu \) value found above 15° is 8000. For these four animals there is in addition quite clear evidence of a change of latitude of variation at about 10°C. with a downward shift; the thermal increment, however, stays unchanged.

One additional animal cannot be brought into any of the preceding groups. The \( \mu \) value found for the temperature range below 15°C. is 13,000 calories, whereas above that critical temperature we obtain 6,760. During the change of temperature going up and down the temperature scale we find a difference in the absolute pulsation frequency. The thermal increment, however, is the same in both cases. The reasons for the change in frequency must be found in some destructive changes at the highest temperatures to which the animal had been exposed (above 30°). Similar cases have been recorded in Notonecta (Crozier and Stier, 1927, a; and Stier, unpublished data).

In most of the cases great difficulty was met in taking readings above 32°C. and below 12°C. Usually the heart stops beating in diastole at temperatures below 10° or 11°C. and at temperatures higher than 31° the pulsation becomes so irregular that no constant readings for pulsation can any longer be obtained. On raising the temperature even higher, the heart stops in diastole until the temperature has been decreased to about 30°C. But even then it is found that in a great many cases the irregular beat does not cease, or that the heart does not come back to function at all for several hours. Similar cases have been described in other tunicates by Hecht, Hunter, Knoll, Wagner, Lingel, Nicolai, Roule. Apparently these extreme temperatures fall too far outside the normal environmental temperature of Ciona intestinalis found in the Gulf of Naples, where temperature of the water stays around 18±°C. throughout the year.

By examination of the data for each individual a variety of \( \mu \) values could be found. In Fig. 2 for example, we have in all animals the same increment for the lower temperature range but above the critical temperature the \( \mu \) values are quite different. If all the data given were plotted en masse without determining the \( \mu \)'s singly a curvilinear
plot would be obtained for which no definite \( \mu \) could be determined (cf. Cole, 1929). This would be due to the different temperature characteristics over the upper range of temperatures (cf. Crozier, 1924; Crozier and Stier, 1925, a; Glaser, 1924).

An attempt was made to use earlier data on pulsation frequency of heart beat in tunicates in relation to temperature. Hecht (1918) has given some data for ten pulsations in ad- and abvisceral direction for *Ascidia atra* at temperatures between 17° and 37°C. The data are relatively few. The approximate \( \mu \) value for the range between 21° and 35°C. is about 12,500 + calories, a value which can be compared easily with values found in *Ciona* in some combination with other magnitudes. In the case of Hecht's observations the temperature characteristic for pulsation in the two directions is the same.

More recently data on *Salpa africana* were published by von Skramlik (1926). In his experiments only readings for pulsation frequencies at temperatures 10° apart were taken for single animals, from which he calculated \( Q_10 = 2.1 \) according to the van't Hoff rule. Computing from his data the time for ten pulsations and plotting the values obtained *en masse* for determination of the temperature characteristic, no definite \( \mu \) value could be determined as the points are too scattered and too few. Furthermore, there might be assumed a change in thermal increment at some critical temperature, just as has been found in *Ciona*, so that a determination of pulsation frequency at only two different temperatures would give us no information at all as to the kind and variety of factors involved in the mechanism controlling pulsation frequency. One fact, however, can be collected from von Skramlik's data, namely that in plotting the frequencies for abvisceral pulsations at different temperatures against the abvisceral ones we find an essentially rectilinear relationship with a slope of 1,—which indicates that in *Salpa africana* for both ad- and abvisceral pulsations the frequencies are the same.

The \( \mu \) values found for abvisceral and abvisceral pulsation frequency of the heart of *Ciona intestinalis* are as follows: 8000, 12,000, and 16,000 in several combinations, and with diverse critical temperatures at 10°, 15°, and 20°C. The fact that the two ends of the heart behave alike in any one individual tested is suggestive for the notion that the same metabolism prevails in the two pace makers at the opposite ends, as is evident also in their identical rates.¹

¹The results of these experiments where the pulsation frequency of ad- and abvisceral heart beat is the same at any temperature in any one individual suggests the desirability of testing a possible relationship between the duration of sequences of ad- and abvisceral heart beat to temperature. For these experiments no particular attention was paid to this possibility.
In this connection it might perhaps be pointed out that according to the results obtained in these experiments we are not permitted to draw any further conclusions about the mechanism controlling pulsation frequency. Known facts are that values like 8000, 16,000, and 22,000 are connected with mechanisms at least partly respiratory in type; \( \mu \) values of 10,000 and 12,000 calories have been associated with neuromuscular activities (Crozier, 1924; Crozier and Stier, 1925, a, b, 1927, a; Wolf, 1928; Steiner, 1932). In experiments with embryonic hearts, where in earlier stages less uniform and more different \( \mu \) values are found than in adult organisms (Crozier and Stier, 1926–27; Glaser, 1929; Parpart and Glaser, 1930), it is tempting to assume that the changes in temperature characteristics are connected with the development of the nervous control of the heart beat. All one can say, however, is that the occurrence of different temperature characteristics for the two cases, embryonic heart and adult, is probably due to the fact that the respective controlling processes are unlike. Only in case one could obtain embryonic preparations of the same state of development but differing concerning the nervous control and then compare their temperature characteristics, might the data perhaps be used for further conclusions (cf. Crozier and Stier, 1927, b).

**SUMMARY**

The frequency of pulsation of the heart of *Ciona intestinalis* increases with temperature in both advisceral and abvisceral direction, according to the Arrhenius equation. The increase in pulsation is the same in both directions. The following \( \mu \) values were obtained: 8,000—, 12,000—, 16,000, in several combinations, with critical temperatures at 10°, 15°, and 20°C. The values found are comparable with earlier findings for activity of the heart in different animals. This quantitative correspondence suggests anew the conception that temperature characteristics may be employed for recognition of controlling processes. The fact that the \( \mu \)'s and the critical temperature are the same for advisceral and abvisceral beats, indicates that the general metabolic condition of the two ends of the heart is the same in any one individual.
CITATIONS

Loeb, J., 1899, Einleitung in die vergleichende Gehirnphysiologie und vergleichende Physiologie, mit besonderer Berücksichtigung der wirbellosen Tiere, Leipsic, Johann Ambrosius Barth.
Navez, A. E., 1931, Protoplasma, 12, 86.
Schultze, L. S., 1901, Jaenische Z. Naturwissensch. 35, 221.
Straub, W., 1901, Arch. ges. Physiol., 86, 504.