

TEMPERATURE CHARACTERISTICS FOR PULSATION FREQUENCY IN GONIONEMUS.

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I.

The analysis of vital activities in terms of specific governing processes through their quantitative control by temperature (*cf.* Crozier, 1924, 1924–25, *b*) requires as an essential preliminary the collection of adequately determined values of the temperature constants or *temperature characteristics* (Crozier, 1924–25, *a*), μ of the Arrhenius formula. As obtained from a variety of experiments with the most varied biological phenomena the magnitudes of this constant are found to be grouped in a manner indicating specific significance (Crozier and Stier, 1926–27, *b*), and this is true also where several values of μ are found associated with the same kind of phenomenon under different circumstances in the same kind of organism (*cf.* Crozier and Stier, 1925–26, *a, b*; 1926–27, *a, c*). The fact that activities of the same general kind in organisms not too distantly related appear to provide concordant magnitudes of μ independently encourages an objective classification of the dynamics of these activities (Crozier, 1924–25, *a*; Crozier and Stier, 1924–25, *a, c*; 1927–28; Glaser, 1925–26, *a, b*; Crozier and Federighi, 1924–25, *a, b*; Fries, 1926–27). The disturbances which may be introduced into the determination of temperature characteristics by the occurrence of critical temperatures at which the quantitative relationship between temperature and rate or frequency is altered, and by the precise sort of alternation encountered, have been discussed in various previous publications (Crozier, 1924–25, *b*; Crozier and Stier, 1924–25, *b*; 1926–27, *a, c*). For certain instances in which there is measured the dependence of frequency of action upon tempera-

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ture, the value of μ at first sight has seemed non-specific. One such case has been recorded by Murray (1925–26) in the beating of explants of embryonic chick heart. In explanation of it the suggestion has been made (Crozier and Stier, 1926–27, *c*) that where more than one local pace maker is functioning, but with differing intrinsic values of μ , there should result distributions of the observations exactly of the general types found. Certain deductions from this interpretation, or connected with it, have been tested (Crozier and Stier, 1926–27, *c*). It was pointed out (*loc. cit.*) that the effect invoked might under certain circumstances be duplicated experimentally with the hearts of tunicates or with the pulsating swimming bell of medusæ. The work of this paper has been devoted to the examination of the relations between temperature and frequency of contraction in *Gonionemus*.

A number of papers have been published in which this question has been discussed, especially for scyphomedusæ (*cf.* Mayer, 1914; Cary, 1917; Schaefer, 1921). It must be said, however, that in no case are the data sufficiently numerous to permit more than suggestive deductions as to the magnitudes of μ which may characterize pulsation frequency, and as to the occurrence of critical temperatures. There are required very numerous readings, upon each of a good number of individuals, in order that the latitude of variation at constant temperature may be adequately dealt with (*cf.* Crozier and Federighi, 1924–25, *a*; 1925; Crozier and Stier, 1924–25, *a*, etc.; Glaser, 1925–26, *a*). This is particularly important when the frequency as measured is high, and the possibility of observational error therefore increased. Before employing these medusæ for the experiments ultimately in view it is necessary to determine if the frequency adheres to the Arrhenius formula, and if there may not be differences among “normal” individuals, as well as to test the effects of certain operations upon the marginal sensory ring. The earlier work upon medusæ has indicated that any of the many marginal sensory organs may be brought to serve as pace maker, the fastest beating region of the margin determining the pulsation of the whole (Loeb, 1900; Mayer, 1906, 1910; Bozler, 1926). The question first to be decided is the degree of uniformity exhibited in the relationship to temperature when there is a possibility that not all the marginal organs are metabolically in exactly comparable states, so that (in terms of the

general theory already discussed) they might not exhibit the same temperature constants.

II.

The material used in these experiments was collected in the Eel Pond at the Marine Biological Laboratory, Woods Hole, during summer months. The individuals varied between 5 and 25 mm. in diameter. In the different experiments animals of all available sizes were used. The activity of these is in general independent of size. There are, however, differences in the number of pulsations per unit time; but this does not affect the way in which the frequency of pulsation changes regularly with the change of temperature. Altogether 76 animals were used during the experiments, 44 during July and August and 32 after a short interval in September, for a reason which is explained later. 5130 stop-watch readings were taken, the greater number for 10 contractions each, a smaller for less than 10, in cases where the animals did not beat regularly enough to get 10 pulsations between pauses. In the figures there are plotted 662 points, representing averages.

The animals were placed in a glass beaker submerged in a large glass vessel in which the temperature could be controlled by adding ice or hot water and kept constant within 0.2°C . during an interval of 10 to 20 minutes. Freshly collected animals are rather active and are in motion most of the time. If however they are kept in the aquarium for several days, beating occurs only seldom. In these cases the animals were touched with a thin glass rod and thus caused to swim actively for awhile. During rainy and cloudy days the activity is only partly as good as on clear days. Animals collected during bad, rainy weather are almost impossible to use for these experiments because the pulsations are not sufficiently regular.

The individuals used are to be divided in six classes, according to the operations made. The first class are normal animals, without any operation or known interference. The second group are animals where the nerve ring was cut twice, on opposite sides of the margin of the bell. The third class are animals where the nerve ring was entirely removed. Class 4 contains animals with one cut through the nerve ring; Class 5, animals where the nerve ring was cut four times; class

6, animals cut in half. The results obtained during July and August were plotted in terms of the Arrhenius equation. In the beginning of September the experiments were repeated. Each of the different groups of animals was tested again. The observations entirely paralleled the results obtained before.

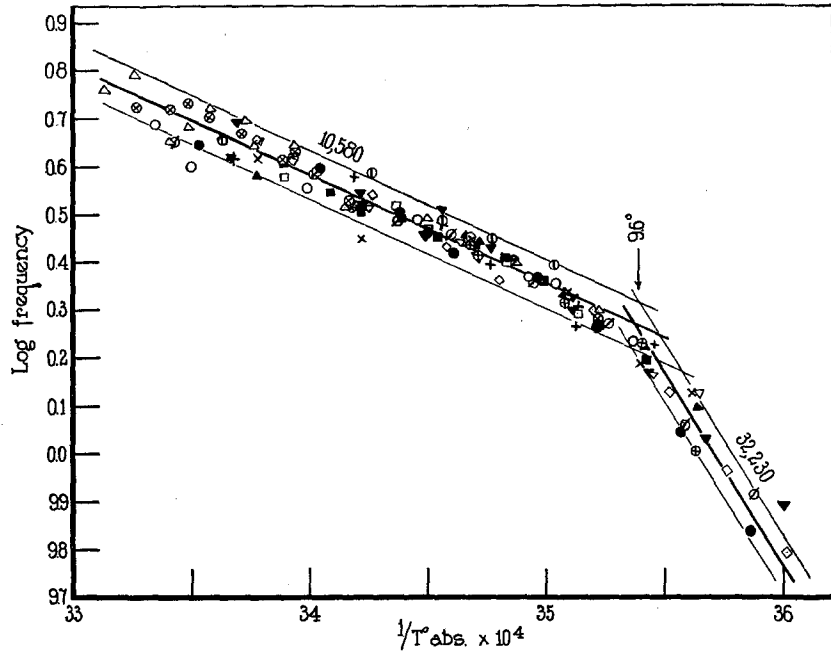


FIG. 1. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data from 18 normal animals, at temperatures between 4° and 28°C., for which $\mu = 10,580$ between 9.6° and 28°C. and $\mu = 32,230$ between 4° and 9.6°C.

III.

The 29 "normal" individuals used for the measurement of the frequency of the contraction must be subdivided into three groups, according to the μ values and the critical temperatures which they show. Most of the animals give $\mu = 10,580$ calories between 29° and 10°C.; below 10°C. $\mu = 32,230$. The break is rather sharp at a temperature of 9.6°C. These data are collected in Fig. 1.

A second group of normal animals gives $\mu = 8,160$ above 14°C. and

22,510 below 14°. Finally, 3 animals were found which could not be brought into either of the preceding two groups. The value of μ for these, taken together, was found to be 11,420 above 12.3°C. and 22,800 below about 12°C.

Figs. 1, 2 and 3 give the plots of the results obtained in these experiments. On comparing the μ values with those already known as typical it is seen that they are similar to values found in a great many other cases and that the critical temperatures happen to be located at places where they are typically found (cf. Crozier, 1925-26, a, b).

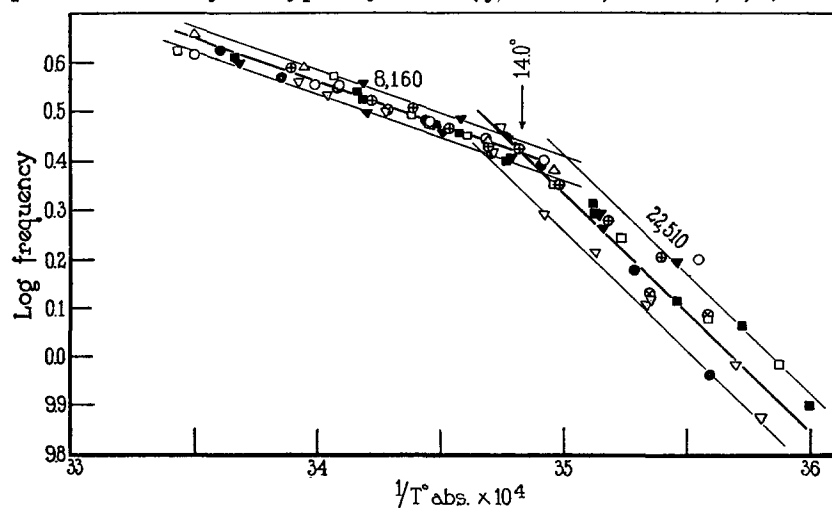


FIG. 2. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data from 9 normal animals at temperatures between 4° and 26°C., for which $\mu = 8,160$ and between 14° and 26°C. and $\mu = 22,510$ between 4° and 14°C.

At first the data from each animal were plotted singly and the value for the temperature characteristic calculated. The scale on the ordinate was chosen very large, therefore even the largest scatter of points represents not more than 1 second difference in the pulsation frequency. According to the values found, and to the location of the "breaks," the animals were brought into the different groups into which they clearly fell. The rate of pulsation in the different individuals is not exactly the same, so that in bringing the groups together the frequency has to be multiplied by a certain small factor in each

case, which changes the relative frequency but not the slope of the line and hence not the μ values. After having brought the different groups together in this way the location of a break is easier to determine precisely (because of the larger number of observations) and the mean value for the temperature characteristics is easily found. Special attention has to be paid to these regions of the plots located around a "break," where one finds a "rounding off" in such a way

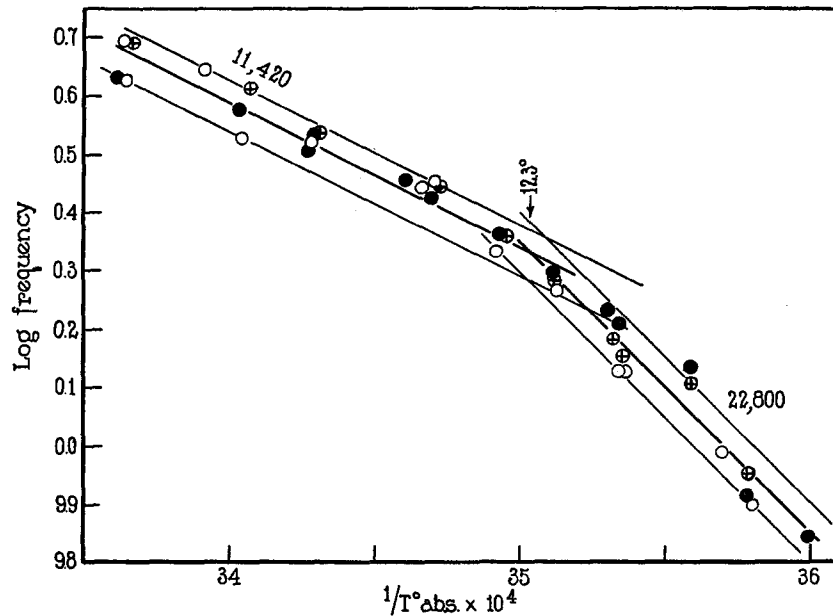


FIG. 3. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data of 3 normal animals at temperatures between 4° and 24°C., for which $\mu = 11,420$ between 12.3° and 24°C. and 22,800 between 4° and 12.3°C.

that most of the points fall nearer to the lower margins of the best fitting ribbons (Figs. 1 and 2). This shows that in going up or down the temperature scale the single readings in the neighborhood of the break may belong sometimes to the higher, sometimes to the lower μ value. Other reasons for this "rounding off" have been mentioned in earlier papers (Crozier and Stier, 1926-27, *a, b, c*). Furthermore, it is to be noticed that in all cases when animals were found constituting a separate group the number of individuals was always greater than

one, and that they recurred at different times during the course of the summer.

IV.

In the second class there are placed the results obtained from 17 animals where the nerve ring was cut in two places, on opposite sides of the margin of the umbrella. This operation has in most of the cases no effect on the rate of pulsation at room temperature. In several cases, however, the beat becomes irregular and retarded for the 1st hour after the operation, which is indicated in the plot (Fig. 7) by a number of points which fall off the normal band at the beginning of the several "runs." Later the pulsation becomes as regular as in normal animals, so that the points fall near together and adhere well to the line describing the course of all. The animals used can be placed together in one group. The μ value for temperatures between 29° and $13.4^{\circ}\text{C.} = 10,510$ calories, and below $13.4^{\circ} = 21,380$. The value for the higher temperature range is the same as for the first group of the normal animals. For lower temperature range we notice a difference, since the animals with two marginal cuts give $\mu = 21,380$ calories, but the normals 32,000. Furthermore, attention has to be paid to the fact that in this case the "break" occurs at 13.4°C. , in the case of the "normals" at 9.6°C.

In the second group of the "normals", however, the "break" occurred at 14°C. , which corresponds sufficiently well to the result for the animals with two cuts.

In the same way, animals with only one cut and others with four cuts were used. The few experiments with animals having one marginal cut were incomplete, but the μ value is approximately the same as in the experiments with the animals with two cuts.

Only 2 animals with four cuts were used. In the activity there is no difference, in general, when compared with the animals used before. The μ value calculated for a high temperature range = 10,660 and for lower temperatures = 21,000, the break occurring at about 13.1°C. These values correspond well with the values for the individuals with two marginal cuts.

Looking over the results of these experiments, it can be said that we get for all the animals, for the temperature range above 15°C. , only

two different values for the temperature characteristics, *i.e.*, $\mu = 11,000 \pm$ and $\mu = 8,000 \pm$; for lower temperatures we get $\mu = 21,000 \pm$ and in one group $\mu = 32,000$. These values are closely comparable with values well established in earlier experiments. The critical temperatures, also, are located at places where they have been found before.

The operation of cutting through the nerve ring might help to show whether the contraction of the umbrella is controlled by only one pace maker or by more. On the supposition that we have to deal with more than one pace maker, it is to be expected that we should get different μ values for animals treated in the same way; or better, we should expect the points as plotted to be largely "sprayed" in the form of a fan, which would indicate that sometimes the contraction is under the control of one pace maker and then under the control of another one for which supposedly different μ values might be obtained. This expectation was not realized. The μ values are rather constant for animals after the different operations.

Finally, there is to be added to the foregoing groups one more, where the medusæ were cut in half. Only four such preparations were used. The contraction of the umbrella becomes rather irregular after the operation but later becomes sufficiently uniform. The beat is occasionally irregular; and the animals may keep quiet for a long time, in which cases they were touched with a glass rod to activate them. The value for μ found for a temperature range between 19° and 4°C . is 16,900, without evidence of any break. In view of the fact that only four half animals were used and not enough points were gotten for the plots, no particular weight can be put on this result, which nevertheless is of interest for the subsequent discussion.

v.

For the remaining experiments the nerve ring was entirely cut off, in 22 animals. After the operation the medusæ stop beating for a long interval, beating recovers but there is a difference between the beating and that in the groups already considered. The contraction of the umbrella is more quick and violent, and the time between two beats accordingly longer. Furthermore, ten beats, one after another, occur only seldom; in many cases only five, six or eight beats can be

counted in a continuous group. The animals are not particularly active, so they had to be touched often with the glass rod; after stimulating in this way the first two or three beats are irregular. Measurements were taken as soon as the beats became regular again. The results of the observations are given in the plot of Figs. 4 and 5. The animals can obviously be arranged in two groups. The first one (8 cases) gives a μ value of 16,000 for the whole temperature range, without break. The second group (10 cases) shows $\mu = 8,100$ above 15°C .,

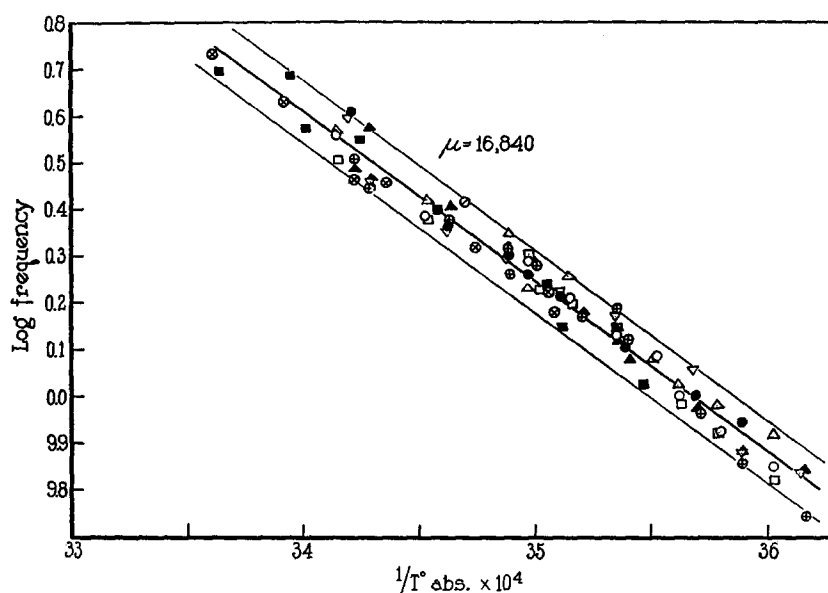


FIG. 4. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data from 8 animals where the nerve ring was entirely taken off, at temperatures between 26° and 4°C ., for which $\mu = 16,840$.

between 15° and 9.4°C . $\mu = 16,530$ and below 9.4°C . a value of 29,000 calories. The "low" readings for the high temperature range, in the case of the second group (*cf.* Fig. 5), are largely due to the fact that apparently the readings were taken too soon after the operation, when the animals were not yet sufficiently recovered. The points are rather irregularly spread over a large range. The value for the middle part corresponds to that for the first group but for the lower range we get a different value from that for the first group. The break is located on

the same place as with the "normal" animals and with the animals having two cuts. Looking over the first group of those without nerve ring we find, however, that in some animals perhaps a slight indication of a break, or of irregularity, can be found at 10°C., but it is not so certain that the animals can be taken out of the $\mu = 16,000$ group.

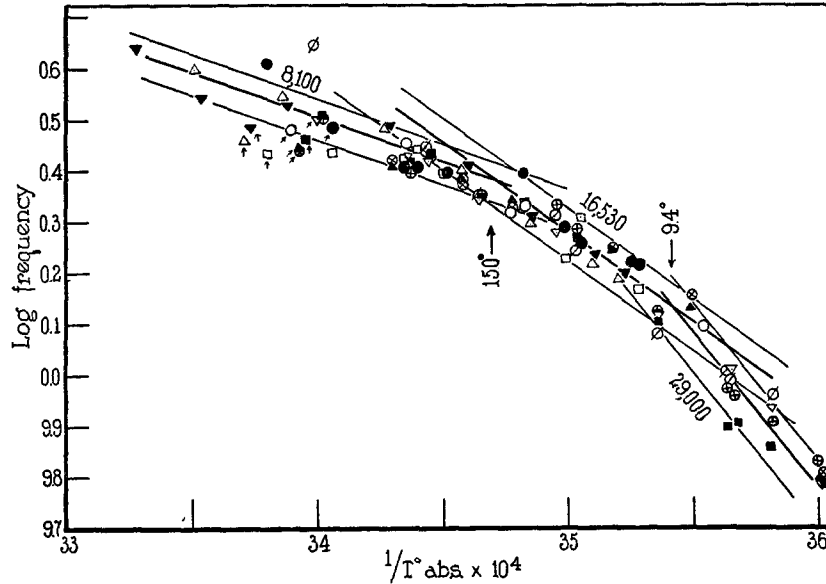


FIG. 5. Observations of the frequency of the contraction of the umbrella of *Gonionemus*. Data from 10 animals in which the nerve ring was taken off. The μ value for a temperature range between 15° and 26°C. = 8,100; for temperatures between 15° and 9.4°C. = 16,530; and $\mu = 29,000$ for temperatures between 9.4° and 4°C.

VI.

The fact that not all the normal animals show the same increments nor the same critical temperatures is parallel to earlier findings in other cases (Crozier and Stier, 1924-25, *c*; 1926-27, *a, c*). On the other hand it does seem true that where different μ 's appear for the same activity in the same kind of organism, either without deliberate experimental interference (and hence "normally," as it would usually be said), or as result of direct operation or treatment, the increments found appear to belong to a consistent system (Crozier and Stier, 1924-25, *a*).

The respiratory phenomena of insects and other animals provide $\mu = 8,000 \pm; 11,000; 16,000 \pm; 32,000$. So also in the present instance, these increments are found, combined in various ways, and with the addition of $22,000 \pm$ for certain individuals at lower temperatures. The interrelations between these values, as in *Notonecta* (Crozier and Stier, 1926-27, *a*), do not encourage the notion that the 2:1 ratio sometimes suggested by the μ magnitudes above and below an intermediate critical temperature is in itself of any significance.

The different classes of "normal" medusæ are not obviously distinguishable on the basis of known accidents of weather, time, size or relative frequency of pulsation. They correspond to those internally existing metabolic differences which have been appealed to in the case of the hearts of embryonic *Limulus* (Crozier and Stier, 1926-27, *c*). It is noteworthy that in no single case is there evidence of the fan-wise distribution of the logarithmic pulsation frequencies, as was conceived possible before the experiments began. The definiteness of the μ classes should make it profitable to investigate in detail the means whereby individuals in one class may be made to exhibit other increments.

Removal of the marginal ring effects profound changes in the thermal relations of pulsation frequency. Two classes of individuals appear. In one, comprising 11 cases, $\mu = 16,500$ in the mid-region, $29,000 \pm$ at low temperatures, $8,100 \pm$ (but irregular) at high, breaks coming at 15° and at 9.4° . In the other, with 8 individuals, $\mu = 16,800$ without break. The absolute rates of pulsation are different before and after the operation, being slower when the marginal ring has been removed. The logarithmic latitude of variation, it is to be noted, is sensibly identical (*cf.* Figs. 1, 4 and 5); this is of interest in relation to the question of a possible interconnection between critical increment and variability of rate. The differences between medusæ with and without marginal ring are so unmistakable that it is difficult or indeed impossible to view the determination of the critical increments in the "normal" individuals as controlled by processes pertaining to the musculature or to the conducting nerve net of the bell. They are obviously to be attributed to the marginal sensoria. It should also be observed that if muscular processes (*i.e.* relaxation viscosity), independently affected by temperature, were superimposed on

the effect of the sense organs we might look for a curvilinear connection between $\log F$ and $1/T$, as found for the speed of progression of tent caterpillars (Crozier and Stier, 1925-26, *a*) and seen also in data of analogous sort from other sources.

The difference between the results of Figs. 4 and 5 are not to be attributed to differences in time elapsing after the operation. Some medusæ in each group were cut 2 hours, or as much as 24 hours, before the experiments began, and the readings were checked by return observations. Therefore the difference must be a real one, although the scatter of the points in Fig. 5, above 15° , suggests the "fan" effect sought when these experiments began; in fact, however, the divergent points (indicated by arrows) were obtained at the beginning of "runs," and are taken to be due to faulty thermal adaptation, which disappears by the time the experiment has gotten well under way. Although the technical procedure with these individuals did not differ from that followed with those in Fig. 4, the presence of the break at 9.4° independently marks these individuals as distinct, and as requiring for best results a more careful handling (although this could not be foreseen while the tests were being made). In a number of instances an individual of each group (Figs. 4 and 5) was under observation at the same time in the same thermostat, so gross technical differences are excluded. The line for $\mu = 8,100$ in Fig. 5 is drawn with the initial observations omitted; its significance is slight, except as fixing the critical temperature at 15° in agreement with inspection. The position at which critical temperatures occur are, for the normal medusæ, 9.6° (12.3°), 14° for those deprived of sensoria, 9.4° , 15° (Fig. 5), or not at all (Fig. 4). This agreement is striking, and may be held to signify that the critical temperatures are not determined by properties of the sensoria, but belong to the rest of the pulsation complex, even in normal medusæ.

The effect of removal of the sensoria of *Gonionemus* is curiously parallel to that obtained in connection with the pulsatile cloaca of holothurians (Crozier, 1916; Crozier and Stier, unpublished experiments) in which the cloacal apparatus gives in the intact animal $\mu = 12,200$ for frequency of contraction, but after amputation $\mu = 20,000$ (*cf.* Crozier, 1916). One difference between these cases is the persistence of members of the "series" of increments $8,000$; $11,000 \pm$;

16,000; 30,000. These might be compared with known increments for nerve net processes (cf. Crozier and Pilz, 1923-24; Crozier, 1924-

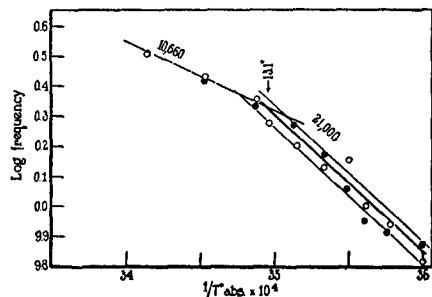


FIG. 6. Observations of the frequency of the contraction of the umbrella of *Gonionemus*. Data from 2 animals in which the nerve ring was cut in four places. For temperatures above 13.1°C. $\mu = 10,660$; and below 13.1°C. $\mu = 21,000$.

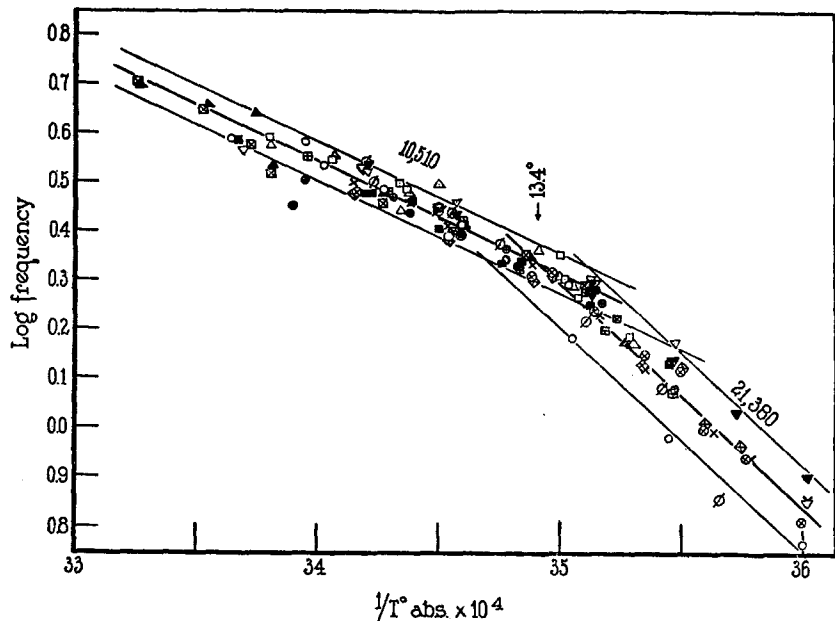


FIG. 7. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data from 17 animals in which the nerve ring was cut on two opposite sides of the margin of the bell. For temperatures above 13.4°C. $\mu = 10,510$ and below 13.4°C. $\mu = 21,380$.

25, *b*; and other measurements since available), and without serious disagreement; but with echinoderms the data on the holothurian cloaca show that no significance can be given to the values of μ as diagnostic for types of tissues (Crozier and Stier, 1924–25, *c*; 1925–26, *b*; 1926–27, *c*; 1927–28).

If this conception be correct, of different mechanisms for control of the frequency of initiation of pulsation as determinable by marginal sense organs and by the rest of the pulsating complex, it ought to be possible to produce a variety of centers of origin for contractions by making cuts in the marginal ring. This experiment is a necessary preliminary to tests in which all but 1 or 2 sensoria are to be removed. It was

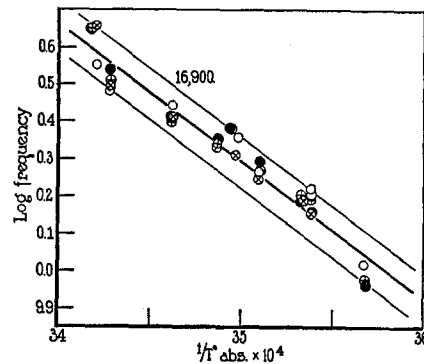


FIG. 8. Observations on the frequency of the contraction of the umbrella of *Gonionemus*. Data from 4 animals which were cut in half. The μ value for the whole temperature range = 16,900 (see text).

carried out in two groups of medusæ, in one of which (2 animals) four equally spaced radial incisions were made, so far as possible through the ring merely (not damaging the velum); in the other group (17 animals) two diametrically opposite cuts were made. All the animals so treated agree in showing $\mu = 10,500 \pm$ above 13° – 15° , $\mu = 21,000 \pm$ below (Figs. 6 and 7). One pretty definitely indicated feature of these measurements is the increased, but constant, relative latitude of variation below 15° . This type of change of logarithmic latitude associated with a change of μ has interesting possibilities for the general theory of these studies, as has been previously indicated (*cf.* Crozier and Stier, 1926–27, *b*).

It is a curious fact that among the normal animals (Figs. 1 and 2) the values $\mu = 10,580$ and $\mu = 32,230$ are found associated, with the critical temperature 9.6° , and in the other group $\mu = 8,160$ and $\mu = 22,500$, with critical temperature 14° ; in the present cases $\mu = 10,500$ is associated with $21,000 \pm$, the "break" being at 14° —, and the increased relative latitude of variation is also unquestionably connected with $\mu = 22,000$. The experiments plotted in Figs. 6 and 7 were made at different times during the summer, and the differences from the results with normal medusæ cannot be attributed to seasonal changes, nor to any obvious differences such as might be associated with size or time of confinement in aquaria. The values of μ are sufficiently definite to show that the operation of merely dividing the nerve ring does not bring about the state of affairs obtained when the whole nerve ring is removed (Figs. 4 and 5). Hence a final attempt to produce animals in which a conflicting mixture of critical increments might be expected to appear was made by cutting medusæ in half. In this way we might look for an intermixing of pulsations originating both from (1) marginal sense organs and from (2) nerve net along the zone of cutting. The visible contractions begin on the bell margin. The data obtained (Fig. 8) show that although the variability is increased above that in uncut animals, the increment is pretty definitely $16,000 \pm$, without breaks, as in the majority of cases without nerve ring (Fig. 4).

SUMMARY.

The frequency of contraction of the bell of *Gonionemus* was studied in relation to temperature, with intact animals and also where different operations were made on the nervous system. A number of values of μ are found for intact animals namely $8,100 \pm$, $10,500 \pm$, $32,000 \pm$ and $22,500 \pm$, with critical temperatures at 9.6° , 12.3° , and 14.0° . Four different classes of operations were used: (1) Animals where the nerve ring was cut on two opposite sides of the bell; the μ values found are $10,500 \pm$ and $21,300 \pm$, with a critical temperature at 13.4° . (2) Animals with four cuts through the nerve ring gave $\mu = 10,600 \pm$ and $\mu = 21,000$, with a critical temperature at 13.1° . (3) In animals where the bell was cut in half the temperature characteristic was found to be $16,900$. And finally (4) in the animals where the nerve ring was

totally removed μ values of 8,100, 16,000 \pm , and 29,000 were found, with critical temperatures at 15.0° and 9.4°.

These results are discussed from the standpoint of the theory which supposes that definite "temperature characteristics" may be associated with the functional activity of particular elements in a complex functional unit, and that these elements may be separately studied and identified by suitable experimental procedures involving the magnitudes of the respective temperature characteristics and the locations of associated critical temperatures. The swimming bell of medusæ with its marginal sense organs permits a fairly direct approach to such questions. It is found that even slight injuries to the marginal nerve ring, for example, produce specific modifications in the temperature relations which are different from those appearing when the organism is cut in half.

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