TEMPERATURE CHARACTERISTICS FOR HEART BEAT FREQUENCY IN MICE

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(Accepted for publication, June 28, 1934)

I

In developing a conception of the probably diverse quantitative relationships between frequencies or speeds of vital processes and temperature in the same or in very similar organisms, studies have been made of the frequencies of breathing movements in inbred strains of mice and in their hybrid offspring (Stier and Pincus, 1928–29; Pincus, 1930–31). The genetic relationships are important as providing a means of testing the definite character of the temperature constants obtained, and are also significant for purposes of genetic analysis (*cf.* Crozier and Pincus, 1928–29, 1929–30 *a*, *b*). We undertook to establish for one of the strains of mice used in previous experiments, the Bagg-Little inbred albino strain, the relationship between frequency of heart beat and temperature of the organism, as a foundation for further experiments. This is the strain of mice also used by Stier (1930, 1933) in experiments concerning "spontaneous activity" and by Pincus, Sterne, and Enzmann (1933) in observations on the development of thermal regulation.

The individuals used for the present observations varied in age from 0.5 to 6 days; they were of the 48th to 51st generation of brother by sister mating.

II

Over the range of external temperatures 15 to 35° the internal temperature of mice of the ages used in these observations is always above that of the surroundings by an amount varying from 0.1° to more than 2°, the difference in temperature being a function of the internal temperature. The results indicate that the difference in temperature between mouse and surroundings does not play a significant part in determining the shape of the heart beat frequency curve.

The experimental procedure was as follows. A mouse was taped back down upon a wooden substratum in a glass chamber which was mounted upon a brass base. The whole was submerged in a water thermostat. Air was passed through the chamber after being drawn through water bottles and a lengthy coil of copper tubing in the thermostat to moisten it and bring it to the temperature of the water bath. One junction of a copper-constantan thermocouple, carried in a fine glass capillary, was inserted into the rectum of the mouse; the other (reference) junction was placed between brass blocks ½ inch thick at the bottom of the chamber. The thermocouple leads were connected to a circuit involving a Leeds and Northrup Type "R" galvanometer, a differential photocell, and a Leeds and Northrup recording potentiometer.[1] In this way the circuit in the original recording potentiometer was so modified that the original full scale sensitivity (10 inches for 5 mv.) was increased so much that 0.5° difference in the temperatures of the junctions of a single copper-constantan couple could be made to record a maximum scale deflection. In the present observations, however, the sensitivity was such that 1 inch deflection was equivalent to 0.473°C. The deflection should be nearly proportional to the temperature difference between the junctions of the thermocouple, and this was found true within the experimental error involved in calibrating the thermocouple over the range of temperature difference 0 to 3.5° by means of standard thermometers. It was necessary to place the reference junction between brass blocks serving as a thermal conductor holding the junction at the mean temperature of the tank; without the blocks, cyclic fluctuations corresponding to the heating and cooling of the tank appeared in the curve drawn by the recorder, even though variations in tank temperature were not usually detectable with the standard thermometer immersed in the tank. The thermostat used has been described by Stier and Crozier (1932–33). The difference between the temperature of the rectum of the mouse and the thermostat tank could be determined with greater precision than the temperature of the tank itself could be measured by a standard thermometer (graduated in 0.10, read to 0.01°). The internal temperature of the mouse was the sum of the tank temperature and the recorded increment. The normal fluctuations of the mouse rectal temperature probably introduced more uncertainty as to the actual temperature of the mouse

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[1] A difference in temperature between the junctions of the thermocouple results in a deflection of the type "R" galvanometer and unequal illumination of the two halves of the photocell. This causes deflection of the galvanometer of the recording potentiometer which in turn introduces a current into the circuit containing the thermocouple and Type "R" galvanometer opposing the current resulting from the temperature difference. This leads to balance in the illumination of the photocell.

over an interval of time than did errors in either of its two experimentally determined components. The heart was assumed to be at the temperature of the rectum. Effort was made to determine whether appreciable temperature differences occur between various parts of the mouse; notably between the heart and rectum. One junction of the thermocouple, encased in a capillary, was inserted into the rectum. The other was placed in a broken-off hypodermic needle which was driven into the region of the heart and withdrawn around the wires, leaving the junction in or near the heart. A drop of collodion prevented the thermocouple leads from working out of the body of the mouse. While it must be understood that the measurements were attended by considerable possibilities for error, the maximum difference found was 0.3°, and the average was only about half of this, the heart temperature being higher. Three of the determinations were made at an environmental temperature of about 23 to 24°, one at about 16°. The mice were about a week old. One determination between the rectum and the brain indicated a small temperature difference. It is probable that the temperature difference between heart and rectum (in mice of the ages used) is not over 0.1°; this difference would not affect the subsequent treatment.

The electrical changes accompanying heart beat were led off by platinum wires inserted under the loose skin of the chest and rump to the input of a 4-stage, resistance-coupled amplifier.[2] The amplified changes passed to a loud speaker or head phones, and by way of a matching transformer to a string oscillograph (General Radio Company, Type 338-L). By the use of a rotating mirror and screen the deflections of the string could be observed, or by a camera attachment they could be photographed at will. The amplitude of the deflections decreased with falling temperature; the lower working limit for detectable electrical disturbances was at about 15°C.

Over the lower portion of the temperature range investigated (15 to 26°C. mouse temperature) the film records were frequently supplemented by a number of readings with a stop-watch graduated in 0.01 second. Above 26°C., the beat rate became too rapid for accurate counting and film records only were used. A procedure frequently employed was to take five sections of film, each lasting several seconds, at approximately 30 second intervals over a period of 2 minutes.

A timing mechanism (Telechron motor) marked $\frac{1}{5}$ second intervals on the film. An integral number of beats were counted. At worst, the position of each beat could be determined on the record to within plus or minus 0.02 second, so that, considering a number of beats occupying a length of film equivalent to about 4 seconds, the maximum error in the determination of the average rate over the interval would be at most 1 per cent, assuming negligible variation in the 60 cycle A.C. supply. This is smaller than the variation which was found to occur, even over such short intervals that little change of rate could have been caused by

[2] We are under obligation to Dr. Morgan Upton for his kind assistance in the construction of this amplifier.

slight, normal variations in the internal temperature of the mouse. At any temperature the variation in rate over a period of several minutes, though smaller than found in many cases for rate of heart beat and other vital processes in various organisms, amounted to about 5 per cent of the mean. The range of variation at any temperature was roughly a constant fraction of the mean, although not enough data were taken at each temperature to warrant statistical analysis (*cf.* Crozier, 1929).

The young mice were generally active at the end of an experimental run of 12 to 18 hours' duration. Only a very few died during the course of the observations, and these deaths may have been caused by the piercing of the rectum by the capillary containing the thermocouple junction. In the few cases where a "hysteresis effect" was observed in the frequency of heart beat, the change was more often an increase than a decrease.

No clear-cut change was observed in frequency of heart beat when the mouse became active in a cycle of "spontaneous movements."

At any constant temperature the frequency of heart beat increases with weight (age) of the mouse. The relationship between frequency of heart beat and weight is approximately parabolic. There is no change, however, in relation of frequency to temperature within the range of weights involved.

As we have found in a number of other cases (*cf.* Pincus, 1930–31), the frequency at the first temperature to which the mouse is adjusted is generally far off the smooth curve connecting the points taken subsequently. This is true even when adequate time is allowed for thermal equilibration. Generally the frequency is too low, and it shows a drift for several hours; if the temperature be changed during this time, the frequency of heart beat at the second or any following temperature to which the animal is subjected does not exhibit this drift.

The precise relationship between frequency and temperature is independent of the order of temperature changes, as was demonstrated by the taking of one or more check points in each experiment.

III

Data from fourteen sets of observations with different young mice of the inbred albino strain are summarized in Fig. 1. In this figure it

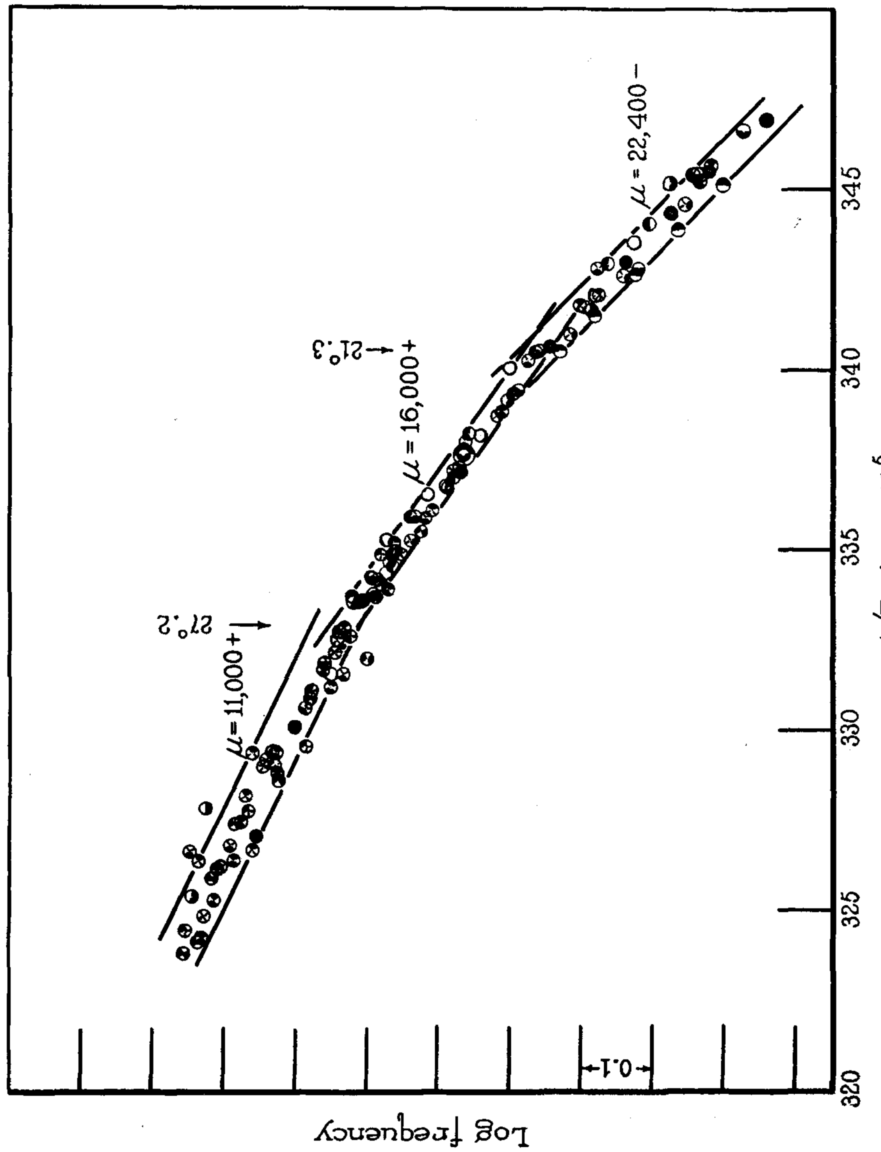

FIG. 1. Mass plot of the data from fourteen individuals of the inbred albino strain. The various symbols denote single individuals. See text.

is intended to compare the *slopes* of the lines drawn through the observations in terms of the Arrhenius equation,

$$\ln \textit{frequency} = -\frac{\mu}{RT} + \text{constant}.$$

The several sets of observations have therefore been brought together by considering that the data in each series may be multiplied by an arbitrary constant so adjusted that the respective curves coincide at 25°. There are apparent two critical temperatures, where the slope constant μ changes rather abruptly; one of these is located at slightly higher than 20°, the other at 27°.

The values of μ (Fig. 1) are 22,400− from 15 to 20°+, 16,000+ from 20+ to 27°, and 11,000± from 27 to 35°+. The apparent greater scatter of the observations over the upper and lower temperature ranges is partly, at least, spurious, since the individual curves were brought together in the middle, and there is some difference in the exact location of the critical temperatures. The breaks in the curves occur at temperatures which have frequently been found as critical (*cf.* Crozier, 1925–26 *a, b*). It is interesting to note that for this same strain of mice 20° − was found to be a critical temperature for frequency of respiratory movements; the values of μ for respiratory movements were, however, 14,000± calories above 20° and 34,000± (or 14,000±) calories below 20° (Pincus, 1930–31). This indicates that in these mice the organism does not determine a uniform temperature characteristic for all processes (see also Stier and Wolf, 1932–33).

These experiments indicate that the Arrhenius equation holds for the temperature relations of heart beat frequency in intact mammalian hearts as has already been shown for isolated hearts (Crozier, 1925–26 *b*). It is rather remarkable that over any given temperature range the value of μ is the same from mouse to mouse. The fact that we are dealing with a highly inbred strain of mice indicates a possible genetic basis for this uniformity. It is hoped that the proper experiments with other strains of mice will elucidate this point.

SUMMARY

An apparatus is described which permits the simultaneous recording of body temperature and heart beat frequency in young mice. When

heart beat frequency is related to body temperature the values of the temperature characteristic for the inbred albino strain used are 22,400− calories over the range 15 to 20°+, 16,000± calories from 20+ to 27°, and 11,000± calories from 27 to 35°+.

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