

Comparisons of Rewarming from Natural Torpidity and Induced Hypothermia in Chipmunks (*Tamias striatus*) with Reference to Heart Rate and Temperature Relationships

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Abstract

Chipmunks live-trapped in the Bloomington area during the summer and early fall of 1968 were housed under four environmental conditions to study their effects on natural torpidity. Beginning in late December and continuing into March, torpid animals were observed in three of the four groups. Depending on the group, 30 to 50% of the animals experienced forms of torpidity ranging from deep to very shallow. In a number of animals provoked to arouse, heart rates and thoracic and colonic temperatures were recorded during the rewarming process. Since a majority of torpid states were so shallow that arousal was provoked before the animals could be fitted with recording leads, individuals from the different groups were subjected to an induced hypothermia by "jar cooling," after which their heart rates and body temperatures were recorded during rewarming.

Animals provoked to arouse from natural torpidity demonstrated intensive shivering, while rewarming with extreme rapidity, generating steep heart rate and thoracic temperature curves with time, which contrasted to lagging colonic temperatures as much as 18.5°C below thoracic temperatures. Rewarming of "jar cooled" animals, attended by varying degrees of shivering, was considerably slower, resulting in heart rate and both temperature curves, with time, of similar sigmoid shape and smaller temperature gradients. The significance of these relationships is discussed.

The eastern chipmunk, *Tamias striatus*, is a member of the tribe *Marmotini* (9). This tribe also includes the woodchuck (*Marmota*) and the ground squirrels (*Citellus*). While physiological investigations of hibernation experienced by the *Marmotini* have mostly concentrated on *Citellus* and *Marmota*, typical deep hibernating forms, only a few preliminary investigations have been conducted on *T. striatus* (4, 7, 8, 10), and their results appear to indicate this animal is not a typical marmotine hibernator.

Although the depths of torpidity have been described by rectal temperature measurements (7, 8, 10), temperature-heart rate relationships of isolated hearts have been reported (4), and oxygen consumption measurements of torpid and arousing animals have been made (7), one important physiological aspect of the torpor experienced by *T. striatus* is missing. No data concerning measurements of heart rates and temperatures during arousals, as exist for the other Marmotine hibernators (3, 5, 6), have been offered. Because of this, it was decided that the first object of this investigation should be a study of heart rates and temperatures during provoked arousals.

Second, since the degree of torpor experienced by *T. striatus* was generally brief and shallow, possibly suggesting either an intolerance to deep hypothermia or insufficient rewarming abilities, from which most animals aroused before they could be fitted with recording leads, the decision was made to attempt to assess their tolerance to hypothermia and rewarming capabilities by subjecting individuals to an induced hypothermia (1) by means of "jar cooling."

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Materials and Methods

Chipmunks were live-trapped in the vicinity of the Indiana University campus from May through October, 1968. All animals were caged individually in two animal rooms with different photo-periods. One room was windowless in which a 12-hour photoperiod of artificial light and a temperature of 19° to 21°C were maintained. The other room contained a window which admitted natural light and had a temperature which fluctuated between 17° and 23°C. All chipmunks were maintained on a diet of Wayne Lab-Blox Shorts (Allied Mills, Inc., Chicago, Ill.), periodically supplemented with sunflower seeds and pieces of fresh lettuce, carrots and apples, and water *ad libitum*.

Early in October, 1968, six chipmunks were placed outdoors on a roof in individual cages provided with hinged-topped nest boxes. These animals were then supplied with nesting materials until additional materials were refused. By mid-October, the chipmunks housed indoors had also been provided with the same type of accommodations.

In mid-December, three chipmunks were placed in a refrigerator. For the first week, the temperature was gradually decreased to 9°C. At the beginning of the second week, the temperature was dropped to 6° to 3°C. The refrigerator was unlighted except for a small amount of 12-hour light entering a 3½-inch diameter ventilation hole near the top of one side of the refrigerator. Humidity was always high, as evidenced by water condensation on the inside of the refrigerator.

All animals were provoked to arouse at room temperature (20° to 24°C) by removing them from their nests, fitting them with EKG and temperature recording leads, and placing them in a rectangular acrylic plastic chamber provided with ventilation ports. Total time elapsed for these manipulations was 3 to 5 minutes.

Electrocardiogram leads were fabricated from highly polished safety pins connected to a Sanborn High Gain Preamplifier (model 150-2700) and Recorder (model 154-100B). The ground was connected to copper screening sandwiched between the walls of the chamber. Prior to subdermal insertion in a Lead II orientation, the tips of the safety pins were wiped with 70% alcohol and dipped in 1% Novocain (Winthrop Laboratories, New York, N. Y.). The EKG was recorded every 1 or 2 minutes and the heart rate calculated by counting from this record. The EMG was recorded as interference on the EKG record.

Temperatures were measured with a YSI Tele-Thermometer, Model 41TS, and 400 and 500 Series thermistor probes (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio). Thoracic temperatures were obtained using a 511 probe inserted into the chest cavity through the barrel of an 18G sterile needle which had penetrated between the third and second last ribs. Previously, the probe had been wiped with 70% alcohol and the needle dipped in 1% Novocain. After the probe had been pushed anteriorly, to loop the tip near the base of the heart, the needle was withdrawn and the lead secured to the animal's back with adhesive tape. Deep colonic temperatures were obtained with a 401 probe, wiped with 70% alcohol and dipped in glycerine, then inserted 3-5 cm into the colon and secured to the base of the tail with adhesive tape. All probes had been previously calibrated $\pm 0.2^\circ\text{C}$ in a well-stirred water bath.

Hypothermia was induced by placing a weighed animal in a glass vessel which had been pre-cooled to 0° to 2°C by surrounding it with crushed ice in a styrofoam chest. The vessel was then sealed air-tight with the animal rebreathing the chilled air as it slowly cooled and lost consciousness. This hypothermia was judged complete when the animal 1) exhibited a shallow, abdominal respiration rate of 30-50 per minute; and 2) was unable to right itself. Approximately 2¾ hours of cooling were required to meet these conditions, after which the animal was removed from the vessel and prepared for recording of rewarming, according to the procedure for provoked arousals.

Results

It was the end of December before any torpor was observed in the chipmunks and this was observed in an outdoor animal. From then on, more torpid animals were observed. The greatest frequency of torpidity occurred from mid-January to mid-March, after which there was an abrupt end. Of six outdoor chipmunks, three were observed in, and provoked from torpor one or more times during this period. Animals in the naturally lighted room began showing signs of lethargy in mid-January with 4 of 12 animals eventually being observed in states of light torpor. While a few of the 12-hour photoperiod animals appeared to show some lethargy during this time, none was observed in any state of torpor. One of the three refrigerator animals was observed twice in deep torpor, the first occurring late in February.

Figure 1 shows the course of the second arousal provoked in a female chipmunk, weighing 132.3g, which had become torpid in the

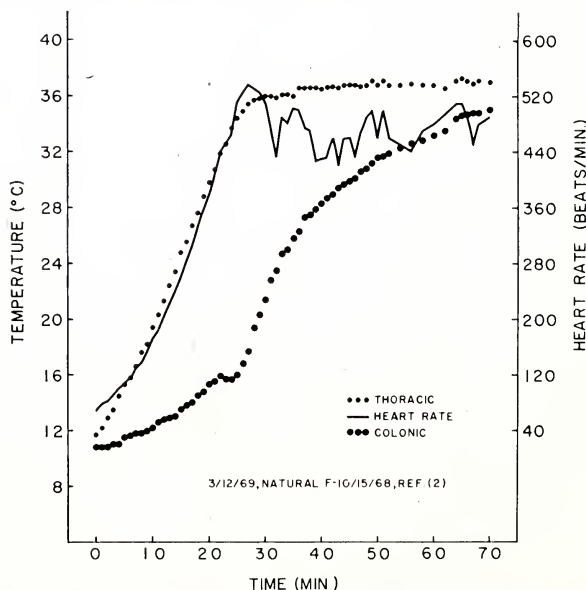


FIGURE 1. Progression of thoracic and colonic temperatures, and heart rate in a refrigerator housed, female chipmunk (132.3g) while rewarming from natural torpor during a provoked arousal.

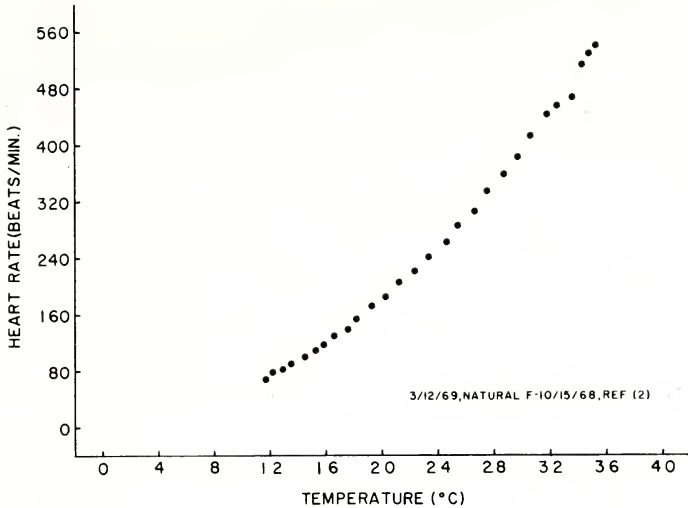


FIGURE 2. Heart rate and thoracic temperature ($^{\circ}\text{C}$) relationships, during acceleration of the heart rate to maximum, for the female chipmunk shown arousing in Figure 1.

refrigerator. From the first recorded heart rate, thoracic and colonic temperatures of 68 beats per minute, 11.7°C and 10.8°C , respectively, this animal completely rewarmed in 70 minutes. Figure 1 also shows how closely heart rates and thoracic temperatures increase together such that by 27 minutes into recording time a maximum heart rate of 535 beats per minute was associated with a thoracic temperature of 35.4°C . The rapid increase in heart rate and thoracic temperature was attended by intense shivering. Shivering was recorded as interference on the EKG from the onset of recording, increasing in intensity, finally ceasing at 28 minutes, when a heart rate of 530 beats per minute and a thoracic temperature of 35.7°C were observed. After shivering ceased, thoracic temperatures increased only slightly over the remaining time and heart rate fluctuated greatly. Colonic temperatures, on the other hand, showed an entirely different progression. While thoracic temperature increased an average of 0.86°C per minute during the period of shivering, colonic temperatures lagged considerably, only increasing an average of 0.31°C per minute. It was during this period (at 25 minutes) that a maximum gradient of 18.4°C existed between the two temperature regions. From this point colonic temperatures increased rapidly, and during the next 12 minutes averaged almost 1°C per minute. Thereafter the rate of colonic temperature increase markedly declined. The colonic temperature curve also shows a "dip" or "plateau" just prior to the development of the maximum gradient. This corresponded to the few minutes after the animal had arisen to its feet (at 21 minutes) and during which shivering was most intense.

Results were similar for other chipmunks provoked to arouse. Rapid increases in heart rates and thoracic temperatures were attended by intensive shivering. Two other animals, one male and one female, for which complete arousals were recorded, showed thoracic temperature

increases of 0.75°C per minute and 0.78°C per minute, respectively, during the rapid phase.

Figure 2 shows the relationship of heart rate to thoracic temperature of the same animal. The relationship is approximately linear in the upper portion of the curve (36° to 24°C). However, at 24° to 22°C there is a turn toward curvilinear which becomes more evident below 22°C . Extrapolation of this curve indicates that the heart should cease to beat at approximately 2°C , much lower than would be predicted (approximately 14°C) for an extrapolation of the upper part of the curve. Such curves for the other provoked chipmunks indicated similar properties. Partial confirmation of these extrapolations exists in the results of a hypothermic cardiac arrest induced in this particular chipmunk. Atrio-ventricular dissociation occurred at 1.3°C with atrial activity ceasing at 0.2°C .

Figure 3 is an example of the results obtained during the rewarming of "jar cooled" animals. The general pattern is quite similar to that of provoked animals. The major exceptions are a more uniform rate of rewarming and a longer time necessary for rewarming to a given level. Thoracic temperatures and heart rates increased less rapidly than, and colonic temperatures increased more rapidly than, their counterparts in animals arousing from natural torpor. The result was the generation of similar sigmoid temperature curves and a smaller temperature gradient. These conditions developed in spite of shivering which, although not beginning immediately, was almost as intense as and persisted longer than that demonstrated by animals provoked to arouse. Again a slight

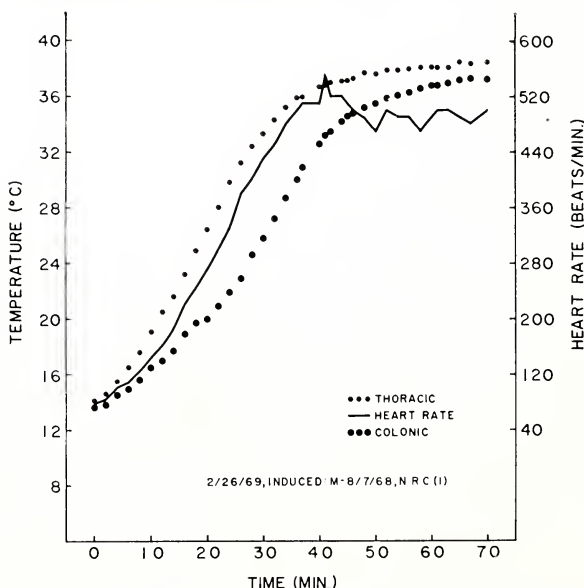


FIGURE 3. Thoracic and colonic temperature and heart rate progressions during re-warming from an induced hypothermia in a male chipmunk (126.8g) of the 12-hour photoperiod group.

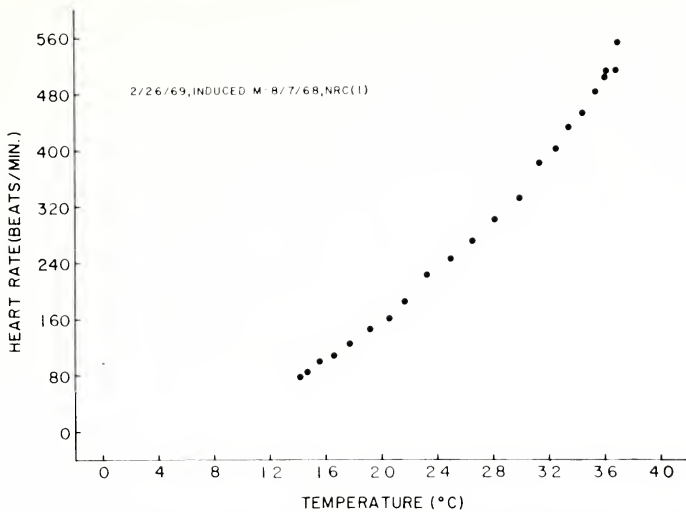


FIGURE 4. Heart rate-thoracic temperature ($^{\circ}\text{C}$) relationships during heart rate acceleration to maximum, for the male chipmunk shown rewarming in Figure 3.

"plateau" was present in the colonic temperature curve when the animal rose to its feet.

A plot of the heart rate-thoracic temperature relationships for this animal (Fig. 4) indicates further differences between the values obtained from an animal provoked to arouse from natural torpor and those obtained from an animal rewarming from an induced hypothermia. The reduced slope for the induced animal is a reflection of comparative heart rates occurring at higher temperatures (approximately 2°C). However, such differences may be due to the different hypothermic states.

While the only example of rewarming from induced hypothermia offered here is that of a 12-hour photoperiod animal, results from further induced hypothermias performed on both male and female chipmunks housed under this and two other conditions indicate a possible difference among the groups. These differences, again, are mainly reflected in the increased times necessary to achieve rewarming to a given heart rate level, in this case the generation of maximum heart rates and the thoracic temperatures corresponding to those heart rates. Table 1 summarizes these data.

An evaluation of Table 1 reveals the animals under the 12-hour photoperiod conditions appear to outperform the other groups of animals in their ability to rewarm after an induced hypothermia, in both the achievement of a maximum heart rate and speed with which it is attained. On the other hand, chipmunks housed in the room with natural illumination exhibited the poorest performance.

Discussion

Confirming the previous reports about *T. striatus* (7, 8, 10) and its western counterpart, *Eutamias* (2), this investigation has also encountered the unpredictable, brief, and generally shallow torpor experienced

TABLE 1. Comparison of mean heart rates and their corresponding temperatures in the different groups of animals. The mean time required to achieve these conditions was measured from a mean heart rate and mean thoracic temperature of 126 and 15.35°C, and 108 and 16.9°C, respectively, in provoked and induced chipmunks.

Group	Provoked from Natural Torpor	Induced Hypothermia		
		12-hr Light	Natural Light	Roof
Number	3	5	4	4
Mean max H.R. $\pm$ S.D.	498 $\pm$ 44	521 $\pm$ 23	478 $\pm$ 21	495 $\pm$ 25
Mean thoracic temp $\pm$ S.D.	31.0 $\pm$ 4.9	35.8 $\pm$ 1.0	36.9 $\pm$ 0.9	37.0 $\pm$ 0.7
Mean time $\pm$ S.D.	19.3 $\pm$ 6.8	42.2 $\pm$ 7.7	65.0 $\pm$ 13.3	51.0 $\pm$ 16.4

by *T. striatus* during the winter months. It has also shown that chipmunks do experience relatively deep torpor. In addition, the explosive nature of this arousal, as indicated by oxygen-consumption experiments (7), has for the first time been complemented by measurements of heart rates, thoracic and deep colonic temperatures during provoked arousals. The results for 3 chipmunks, in which complete measurements during provoked arousal have been obtained, indicate the ability to rapidly increase heart rates and thoracic temperatures, reaching maximum heart rates, in less than 30 minutes. During this period of rapid acceleration, thoracic temperatures increased at an average rate of 0.8°C per minute, while colonic temperatures lagged those of the thoracic-heart region by as much as 18.4°C. However, thoracic and colonic temperatures were only as low as 11.7° to 18.7°C. The average rate of thoracic temperature acceleration is considerably larger than the 0.5°C per minute rate in rectal temperatures observed by Cade (2) in a fasted, refrigerator-housed *Eutamias amoenus*. The development of large temperature gradients between thoracic and colonic regions indicates the physiological ability of circulatory shunting of heat to critical organs (3), a method of differential rewarming demonstrated by all hibernators which have been studied. The apparent "plateaus," followed by an increased rate of increase, in the colonic temperatures curves may indicate a rapid vasoconstriction, followed by vasodilation, of the vasculature to the posterior portion of the animal.

Because the majority of torpid animals experienced a hypothermia of a very brief and shallow nature, it appears that a physiological limitation may govern the depth of torpor. Evidence for this has been presented by Lyman and Blinks (4) who have demonstrated that the ventricles of the isolated chipmunk heart cease activity between 7° and 5°C, with the atria stopping between 3.3° and 0.7°C. These results were compared to those of *Citellus tridecemlineatus* hearts which continued beating as low as -1°C. Their isolated chipmunk hearts also showed a linear temperature-heart rate relationship from 30° to 12°C, with an abrupt curvilinear relation below this point, such that the hearts continued to function at a lower temperature than would be predicted from an extrapolation of the linear portion of the curve. The heart rate-temperature relationships presented here, while not as linear as those for the isolated hearts (4) do indicate that the chipmunk heart in the

whole, intact animal also continues to function at a lower temperature than predicted. The finding here that one heart ceased normal activity in the whole animal at 1.3°C is offered in support.

It was hoped that further insight into possible physiological limitations, either in the form of reduced hypothermic tolerance or rewarming capabilities, could be obtained by subjecting individuals to a suitable hypothermic stress. This was accomplished by "jar cooling" in which the animals experienced a state of "artificial hibernation" (1). At this time it should be emphatically pointed out that natural hibernation and induced hypothermia are distinctly different (1, 3, 5). The most important factor characterizing natural hibernation is the hibernator's capability of rewarming from this state without the aid of external heat (1, 3, 5). Animals under induced hypothermia usually show only vestiges of rewarming capabilities (1). Accordingly, then, the chipmunks in this investigation performed rather well. They showed both the ability to withstand and rewarm from a hypothermia which would have produced dire consequences to most homotherms, unless artificially rewarmed. The fact that these animals were capable of rewarming from body temperatures of 11° to 17°C, in a relatively rapid period of time and without artificial rewarming is significant. The differences in rewarming capabilities between the different groups of animals seems to suggest environmental and possible seasonal influences, for which studies are now in progress.

Literature Cited

1. ADOLPH, E. F., and J. RICHMOND. 1955. Rewarming from natural hibernation and from artificial cooling. *J. Appl. Physiol.* 8:48-58.
2. CADE, T. J. 1963. Observations on torpidity in captive chipmunks of the genus *Eutamias*. *Ecology*. 44:255-261.
3. LYMAN, C. P. 1965. Circulation in mammalian hibernation. In W. F. Hamilton [ed.] *Handbook of Physiology, Section 2, Circulation III*. American Physiological Society, Washington, D. C.
4. LYMAN, C. P., and D. C. BLINKS. 1959. The effect of temperatures on the isolated hearts of closely related hibernators and non-hibernators. *J. Cell. Comp. Physiol.* 54:53-64.
5. LYMAN, C. P., and P. O. CHATFIELD. 1955. Physiology of hibernation in mammals. *Physiol. Rev.* 35:403-425.
6. LYMAN, C. P., and R. C. O'BRIEN. 1963. Autonomic control of circulation during the hibernating cycle in ground squirrels. *J. Physiol.* 168:477-499.
7. NEUMANN, R. L. 1967. Metabolism in the eastern chipmunk (*Tamias striatus*) and the southern flying squirrel (*Glaucomys volans*) during the winter and summer. In K. C. Fisher, A. R. Dase, C. P. Lyman, E. Schonbaum, F. E. South, Jr. [eds.] *Mammalian Hibernation III*. American Elsevier Publishing Co, Inc., New York, N. Y.
8. PANUSKA, J. A. 1959. Weight patterns and hibernation in *Tamias striatus*. *J. Mammal.* 40:554-556.
9. SIMPSON, G. G. 1945. The principles of classification and a classification of the mammals. *Bull. Amer. Mus. Natur. Hist.* 85:1-350.
10. WOODWARD, A. E., and J. M. CONDIN. 1945. Physiological studies on hibernation in the chipmunk. *Physiol. Zool.* 18:162-167.