Respiration in the Hibernation of the 13-Lined Ground Squirrel

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ABSTRACT


—Respiratory rate, heart rate and body temperatures were recorded simultaneously in the 13-lined ground squirrel (Citellus tridecemlineatus) throughout the hibernating cycle. An induction trend may appear at any time during the latter part of arousal. It may lead to the hibernating state, or it may give way to the continuation of arousal, hence neither induction nor arousal appears to be an 'all-or-none' phenomenon. In hibernation, respiration consists of one to two deep sighs followed by apnea of variable duration, averaging 1-3 breaths/min. The heart rate is often arrhythmic, but averages 7-8 beats/min. As the frequency of the sighs change, other shallow rhythmic breaths appear very early in arousal, and increase in rate and amplitude to marked hyperventilation. Heart rate also increases rapidly and without arrhythmia, but in late arousal various stimuli will elicit momentary respiratory and cardiac arrest. In transition stages, respiratory lead heart rate changes, except during early arousal and late induction when respiratory and heart rates are linearly related. The apparent initiatory action of respiration further implicates the respiratory muscles in the heat production of arousal.

The term 'hibernation' has been applied to many and varied conditions, including surgical hypothermia, the stuporous sleep of bears, and the winter condition of poikilotherms, as well as the state of torpor which obtains in the true hibernators. This paper is concerned with the latter response, termed 'deep hibernation' by some (1, 2). The authors of this paper prefer the term 'natural hibernation.' Essential features of such a response include a profound drop in heat production and rate of metabolic processes, reflected in a corresponding decrease in body temperature. A distinction is made between natural hibernation and hypothermia, in that animals which are in a state of hibernation are capable of rewarming themselves without external application of heat. This ability is not possessed by animals (hibernators or nonhibernators) which are cooled artificially, with or without anesthesia.

As used in this paper, the term 'hibernation' will describe that condition which obtains in certain mammals when heat production and metabolism drop to very low levels, but from which the animal is capable of arousing without external application of heat. Natural hibernation in the 13-lined ground squirrel is seasonal, occurring largely between late October and early April. It is a cyclic phenomenon, with periods of torpor alternating with periods in which the animal is 'aroused' or 'active.' (An animal may be said to be 'aroused' in the nonhibernating season, but the term 'aroused' is reserved for the period of activity between periods of hibernation during the hibernating season.) 'Arousal' refers to the process by which an animal in natural hibernation increases metabolic activity and raises body temperature to the levels of the nonhibernating state. 'Induction' is the reverse process of transition into the hibernating state.
The terms ‘asleep’ and ‘awake’ have been used by many to designate the hibernating and aroused states, but in the opinion of the authors, these terms should apply only to the usual diurnal sleep-wake cycle.

A descriptive narrative was chosen as the most effective means of presenting respiration throughout the hibernation cycle. Visual observation, however, is far more satisfactory. A film is therefore recommended to the reader showing the breathing phenomena in hibernation and arousal.

METHODS

Ground squirrels (*Citellus tridecemlineatus*) were caught in the spring, the summer and the fall. They were housed in individual cages in a general animal quarters until they were placed in a cold room.

An insulated metal box with a removable lid having a wire gauze window served as a ‘burrow.’ The sides and bottom were lined with ½-inch Celotex insulation. Cotton waste was used for nesting. A small dish of water was placed in the ‘burrow’ and sunflower seeds constituted the main item of diet. The burrow boxes were placed on shelves in enclosed cabinets. During the hibernating season the cabinets were kept in a cold room where temperature was allowed limited fluctuation with outside temperature. At no time did the cold room temperature fall below -1°C, and it exceeded +12°C only twice between late November and the first of April, after which the outside temperature rose.

For experiments, a hibernating animal was transferred from its ‘burrow’ to a cold desiccator which had an opening in the top providing an open airway as well as providing an outlet for electrocardiographic and thermocouple leads. There was soda lime in the bottom, and some nesting was provided.

The desiccator containing the animal was immediately transferred to a cold chamber. A center divider in the chamber made two compartments, each of which would accommodate a desiccator or burrow box. A plastic lid, in two sections, screwed down firmly, and if a suitable lubricant was used, made a good seal against water or pressure. The extended ends of the cold chamber provided outlets for recording leads, and served as handles. The cold chamber was kept in a large, moveable refrigerated water bath. The temperature of the bath was controlled so that an environmental temperature close to 5°C was provided for the animal in the desiccator.

Breathing rate was recorded by means of a device designed and constructed by Mr. Samuel Battista and Dr. Albert Dawe in this laboratory. It consisted chiefly of one thermistor mounted in an airway leading from the desiccator and a second thermistor thermally contained in a water jacket around the airway. Each thermistor was wired into one arm of a Wheatstone bridge circuit. The temperature in the airway was stabilized by the water jacket. Breathing by the animal caused air movement, cooling the temperature-sensitive airway thermistor sufficiently to change its resistance. This change in resistance upset the balance of the Wheatstone bridge circuit, and this was amplified and recorded by an electrocardiograph. A four-channel Sanborn Poly-Viso, or a single channel Burdick electrocardiograph was used as amplifier and recorder in these experiments. With the former (a 4-channel instrument), breathing and cardiac activity could be recorded simultaneously, but with the latter, a switching device was used in order to provide rapid changeover from one measurement to the other.

Heart rate was picked up by stainless steel electrodes which had been previously imbedded under small bits of skin in the right shoulder and right and left flanks. Wires clipped to the exposed ends of the electrodes led out through the top of the desiccator, the openings in the cold chamber, and through a switch to the electrocardiograph.

Chronically implanted iron-constantan thermocouples were used for pick-up of body temperatures. The thermocouples, imbedded under small bits of skin in the right back near the shoulders, recording front temperature, (T_f), and near the base of the tail, recording hind temperature, (T_h). The bare ends of wire protruded posteriorly from the skin. Ambient temperature was picked...
upt by a thermocouple fixed in the desiccator. Shielded wires which led to a Brown thermocouple recorder were clipped to the wire tips protruding from the animal and also clipped to the thermocouple fixed in the desiccator.

RESULTS

In the experiments, simultaneous recordings were obtained of respirations, electrocardiograms and body temperatures of ground squirrels during induction, in the hibernating state and during arousal. Data were obtained on 13 partial or complete inductions, and all or part of 30 arousals.

Induction. Induction is the least understood part of the entire hibernating cycle. Little is known except that the rates of the metabolic processes decrease, and body temperatures fall toward that of the environment. It has been stated that once arousal begins, it evidently proceeds to completion in all species of hibernators (2) but in this series of experiments, induction immediately followed the arousal process on five occasions. Arousal had gone nearly to completion in only two of these (Tf = 33°C, HIR = 410; Tf = 31°C, HR = 380). In the other three experiments induction began well before the maximum values for body temperature and heart rate had been reached (Tf = 25°C, HR = 260; Tf = 22°C, HR = 200; Tf (not taken), HR = 280.) Thus it is apparent that induction can begin during the arousal period, dropping the animal back into hibernation, or merely acting to slow down the arousal process.

Under ordinary conditions, induction proceeded slowly, requiring 5-7 hours in experiments in which it went to completion. Breathing rate, heart rate and body temperature declined slowly and somewhat irregularly. The rate of decline was rapid at first and later slow. The fall of body temperature lagged behind the drop of breathing and heart rates. In view of the difficulty of recording induction, little more can be described.

Hibernating State. After 5-7 hours of induction the animal had ordinarily attained the hibernating state. Frequently it was possible to prepare an animal in the hibernating state for recording without initiating arousal. The animal remained in this condition for hours, and in one experiment data were obtained intermittently from a hibernating animal for eighteen hours.

Breathing rate records from hibernating ground squirrels showed that the breaths, which were very deep, usually occurred singly or in pairs. The intervals between breaths were seen to be extremely variable, ranging from a few seconds to about 5 minutes, but at an average rate of 1-3 breaths a minute. A single breath consisted of an abrupt inspiration followed immediately by a slower expiration. The whole breath usually required 1-2 seconds. Sometimes the expiration was greater than the inspiration, so that the record showed a dip below the base line, especially when there were several breaths in succession. Respiration was primarily diaphragmatic during the hibernating state. A stimulus such as moderate tactile pressure to the animal usually precipitated a response of one or two deep breaths. The stimulation of connecting an animal for recording also often resulted in an increase in respiration to about 20/min. Unless arousal followed, this speed-up in respiration returned to the lower rate within about one hour. A typical breathing record of an animal in the deeply hibernating state is shown in figure 1A.

When an animal in the hibernating state was decapitated, the stump of the corpus showed bright cherry-red blood. Similarly, blood obtained by cardiac puncture from several animals in the hibernating state also showed bright red blood. The tongue, roof of the mouth and feet of a hibernating animal were very pink. If the skin was cut, the wound did not bleed.

The heart rate of hibernating ground squirrels varied between 3 and 15 beats/min., an average of 7 or 8/min. The rhythm of the heart was extremely variable. Oftentimes it was very irregular, with beats occurring in pairs, in bursts or at random. Arrhythmias could be correlated with respiration. Other arrhythmias correlated with the slow muscular movements that characterize the hibernating animal.

The body temperatures recorded from hibernating ground squirrels at an ambient temperature of 2.5-8°C ranged from 3°C to 9°C at the fore part of the animal, and 3°C
Fig. 1. Respiration (upper trace) and heart rate (lower trace) during hibernation and arousal (exp. C, 37). A, during hibernation: respiration consists of occasional 'sighs' at about 1/min. Heart rate is irregular and slow, at about 6/min. B–H, successive stages of arousal: respiration shows appearance of small breaths which become established as the sighs disappear. Heart rate is regular and steadily increases. Noncardiac muscle action potentials appear on some of the electrocardiograph records. Respiratory trace in G and H shows 2 peaks for each breath. Base line shifts on the respiratory record as well as the noncardiac muscle action potentials on the heart record are caused by the animal moving about. The late arousal record H indicates a respiratory rate of approximately 160/min., and a heart rate of 420/min.

Fig. 2. 'Startle' response in late arousal. Respiratory and cardiac arrest in response to auditory stimuli (hand claps) applied at times indicated by X’s on the record. Base line shifts in the respiratory trace reflect sudden muscle contraction.

to 8°C at the rear. The averages were 5.5°C in front and 4°C in the rear. The body temperature was seldom more than 1°C above that of the environment.

Arousal. After a variable period of time the hibernating state was broken by the arousal process. When arousal began, either spontaneously or in response to external stimulation, there was a striking mobilization of resources to increase metabolism and produce enough heat to raise body temperature, even though the animal was still in a cold environment.

Arousal was seen to begin slowly and proceed at an ever-increasing rate. The metabolic processes increased many fold during a period of about 2 hours. It should be noted that this is much faster than induction. Respiration increased from 1–3 breaths a minute to near 200/min., heart rate from 7–8 beats/min. to about 400/min., and body temperature rose from about 5°C to nearly 35°C. The breathing and heart rates overshot, after which they declined to an active resting level. (In the 13-lined ground squirrel, the resting level is a breathing rate of between 100 and 125 breaths/min. and a heart rate of 200–250 beats/min.) As heat production increased, the fore part of the animal warmed to about 20°C before the hind quarters began to warm noticeably. When arousal was well underway the animal augmented heat production by violent shivering, especially of the fore parts.

In the hibernating state and before arousal was triggered, the respiratory record consisted of a relatively straight base line, interrupted at intervals by occasional large slow deflections, caused by sighs. The first sign of
arousal was a temporary slight increase in the frequency of these sighs. Very small rapid deflections of the base line then began to appear. It is not possible to state exactly when these first appeared, because the onset was quite early and very gradual. They increased in amplitude and frequency throughout arousal (fig. 1, B-H). The small breaths appeared on the record before they could be visually distinguished by an observer. As they became established, the interval between the large sighs became longer, until the sighs finally disappeared. Although there was considerable variation, the last of the deep breaths was noted when the rate of the small breathing movements was up to about 30/min., and the heart rate up to 50/min. The small breaths were quite regular when they appeared and remained regular until the late stages of arousal.

By visually observing the animal, it was seen that early arousal breathing was diaphragmatic, but as arousal proceeded a thoracic component appeared. The thoracic and diaphragmatic components were dissociated at first. This dissociation, coupled with the violent shivering which soon developed, made it virtually impossible to count respirations, either visually, or by mechanical contact methods which depend upon the movements associated with respiration. However, with the thermistor device described above, breathing movements were separable from other movements on the record.

When arousal was well underway the increase in amplitude of respiration became very apparent, developing into a marked hyperventilation. An increased level of carbon dioxide in the blood undoubtedly was acting as a stimulus for this response. Possible rise in carbon dioxide content inside the desiccator was not responsible for the hyperventilation, since it also was observed in those animals which were allowed to arouse outside of the desiccator, and at room temperature. However, hyperventilation was less apparent when animals were handled or stimulated to move about.

At the onset of arousal the initial increase of respiration and heart rate apparently began simultaneously. Respiration, however, usually reached its maximum rate and began to decline 5-15 minutes before the heart rate had reached its maximum.

When an animal was decapitated after partial arousal from hibernation, the stump of the corpus showed very dark, almost black blood. Similarly, blood withdrawn by cardiac puncture from the heart of a partially aroused animal \((T_r = 15^\circ C)\) was very dark. The tongue and membranes of the mouth of arousing animals were also dark. These observations are interpreted as signs of an increase in concentration of reduced hemoglobin, evidently due to a rapidly accelerating demand for oxygen by the cells, and the associated release of carbon dioxide into the blood. A cut in the skin of an arousing animal, which had not bled when it was in the hibernating state, bled slightly as it warmed. After a time a considerable amount of blood appeared at the site of the wound, indicative of a rising blood pressure.

Figure 2 shows a phenomenon which was frequently observed in the late stages of arousal. The animal here responded to external stimulation by what seemed to be a fright response. There was a sudden muscular contraction accompanied by momentary cessation of respiration and heart rate, in a manner resembling 'startle' to sudden noise. This response has been obtained in ground squirrels at this stage of arousal by employing auditory, visual or tactile stimuli.

A large temperature gradient between the fore and hind parts of the animal was apparent in all experiments in which the animal aroused in the cold. The maximum gradient seen was 15\(^\circ C\) \((T_r = 25^\circ C, T_h = 10^\circ C)\). The gradient was considerably less when the animal aroused at room temperatures where the environment was considerably warmer than the animal. Since the rise in body temperature is largely a result of increasing heat production, body temperatures lagged behind the increase in metabolic activity, both as to onset, and as to the time of reaching peak value. When the hind quarters began to warm, it was often noted that warming of the fore parts was temporarily arrested or slowed down until the fore-to-hind gradient had been decreased.

Relationship Between Breathing Rate, Heart Rate and Fore Temperature. Figure 3
consists of smooth curves showing the relation between respiratory rate and heart rate in several experiments. There was little spread between individual curves, until breathing rate reached about 80/min., and heart rate reached about 120/min. The course of the curves beyond this point varies considerably, but in most experiments they tend to form a loop which sweeps in a counterclockwise direction. This loop is an expression of the fact that respiration reached its peak and began to decline before the heart rate, that is, respiratory change led heart rate change. It can be mathematically shown that when a loop is formed in a counterclockwise direction by such a system of plotting, changes in the parameter indicated by the abscissa lead changes in the parameter measured on the ordinate. In each case in which induction was completed, (exp. C46 and C57), the curve terminated with the same relationship existing between respiratory and heart rate as had existed in early arousal.

Data from several experiments, with breathing rate related to body temperature during arousal is shown in figure 4. There was considerable variation between experiments, and although the curves were somewhat similar, a clear-cut relationship was not apparent. The relationship between heart rate and body temperature was found to be essentially in agreement with the data of Lyman and Chatfield (2).

DISCUSSION

It was not possible to obtain records of a natural induction, such as occurs several days after arousal. Records obtained from partial or complete inductions during or immediately following arousal provide the meager information available. In a recent review, Lyman and Chatfield (2) discussing induction, mention only that body temperature and metabolic rate decline, with metabolic rate reaching its minimum before body temperature. It is of interest that 5 of 30 arousals reported here were followed immediately by induction into the hibernating state, 3 of which began before arousal was complete. In eight other arousals induction appeared to have started, only to give way to arousal.

During hibernation the pattern of respiration varies between species, but usually consists of several deep breaths followed by a prolonged apnea (3-7). In hibernating ground squirrels it consists of single or grouped deep sighs, with an interval of variable duration between. The average rate over a period of time of 1-3 breaths/min. is virtually identical with the rates in other species. There is also
species variation in the heart rate, with all except the hedgehog considered to have fewer than 15 beats/min. while hibernating (2). The average of 7–8 beats/min. found in this series is consistent with these reports. In the case of hibernating heart rate, an electrocardiographic study in three species points to similar mechanisms operating to maintain function in the cold (8).

Many methods have been used to determine respiration during arousal. Hall (9) in 1832, placed a wand through a hole in a box containing the animal in such a way that one end rested on the animal and the other end moved in the air. This technique, with the addition of a kymograph has been used by others (5–7). Some have used direct observation (4, 10), or the muscle action potentials associated with respiration which often appear on the electrocardiogram (4). Such methods are not wholly satisfactory, for when the animal begins to arouse, shivering and other bodily movements mask those of respiration.

Pembrey (5) and Pembrey and Pitts (6) were of the opinion that continuous rhythmic breathing did not appear until late arousal in the dormouse, hedgehog and marmot. This may have been due to failure to detect the early onset of the small respirations, or to species differences. Johnson (10) reported that breathing rate in the 13-lined ground squirrel did not reach maximum until several minutes after heart rate. This was based upon direct observation, and in many cases, experiments (observations) were discontinued before peaks were apparent. In the current series however, respiration almost invariably reached its maximum and began to decline before heart rate.

There is evidence that the oxygen saturation of hemoglobin in the venous blood of a hibernating animal is essentially normal (11). This, plus the arterial appearance indicates that the diminished amount of oxygen supplied to the tissues is sufficient. During hibernation the total carbon dioxide is raised, but carbon dioxide tension is lowered (12). It is not known whether blood withdrawn by cardiac puncture is venous or arterial (right or left heart), but if it is arterial, which seems likely, the blood is not being adequately oxygenated by passage through the lungs during arousal. Data are lacking on the blood gas content during arousal, but it would seem that oxygen saturation of hemoglobin in venous blood would be lowered, and that both total carbon dioxide and carbon dioxide tension would be raised. Much confusion in the literature about blood gas values in hibernation may be due to the fact that blood samples taken from 'hibernating' animals actually were taken from animals in the early stages of arousal.

The integrating force during arousal seems to be nervous, in view of the rapid onset when the animal is disturbed. Apparently there is stimulation of all metabolic processes, largely sympathetic (13), especially to cardiac and respiratory centers. The absence of thoracic breathing during hibernation, and the lack of coordination with the diaphragm when it appears in arousal is similar to the respiratory pattern during recovery from surgical anesthesia.

Numerous suggestions have been made as to the source of the heat necessary to rewarm the body of the hibernating animal. These include the liver (14), shivering and increased muscle tension (15), the heart (2), and respiratory muscles (10). In view of the early onset of respiratory acceleration and the hyperventilation which develops, it would seem that the muscles of respiration are of considerable importance in the production of heat for rewarming. Oxygen consumption accelerates greatly during arousal, but the dark color of the blood and membranes of the mouth during arousal suggest that metabolism increases faster than oxygen is supplied. An accompanying rise in carbon dioxide tension of the blood would constitute a powerful stimulus to respiration, and could account for the fact that respiratory rate usually peaks before heart rate. The prompt decline in the rate of respiration also suggests that the accumulated oxygen debt is being repaid rapidly, even while metabolic activity is still at a high level. In induction, a diminishing metabolism and carbon dioxide production would decrease stimulation to the respiratory centers. Studies to relate respiratory activity with metabolic rate, as well as with blood oxygen and carbon dioxide during arousal are needed to clarify this mechanism.
REFERENCES