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AUTONOMIC CONTROL OF CIRCULATION DURING THE HIBERNATING CYCLE IN GROUND SQUIRRELS

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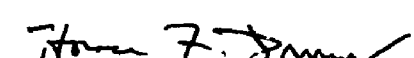
ABSTRACT

The control of circulation in undisturbed Citellus tridecemlineatus and C. lateralis has been studied during all phases of the hibernating cycle by infusing drugs of known pharmacological effect into the blood stream via chronically implanted aortic cannulae and by monitoring pulse pressure. It is concluded that the parasympathetic system has a regulatory, but not essential, effect on the heart rate as the animal enters hibernation. During hibernation the heart and circulation are virtually impervious to parasympathetic influence and during arousal the parasympathetic system has no detectable effect. Decline in sympathetic activity contributes to the slowing of the heart during entrance into hibernation, but sympathetically mediated vasoconstriction maintains peripheral resistance which is essential for life in hibernation. During hibernation skeletal muscle is extremely sensitive to the nicotinic effects of acetylcholine and methacholine, but the heart is impervious to parasympathetic effects. Muscle action potentials, either induced nicotinically or by physical stimulation, may initiate the cardioacceleration of arousal via sympathetic fibers. During arousal, sympathetic activity is essential to mediate the precisely timed vascular changes which aid in restoring the animal to the warm-blooded state.

PUBLICATION REVIEW



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SECTION 1. INTRODUCTION

It has been assumed for several decades that the autonomic nervous system plays an important role in the phenomenon of hibernation in mammals but definitive evidence has not been available. Until recently, investigations of the entrance into hibernation and the hibernating state were perforce descriptive, for any physical disturbance of the animal usually started the process of arousal. During arousal various physiological manipulations could be carried out and the effects noted, so that this phase of the hibernating cycle is moderately well documented (Lyman and Chatfield, 1955). From these data it has been postulated that the sympathetic nervous system is importantly involved in the process of arousal. On the other hand, it has been suggested that entrance into hibernation is brought about by parasympathetic influence (Strumwasser, 1960) or lack of sympathetic influence (Britton, 1928), but evidence to substantiate this is scanty.

Techniques of chronic intubation of major blood vessels (Still and Whitcomb, 1956) have opened a new approach to the study of hibernation. By means of an indwelling catheter, drugs of known pharmacological effect can be introduced into the blood stream in minute amounts and the effect noted with the assurance that the changes observed are due to the drug alone. Throughout the experiment blood pressure, pulse pressure, heart rate, heart temperature and the electrocardiogram can be obtained at will. With these techniques we have attempted a partial dissection of the role of the autonomic nervous system throughout the hibernating cycle.

SECTION 2. SUMMARY

The control of circulation in undisturbed Citellus tridecemlineatus and C. lateralis has been studied during all phases of the hibernating cycle by infusing drugs of known pharmacological effect into the blood stream via chronically implanted aortic cannulae and by monitoring pulse pressure.

The onset of hibernation is not hastened by infusion of a sympatholytic agent. Asystoles, which are typical of the animal entering hibernation,

serve to maintain a more even temperature-heart rate curve as the animal enters hibernation. Parasympathetic blockade abolishes these asystoles. Nonetheless the atropinized animal will enter hibernation. Extremely prolonged asystoles sometimes occur in atropinized animals. These are believed to reflect the cessation of sympathetic activity on the fatigued heart.

During deep hibernation, parasympathetic blockade has little or no effect on the heart rate. Veratramine does not alter the inherent rhythmicity of the pacemaker. The heart cannot be slowed by vagal stimulation, and acetylcholine and methacholine cause cardioacceleration. This cardioacceleration is only partially blocked by atropine and is potentiated by eserine. Ganglionic or sympathetic blockade causes a decline in peripheral resistance and a drop in blood pressure and abolishes the cardioacceleratory action of parasympathomimetics. Therefore this action is probably mediated through the cardioaccelerator fibers of the sympathetic system.

Even in very low doses, acetylcholine and methacholine cause a long-lasting burst of muscle action potentials, which is immediately followed by cardioacceleration. The same effect is seen if the hibernating animal is physically disturbed. Curarization blocks the muscle action potentials and the cardioacceleration caused by these parasympathomimetics.

The heart of the animal in hibernation is not slowed by parasympathomimetics or by vagal stimulation until it has accelerated and beat at a faster rate for some minutes. This change in sensitivity is due to the continuance of a faster rate, for heart temperature may remain unchanged.

During arousal sympatholytics abolish the vasoconstriction of the posterior portion of the body which is typical of all hibernators and this cannot be re-established by norepinephrine. This indicates that the differential vasoconstriction is not due to a lower threshold for norepinephrine in the posterior. Acetylcholine causes a vasodilation of the posterior so that this portion warms briefly while the anterior cools. In normal arousal, when the anterior has warmed and the vasodilated posterior is warming rapidly, vasoconstriction of this area can be reimposed briefly by norepinephrine. Atropinization has no effect on heart rate or the circulation during arousal.

It is concluded that the parasympathetic system has a regulatory, but not essential, effect on the heart rate as the animal enters hibernation. During hibernation the heart and circulation are virtually impervious to parasympathetic influence and during arousal the parasympathetic system has no detectable effect.

Decline in sympathetic activity contributes to the slowing of the heart during entrance into hibernation, but sympathetically mediated vasoconstriction maintains peripheral resistance which is essential for life in hibernation. During hibernation, skeletal muscle is extremely sensitive to the nicotinic effects of acetylcholine and methacholine, but the heart is impervious to parasympathetic effects. Muscle action potentials, either induced nicotinically or by physical stimulation, may initiate the cardioacceleration of arousal via sympathetic fibers. During arousal, sympathetic activity is essential to mediate the precisely timed vascular changes which aid in restoring the animal to the warm-blooded state.

SECTION 3. METHODS

The thirteen-lined ground squirrel (Citellus tridecemlineatus) was the principal experimental animal used in this study, but comparative observations were made on the golden-mantled ground squirrel (Citellus lateralis). A total of 116 animals were intubated, of which 57 C. tridecemlineatus and 2 C. lateralis supplied useful data in a total of 323 definitive experiments.

Only animals which had been hibernating were intubated. The technique for intubation has been given elsewhere (Lyman and O'Brien, 1960). A thin polyethylene tube (PE 10, OD 0.61 mm, ID 0.28 mm), sealed at one end, was implanted in the abdominal aorta with the open end facing upstream about one cm above the renal arteries and the sealed end protruding from the skin of the mid-back. In a few animals the external jugular vein was also intubated so that a drug could be introduced on the venous side. After intubation the animal was returned to the hibernaculum and usually re-entered the hibernating state. It was then fitted with an iron-constantan thermocouple which was sewed subcutaneously in the region of the heart, with the wires making their exit beside the aortic tube on the mid-dorsum. The aortic tube was spliced to a length of PE 10 tubing using a section of 27 g hypodermic needle. This tube and the two thermocouple wires were protected by a light helical spring which was attached to the back of the animal. In some animals three silver wire electrodes were sewed into the skin of the back for EKG and EMG recording.

The animal was placed in a round battery jar (23 cm diameter) with ample bedding, food and water. The helical spring was led through a screen covering the top of the jar and suspended at the end with an elastic band and a fishing swivel so that the animal could move freely. Usually five or six intubated ground squirrels were kept in this manner at $5^{\circ} \pm 2^{\circ}$ C in a cooled, insulated box.

Body temperature, called hereafter "heart temperature," was monitored on a Speedomax thermoelectric recorder, type G, with an accuracy of ± 0.25° C (Leeds and Northrup, Philadelphia, Pa.). The indwelling thermocouples were made of 36 g wires, each protected throughout its length with PE 10 tubing. Corrosion of the iron wire was reduced by filling this tube with a flexible epoxy resin. For acute colonic temperatures, a thermocouple was inserted to a depth of 2 to 3 cm into the rectum.

When measurements were made, the protective helical spring was steadied with a clamp, the thermocouple wires were attached to the potentiometer, and another length of PE 10 tube was spliced to the aortic tube. Polyethylene was used routinely as a splice because comparisons revealed no difference in pulse pressure when this tubing was used instead of the more inconvenient Cournand woven catheter. The tube led to an infusion apparatus consisting of a 1 ml syringe fitted with a motor-driven screw drive. Using this apparatus, the animal could be infused steadily at the rate of 0.2 ml per hour. By manually turning the drive the perfusion rate could be as high as 0.1 ml in four seconds, with an accuracy of 0.001 ml. A Statham pressure transducer, model P23D (Statham Instruments Inc., Los Angeles, Calif.) was attached to a T in the copper tubing which led from the infusion apparatus to the attachment of the polyethylene aortic splice. The transducer was amplified with a Grass low-level DC preamplifier, model 5 P1A, and a Polygraph DC driven amplifier, model 5 (Grass Instrument Co., Quincy, Mass.) and the excursions recorded on an ink-writing oscillograph. A mercury manometer, set at the level of the animal and attached to another T in the tubing, was used for calibration.

In testing the effect of a drug a record was made of the pulse pressure until a typical pattern was established. A measured amount of the drug was then introduced into the polyethylene splice using a Krogh-Keyes pipette. The drug-containing fluid was isolated on each side from the neutral infusion fluid by a short (5 mm) column of air. Tests using a colored liquid and heparin saline showed that the air columns prevented mixing of the two liquids much more effectively than oils or other substances. The short air columns did not affect the contour of the pulse pressure.

Prior to infusion of the drug, the same amount of neutral fluid (7.5 mg heparin/100 ml physiological saline) was infused into the animal as a control. The drug could then be introduced either as a whole or by small increments using the screw drive of the infusion apparatus. In the latter case the first dose of the drug could not be exact because the precise volume of the whole tube was unknown and because some mixing of the two fluids always occurred, but once there was a pure column of the drug at the open end of the cannula, as little as 0.001 ml of the drug-containing

fluid could be introduced with reproducible physiological results. The results of infusing the various drugs indicated that the course of the liquid was almost invariably in the direction of the blood flow, i. e., caudally. Very occasionally, however, when the heart rate was slow and the drug was infused quickly, the rapidity of the result indicated that the drug had been forced upstream into the coronary circulation.

Doses throughout this paper are reported in mg/kg. They are calculated using 150 gms as the average weight of the ground squirrel. Because weighing disturbed the active animals and aroused the hibernators, they were not weighed routinely, unless an animal was obviously over- or underweight.

In determining the drug dosage for animals in the various stages of hibernation, the substance was first introduced via the aortic tube into animals which were not hibernating but in the "active"* state, and the dose was established for the required result. With exceptions which will be noted, doses of the same magnitude were used for animals in deep hibernation.

The effect of most of the drugs could easily be assessed in the hibernating animal. However, for reasons to be explained below, it was difficult to be sure that the parasympatholytic action of atropine (atropine sulfate; Mallinckrodt) had been established in the hibernator, and the variable heart rate of the active animal masked the effect of the drug. Asphyxia causes a reflex slowing of the heart of the hamster arousing from hibernation and this reflex is abolished by atropine (Chatfield and Lyman, 1950). The same reflex was found to occur in the ground squirrel, and its abolishment when the animal was manually choked was considered a sign of total atropinization. The choking test was also used on active animals, but the abolishment of the reflex slowing of the heart when the animal was startled by noise or vibration was the more usual test. No vagotomized animals were used, because this operation also divides the recurrent laryngeal nerve and respiration becomes blocked by mucus.

In order to stimulate the vagus nerve of hibernating animals, electrodes were fashioned of fine stainless steel wire, each in a polyethylene tube (ID 0.23 mm). Near each tip, one side of the tube was cut away, exposing the wire. The right vagus nerve of previously intubated animals was dissected free and the exposed part of each wire was wrapped around the nerve, with the intact portion of the polyethylene serving as a shield. The

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* Because of semantic difficulties, we use this word to describe a potential hibernator when not in any phase of actual hibernation. Used in this sense, the "active" animal could be actually asleep or immobile.

electrodes were stitched in place, with a polyethylene spreader separating them by about 0.5 cm. The two insulated wires were led to the mid-dorsum and passed through the protective helical spring. Necrosis of the nerve always occurred and the useful duration of such a preparation was never more than a week. A Grass stimulator (Grass Instrument Co., Quincy, Mass.) was used to produce biphasic shocks of 20 msec duration at a frequency of 25/sec.

Using the techniques outlined above, studies were made on active animals, and on animals entering hibernation, in deep hibernation, and arousing from hibernation. Of the four phases, the second was most difficult to study, because the onset of hibernation is unpredictable. Furthermore, the animals are extremely sensitive at this time, so that stimulation, even by infusion of isotonic saline, is apt to change this phase into the very different phase of arousal. For these reasons, investigations on entering hibernation have been necessarily limited.

SECTION 4. RESULTS

Entering Hibernation

When the ground squirrel enters hibernation a fall in heart rate and blood pressure always precedes the decline in body temperature. The slowing of the rate is accomplished by a lengthening of the period between the even beats and by actual skipped beats which appear at fairly regular intervals (Lyman and O'Brien, 1960). As the temperature declines, the even beats become slower, followed by longer periods of asystole (Figure 1a). This "interrupted saw-tooth" type of pulse pressure may result in a very low average heart rate as the animal approaches the deeply hibernating state, in spite of the intermittently rapid heart. Heart rate plotted against either time or heart temperature (Figure 2) results in a smooth curve. If only the periods of even heart rate are counted, the rate is, of course, faster for any given temperature, and the temperature-rate relationship is much less exact (Figure 2). Thus, the periods of skipped beats serve to smooth the overall heart rate curve as the animal enters hibernation.

Parasympathetic Blockade. The beginning of hibernation is presaged by fluctuations of body temperature and onset of skipped heart beats. Both of these are abolished by atropine and the animal becomes more alert. Thus, although the heavily atropinized animal will enter hibernation, only one complete record, out of many attempts, was obtained. Once the body temperature has declined a few degrees, the animal may be atropinized or

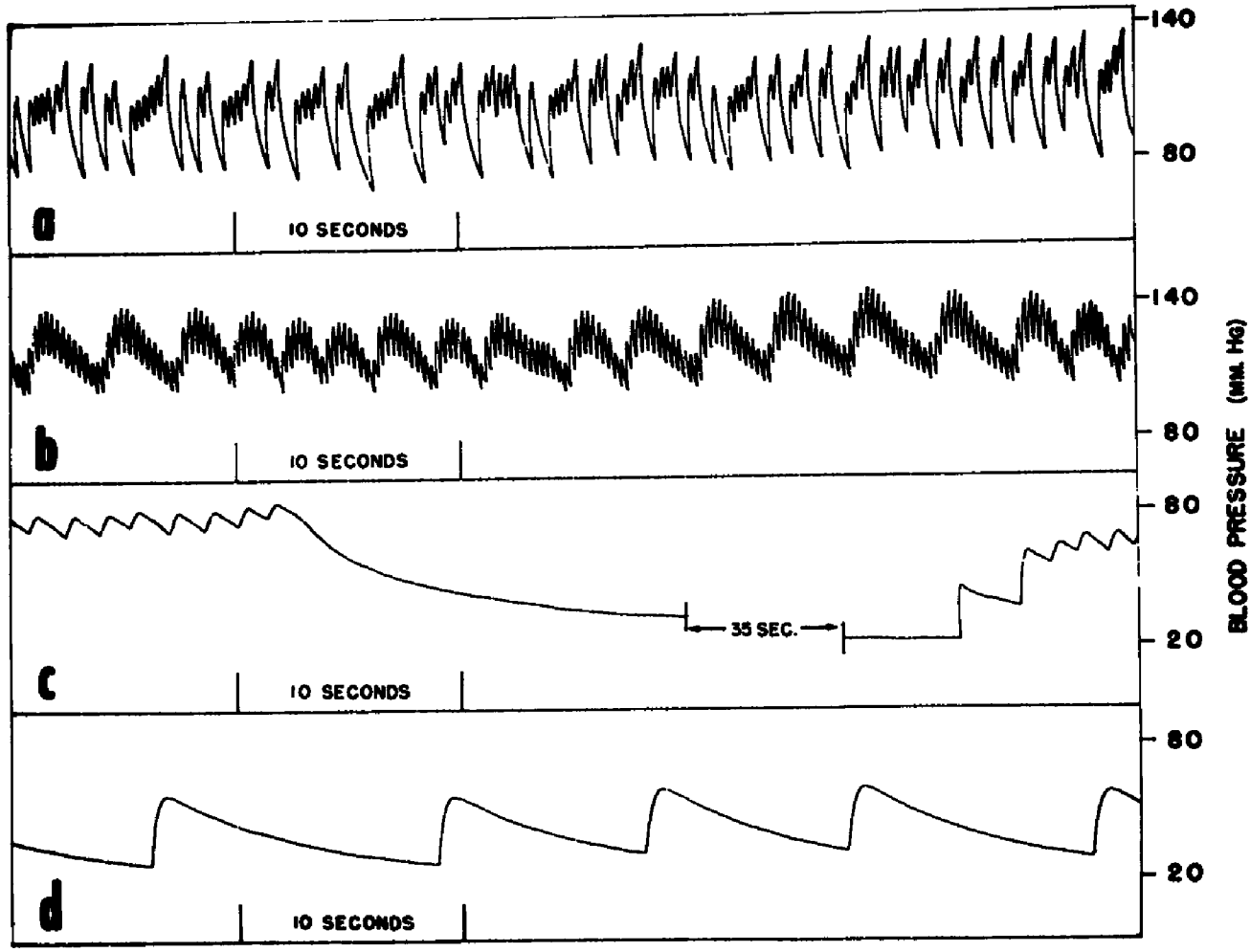

FIGURE 1. (a) Typical pulse pressure pattern of thirteen-lined ground squirrel entering hibernation, showing skipped beats. Heart temperature 29.5° C.

(b) Same animal 10 minutes after treatment with atropine, which abolishes skipped beats. Heart temperature 29.0° C.

(c) Long asystole in atropinized animal entering hibernation. Asystole lasted 58 seconds in this case. Heart temperature 14.5° C.

(d) Atropinized animal (18.5 mg/kg) with pulse pressure pattern which is typical of untreated ground squirrel in deep hibernation. Heart temperature 10.6° C.

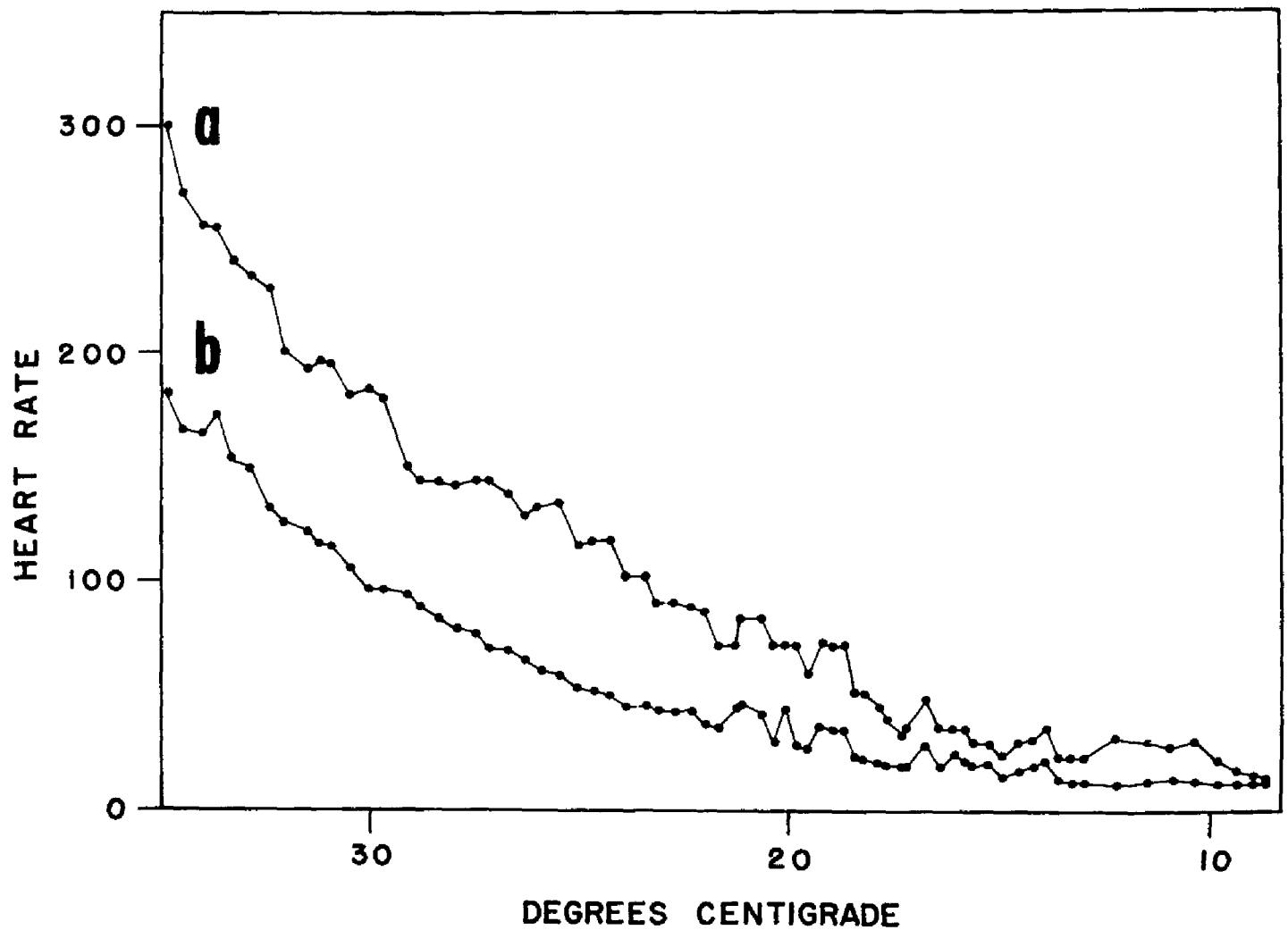

FIGURE 2. Temperature-rate curves of a normal ground squirrel entering hibernation.

(a) Rate calculated by counting only the evenly occurring beats.
(b) Actual heart rate. Note fewer irregularities in curve b. Heart rate in both cases registered for a one-minute period at five-minute intervals.

treated with methantheline bromide 1.3 mg/kg (Banthine bromide; G. D. Searle and Co.) and usually continues into hibernation. Under these conditions the skipped beats are abolished for long periods, the evenly spaced beats occur more rapidly than they do at the same temperature in the untreated animal, and the blood pressure rises (Figure 1b). In one ground squirrel, atropinized when the body temperature had reached 23° C, the heart rate remained elevated throughout the night; the body temperature ceased its decline at 13° C and remained between 6 and 10° C above the environmental temperature for 15 hours, when a slight disturbance caused the animal to arouse. Usually, however, in spite of long periods of absolutely even heart rate, asystoles occurred. These asystoles were much longer than those observed in the unatropinized animals and lasted as long as 86 seconds at a heart temperature of 15.6° C (Figure 1c). As the asystole continued, respiratory rate increased. Before the heart resumed beating, a bodily jerk often occurred which was too violent to be caused by respiration alone. When the heart beat resumed, the rate was very rapid but decreased as respiratory rate again declined. As the animals grew colder the bodily jerks ceased, the periods of asystole became more frequent and the periods of tachycardia shorter, until the typical pulse pressure pattern of deep hibernation was reached (Figure 1d). The occurrence of prolonged asystoles in the atropine-treated animal cast doubt on the effectiveness of the dose which had been employed. However, it was found that the asystoles occurred when doses of atropine as high as 24 mg/kg were administered, while less than one-half this dose will completely abolish the reflex bradycardia produced by choking the arousing hibernator and only 3 mg/kg will abolish vagal slowing caused by startling the active animal. Thus, it seems assured this type of asystole is not produced by parasympathetic action, and is a different phenomenon than the atropine-sensitive skipped beats which occur in the untreated animal as it enters hibernation.

Parasympathetic blockade caused a marked increase in heart rate in the later stages of entering hibernation when the heart rate was at the level of deep hibernation but the body temperature was still declining. Thus two animals, with rates varying between 6 and 10 and heart temperatures of 10° C, increased the heart rates to 22-32 beats/min when given 4.1 mg atropine/kg. Another animal with the same rate and a temperature of 14° C increased the heart rate to 63 after 3.6 mg atropine/kg. In these and other cases the body temperatures continued to decline, in spite of a faster heart rate and a consequent higher blood pressure.

Because of the absence of skipped beats, the heart rate of an atropinized animal entering hibernation is usually faster than the rate of an unatropinized animal at the same heart temperature. However, if the even rate of an atropinized ground squirrel is compared to the even rate of the same animal entering hibernation without treatment, it is found that this rate in the atropinized animal is not necessarily faster at the same temperature.

In atropinized animals where prolonged asystoles occur, the average heart rate approaches that of the control, though a rate-temperature curve cannot be constructed because the rate is so variable.

Parasympathetic Stimulation. The study of the effect of parasympathomimetic drugs on the heart rates of C. tridecemlineatus and C. lateralis while entering hibernation or in the hibernating state was complicated by the fact that infusion of either acetylcholine (acetylcholine chloride; Merck) or methacholine (Mecholyl chloride; Merck) in a wide variety of doses caused cardioacceleration before either drug could circulate to the heart (see In Hibernation; Acetylcholine, Methacholine). We had no opportunity to test the effect of either drug on an animal entering hibernation when fitted with a jugular cannula, nor were we able to stimulate the vagus electrically during this phase of the hibernating cycle.

Sympathetic Blockade. The infusion of the sympatholytic agent β-TM10 ([2-(2, 6-Dimethylphenoxy) propyl]-trimethylammonium chloride hydrate; Smith, Kline and French) in doses as high as 13.8 mg/kg did not hasten the onset of hibernation in active ground squirrels which recently had been hibernating. Because the initial effect of this drug is a transient but marked cardioacceleration, it could not be used to study the effect of sympathetic blockade during the process of entering hibernation, for infusion resulted in immediate arousal.

In Hibernation

Even when only temperature and heart rate are considered, the transition from entering hibernation to the deeply hibernating state is not clear-cut. The heart slows to the rates found in deep hibernation while the body temperature is still several degrees above the environmental temperature, yet body temperature continues to decline for four or more hours thereafter (Lyman and O'Brien, 1960). Finally, the body temperature becomes stable two or three degrees above the environmental temperature.

It may be categorically stated that deep hibernation has been attained under these conditions, yet the interrupted saw-tooth type of pulse pressure, which is typical of entering hibernation, can persist for many hours at average rates which are as slow as the relatively even rates of hibernation. Throughout the hibernating period, the heart rate varies from day to day and sometimes from hour to hour, with rates usually falling between 5 and 8 beats/min at heart temperatures of 3° to 8° C. However, rates as slow as 2 and higher than 10 beats/min have been recorded in the normal hibernating ground squirrel.

Parasympathetic Control. The effect of parasympathetic blockade during deep hibernation contrasted with its effect during entrance into hibernation. In animals with heart rates of 2 to 12 beats/min and steady heart temperatures between 3.5° and 8° C, atropine (4.1 to 41.0 mg/kg) often did not increase the rate at all, though if there were unevenly occurring periods of asystole they were abolished, causing a more even rate. Blood pressure remained unchanged. Extremely slow heart rates (2-4/min) showed no more tendency to increase after atropinization than faster rates. Methantheline bromide (1.5 to 6.4 mg/kg) usually, but not always, caused transient cardioacceleration lasting about one hour in deeply hibernating animals, the rate then returning to below 10/min.

The initial cardioacceleratory action of acetylcholine and methacholine complicated the study of these drugs on hibernating animals. However, in a large series of experiments on over 45 C. tridecemlineatus and C. lateralis, infusion of acetylcholine or methacholine never slowed the heart rate of the undisturbed hibernating animal in spite of a wide variety of volumes, infusion rates and doses (acetylcholine 0.013 to 8.2 mg/kg and methacholine 0.1 to 1.0 mg/kg). Pretreatment with physostigmine 0.16 mg/kg (physostigmine sulfate; Merck) also failed to produce the typical parasympathomimetic effect of acetylcholine. Some time after either acetylcholine or methacholine had accelerated the heart, infusion of the same drug caused a series of prolonged asystoles. These asystoles appeared 9 to 15 seconds after the infusion, and must reflect the circulation time from the end of the catheter to the heart. Sometimes another asystole occurred 20 or more seconds after the first, probably caused by the drug circulating to the heart a second time. On occasion, slowing of the accelerated heart occurred almost immediately after infusion of the drug. In these cases it was assumed that some infusion fluid had been forced upstream into the coronary arteries.

In animals which had been in hibernation for a day or more, the change in threshold to the parasympathomimetic effect of acetylcholine or methacholine took place at least fifteen minutes after the first infusion of the drug had caused cardioacceleration, but often occurred more rapidly if the animal had just entered deep hibernation. This change took place even if the heart temperature and faster rate remained static, indicating that time and an accelerated rate were important factors. Thus, in a typical case, 6.2 mg acetylcholine/kg caused the heart of a hibernating C. tridecemlineatus to increase from 7 to 30 beats/min in twelve minutes. At this point infusion of the same dose had no effect. Seven minutes later, with the heart rate still at 30, the identical dose caused transient slowing of the heart. Heart temperature remained at 8.5° C throughout this time. The infusion of acetylcholine with its accompanying cardioacceleration often caused the ground squirrel to arouse from hibernation. At various

periods during arousal, the minimum dose of acetylcholine necessary to produce asystoles could be determined. It was found that once the typical parasympathomimetic effect was established, the threshold to the drug dropped within a few minutes to one-tenth or less of the concentration needed originally to produce cardiac slowing. Thereafter the threshold was changed very little as the animal warmed. It should be pointed out, however, that such a preparation does not truly test the threshold response of the heart with changing temperature, for the blood-borne drug must circulate to the heart, and the change in activity of cholinesterase during arousal from hibernation is unknown.

The effect of the parasympathetic system on the heart rate during deep hibernation was explored further using chronically implanted vagal electrodes. In all cases stimulation with up to 140 volts for 10 to 60 seconds failed to produce slowing of the heart during deep hibernation. The stimulation produced a contraction of the neck muscles, and the heart rate increased as if the animal had been disturbed manually. Stimulation with the same voltage immediately after the heart rate had increased failed to cause slowing, but after about one hour a prolonged asystole could be produced even in cases when the heart temperature had risen to only 9° C. As the animal warmed and the heart rate increased further, progressively lower voltages were needed to produce asystole until the absolute threshold was reached when arousal was about two-thirds complete.

A similar result often occurs if a deeply hibernating animal is lightly stimulated mechanically or infused with a small amount of a cardio-accelerating drug. The heart immediately accelerates, sometimes with no change in heart temperature, and remains at the new rate for some time without advancing further toward arousal. After a period of at least one-half hour, prolonged asystoles begin to occur quite regularly. The asystoles become more frequent with time and the pulse pressure record assumes the typical interrupted saw-tooth appearance of the normal animal entering hibernation with the heart responding to vagal influence. After some hours the pulse pressure returns to the typical pattern of deep hibernation.

A cruder, but effective, test of the ability of the parasympathetic system to slow the heart in hibernation made use of the reflex slowing of the heart during asphyxia. If deeply hibernating ground squirrels were manually choked (using a glove in order not to warm the neck region) the heart was not slowed. Choking elicited arousal, with cardioacceleration and warming. A series of prolonged asystoles could be produced by choking, fifteen or more minutes after the initial stimulus. As with acetylcholine, there was no precise correlation with heart temperatures. One heart failed to slow at 10.6° C while another slowed at 8° C.

Pacemaker. Veratramine 0.98 mg/kg was infused into hibernating animals in an attempt to slow the heart by altering the inherent rhythmicity of the pacemaker (Krayer, 1949). After infusion of the drug the respirations became deeper, but there was no alteration of heart rate. The animals were then disturbed, and aroused from hibernation with normal cardioacceleration. As they warmed they underwent violent convulsions indicating that the dose, at least during the waking process, was sufficient to affect the heart, for the amount of drug which produces convulsions is considerably higher than the amount which influences the heart (Krayer, 1949).

Acetylcholine, Methacholine. The cardioacceleration produced by parasympathomimetic drugs was noted early in this study and considerable effort was expended in attempting to explain this anomalous effect. Three types of cardioacceleration could occur after infusion of either acetylcholine or methacholine. In the most common type of response a wide range of doses of acetylcholine (0.01 to 82.0 mg/kg) caused an immediate increase in heart rate and rise in blood pressure. The cardioacceleration usually occurred within two heart beats after infusion of the drug, and there was no evidence of change in peripheral resistance prior to the acceleration. A similar result occurred with methacholine (0.1 to 1.0 mg/kg) but the cardioacceleration was delayed at least 24 seconds after infusion. During this time a slight decline in diastolic pressure sometimes, but not invariably, occurred. With either drug the increased rate always lasted many minutes and was often followed by arousal from hibernation, particularly when large doses were employed.

A second common response to either acetylcholine or methacholine in low doses consisted in a drop in peripheral resistance and blood pressure during the next half-minute or more, followed by an increase in heart rate which brought the blood pressure above its initial value. Usually the heart rate and blood pressure slowly decreased to their original state, but occasionally the heart rate continued to increase and the animal aroused from hibernation. The third type of response occurred but rarely. After infusion of acetylcholine or methacholine no change in pulse pressure was observed for several minutes. This was followed by a transient increase in heart rate and blood pressure.

Though the cardioacceleration which followed a drop in blood pressure has been considered to be a compensatory response (Lyman and O'Brien, 1960), there was no obvious explanation for the increase in heart rate which occurred almost immediately after the infusion of a parasympathomimetic. It seemed possible that this anomalous response might be an indication of an unusual type of circulatory control in the deeply hibernating animal, and for this reason it was examined in some detail. No

effort was made to study the long-delayed type of cardioacceleration because its occurrence was so rare and unpredictable.

By starting with low doses, it was possible to determine the minimum amount of parasympathomimetic drug necessary to produce an immediate increase in heart rate in the deeply hibernating animal. This minimum dose often varied from day to day in the same animal with the same heart temperatures and comparable heart rates, and was as low as 0.01 mg acetylcholine/kg or 0.1 mg methacholine/kg. There was little difference between a dose which caused a speeding of the heart with subsequent return to the slower rate of deep hibernation, and a dose which initiated complete arousal, so that many animals were awakened from hibernation unintentionally. Pretreatment with physostigmine 0.16 mg/kg potentiated the reaction, reducing the minimum effective dose of acetylcholine by about one-half, and causing the cardioacceleration to continue for longer periods.

Hibernating animals were tested for their response to low doses of acetylcholine before and after treatment with atropine 6.15 mg/kg. Although this dose is sufficient to block vagal slowing in the active animal for several hours, it only partially blocks the cardioaccelerating effect of acetylcholine in hibernating ground squirrels, for doubling the dose of acetylcholine overrode the effect of atropinization.

Ganglionic blockade was produced by infusing the hibernating C. tridecemlineatus or C. lateralis with hexamethonium chloride 64.1 mg/kg ('Hexameton' chloride; Burroughs, Wellcome and Co.). This caused a drop in peripheral resistance and a marked drop in blood pressure with a transient compensatory increase in heart rate. When acetylcholine (0.08 to 0.62 mg/kg) was infused, the heart rate either remained unchanged or dropped to a lower rate after a time commensurate with the circulation time to the heart. The blood pressure dropped even lower, so that despite artificial respiration, heat and infusion of l-norepinephrine (Levophed bitartrate; Winthrop) one animal failed to survive. Lower doses of hexamethonium caused a drop in blood pressure but failed to block the cardioaccelerating action of acetylcholine.

The use of β-TM10 as a sympatholytic agent was complicated because infusion with a variety of doses and rates caused a cardioacceleration and rise in blood pressure which often resulted in arousal from hibernation, particularly if the infusion rate was slow. In some animals treated with β-TM10 9.2 mg/kg, the heart rate returned to its original level within 24 hours. Infusion of acetylcholine 6.2 mg/kg always caused cardioacceleration in these cases. However, in three animals the cardioacceleration caused by the same dose of β-TM10 lasted only one to three hours and

the rate then dropped to 4 to 12/min with a lower blood pressure and peripheral resistance. Infusion of acetylcholine then caused a further reduction in blood pressure, but no cardioacceleration, and prolonged asystoles were produced when the drug reached the heart. As with ganglionic blockade, one animal died without arousing and the others recovered only after treatment with norepinephrine, heat and artificial respiration.

Although these results indicated that the heart was being speeded via the sympathetic cardioaccelerator fibers, they did not explain why acetylcholine and methacholine invariably increase the heart rate of the hibernator. We therefore turned our attention to active, anesthetized animals in the hope of discovering some pharmacological peculiarity in the response of animals which hibernate to the infusion of parasympathomimetic drugs.

In ground squirrels anesthetized with pentobarbital sodium 80 mg/kg (Nembutal sodium; Abbott) rapid aortic infusion of acetylcholine (1.0 mg/kg or more) resulted in typical parasympathomimetically induced asystoles 5 to 6 seconds after infusion, often with a drop and subsequent recovery of blood pressure. The recovery of blood pressure was sometimes accompanied by cardioacceleration, which could be abolished by β-TM10 9.2 mg/kg. Pretreatment with physostigmine 0.16 mg/kg reduced the amount of acetylcholine necessary to produce cardiac slowing by about one-half. Unlike the case during hibernation, cardioacceleration was never a primary result of acetylcholine infusion.

When the acute preparation was observed with viscera exposed, it was seen that the first effect of infusion of acetylcholine in a variety of doses (0.75 to 9.2 mg/kg) was a twitch of the hind legs which occurred within 1.5 seconds after infusion of the drug. This was followed first by a slowing of the heart and piloerection of the tail, then by contraction of the bladder, increased peristalsis and a respiratory gasp. Atropine (9.2 to 24.6 mg/kg) abolished the cardiovascular and visceral effects produced by acetylcholine. Three or four times as much acetylcholine was needed to produce the nicotinic effects after atropinization, but these effects could not be completely blocked.

The nicotinic effects were examined further using hibernating thirteen-lined ground squirrels which had been previously fitted with three electrodes sewed to the skin of the back. Infusion of acetylcholine in doses as low as 0.02 mg/kg resulted in a long-lasting burst of muscle action potentials but no visible movement of the animal. This was followed at once by cardioacceleration (Figure 3a). An almost imperceptible respiratory-like dorsal flexing occurred 16 or more seconds after the infusion,

and, as the faster heart rate continued, respiratory rate also increased. Identical bursts of muscle action potentials followed by cardioacceleration and increased respiratory rate could be induced by physically poking the animal with a stiff wire, and usually resulted in arousal from hibernation (Figure 3b).

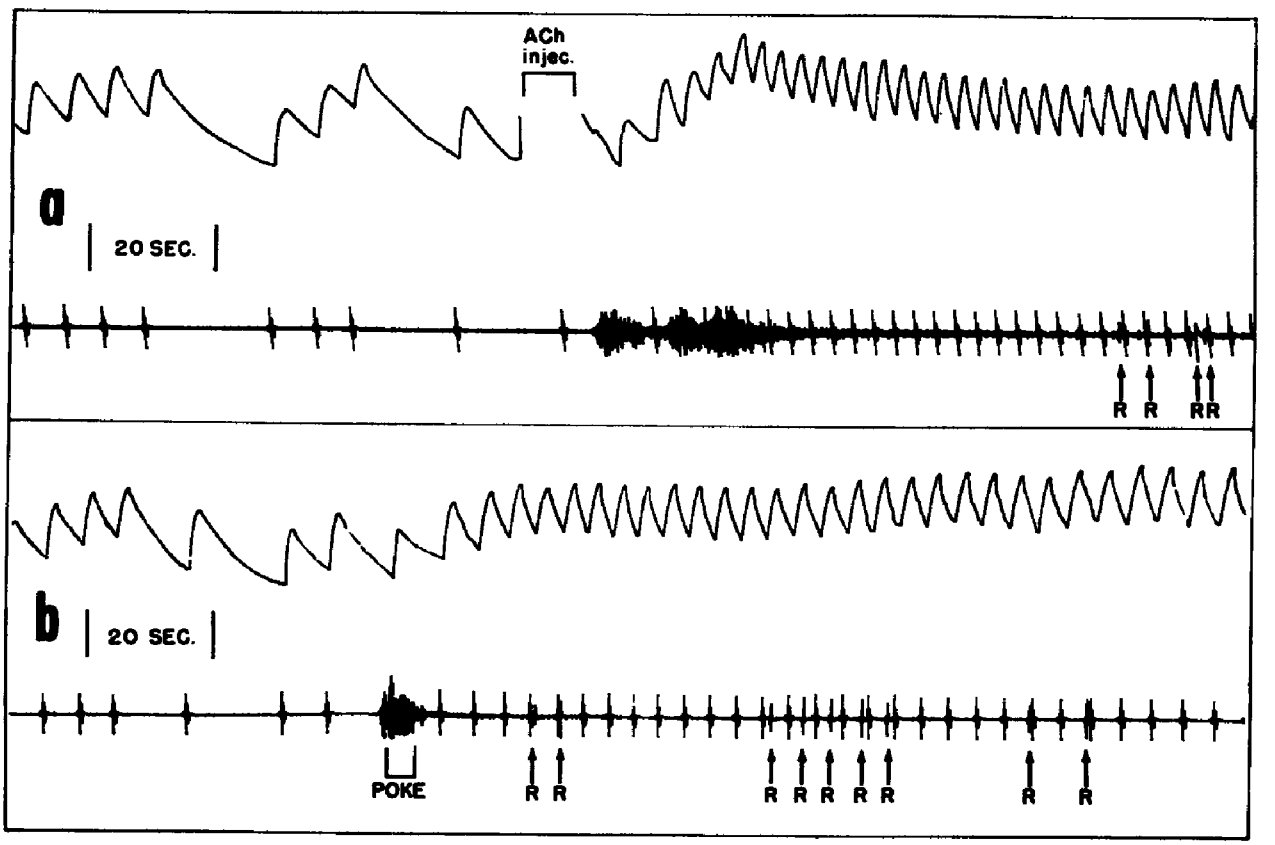

FIGURE 3

(a) Muscle action potentials and cardioacceleration caused by infusion of acetylcholine (0.205 mg/kg). Note increase of respiration (R) after the stimulus. Heart temperature 9° C. Animal did not arouse from hibernation with this stimulus.

(b) Muscle action potentials and cardioacceleration caused by poking same animal with a stiff wire four hours after (a), with very similar results. Heart temperature 8.6° C.

Methacholine 0.31 mg/kg gave rise to no muscle action potentials immediately after infusion into the hibernating animal but caused one or more respiratory-like movements 24 to 180 seconds later, which could be seen grossly and appeared on the EKG as bursts of muscle action potentials. These were followed by cardioacceleration.

The time of onset of the nicotinic effects of acetylcholine in contrast to methacholine coincided with the difference in the onset of cardioacceleration caused by these two drugs in the hibernating ground squirrel. This suggested that induced muscular activity might be associated with cardioacceleration during hibernation, and the invariable appearance of muscle action potentials prior to cardioacceleration, whether the animal was stimulated pharmacologically or physically, supported this concept. As a further test, hibernating ground squirrels were curarized (0.7 to 0.9 mg/kg) (d-tubocurarine chloride pentahydrate; Abbott) prior to treatment with acetylcholine. Unfortunately, this caused a transient increase in heart rate, so that it was necessary to wait 30 to 55 minutes after infusion of the drug before the heart returned to its original slow rate. By that time the respiratory muscles were at least partially paralyzed, and with the dose of 0.9 mg curare/kg the development of A-V dissociation indicated that the heart was anoxemic. Under these conditions, infusion of acetylcholine or physical stimulation of the animal produced no muscle action potentials and no cardioacceleration. Using 0.7 mg curare/kg, muscle action potentials could be reduced, but not abolished, when acetylcholine was infused. However, acetylcholine (0.05 to 6.2 mg/kg) never (six cases) caused cardioacceleration and never produced the typical respiratory-like movement observed in the uncurarized animal even though both of these effects were produced by 0.05 mg acetylcholine/kg prior to curarization. With 6.2 mg acetylcholine/kg the heart slowed briefly thirty or more seconds after infusion, evidently due to the direct action of acetylcholine. In all cases warming and artificial respiration were necessary to rouse the animals.

Arousal from Hibernation

As has been described previously (Lyman and O'Brien, 1960) an increase in heart rate and often a decrease in peripheral resistance are among the first changes observed when the hibernating animal starts the arousal process. Because of the decrease in peripheral resistance, the blood pressure does not necessarily increase at once, but eventually it rises as the heart beats faster. With the increase in heart rate and blood pressure, the anterior part of the body warms while the posterior remains cool. The blood pressure is at its peak value at about the time the heart temperature reaches 37° C. At this point, the post-thoracic portion of the body warms quickly while the heart rate remains rapid and the blood pressure usually declines somewhat.

The variety of drugs which produced cardioacceleration in the hibernating ground squirrel has been detailed in the previous section. The initial effect of most of these drugs was simply an increase in rate followed by an immediate rise in blood pressure. A decline in peripheral resistance and cardioacceleration occurred only with infusion of acetylcholine or methacholine and this was not invariable. Although the action of these two drugs most closely mimicked either natural arousal or arousal caused by physical disturbance, still any drug which produced cardioacceleration could cause the animal to arouse from the hibernating state.

Infusion of various drugs confirmed the view (Lyman and Chatfield, 1955) that the differential rewarming was caused by vasoconstriction. Hibernating ground squirrels were infused with β-TM10 9.2 mg/kg and mechanically stimulated to arouse 24 hours later. Under these circumstances normal cardioacceleration occurred, but the deep rectal temperature increased almost as rapidly as the heart temperature and remained no more than 4 to 5° C colder than the heart temperature during the whole arousal. With vasoconstriction absent, the warming process was greatly prolonged (Figure 4).

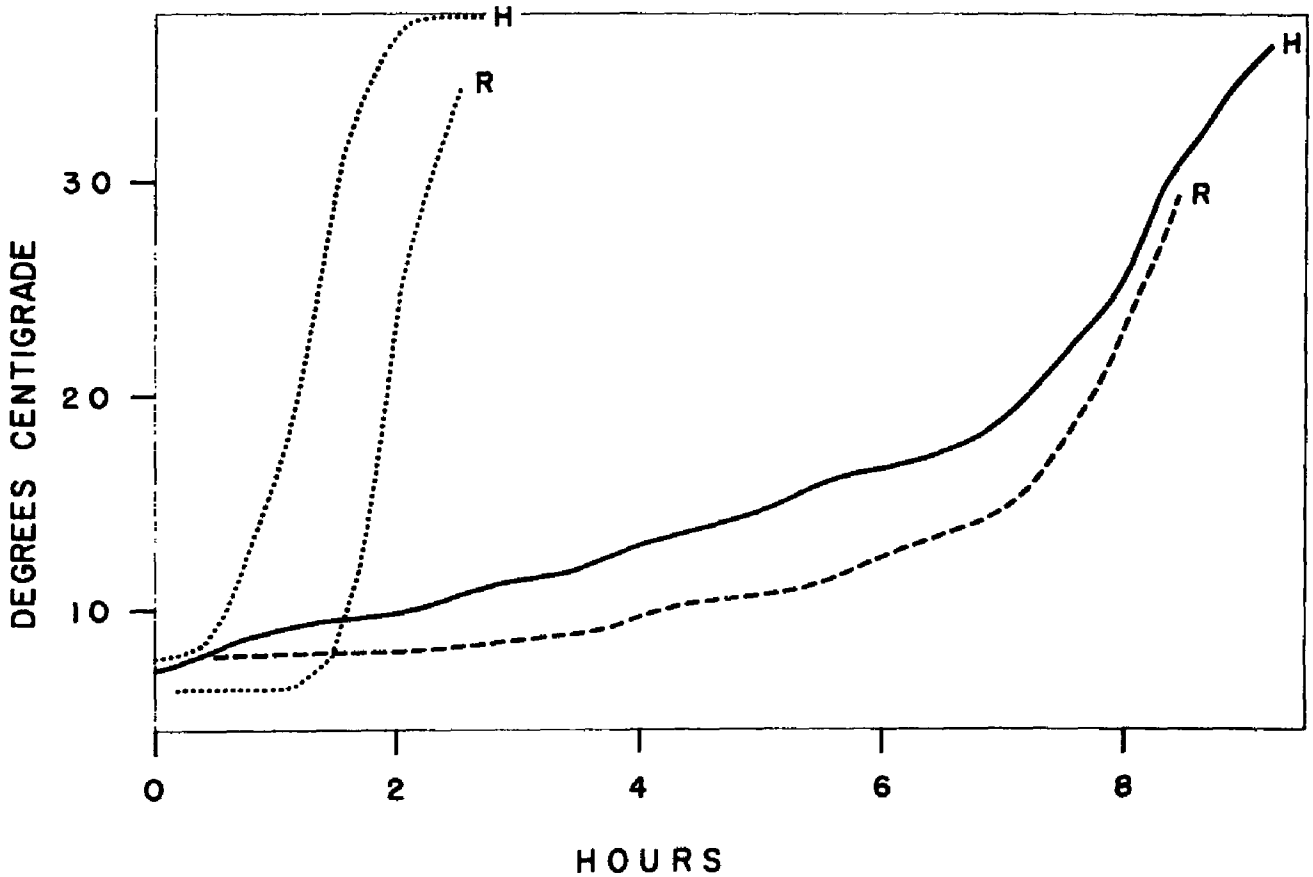

FIGURE 4. Normal rapid arousal from hibernation (dotted lines). Arousal after treatment with sympatholytic β-TM10 9.2 mg/kg (solid and dashed lines).
H = heart temperature; R = rectal temperature.

During normal arousals, animals were quickly infused with acetylcholine. Immediately after infusion the heart temperature stopped its rapid rise and the rectal temperature began to increase. Typical prolonged asystoles sometimes occurred as the acetylcholine reached the heart. When the vasodilatory effect of the drug was dissipated, the rectal temperature ceased to rise, and the heart again warmed rapidly. Repeated injections caused a rise and a plateau of heart and rectal temperatures, each a rough mirrored image of the other (Figure 5).

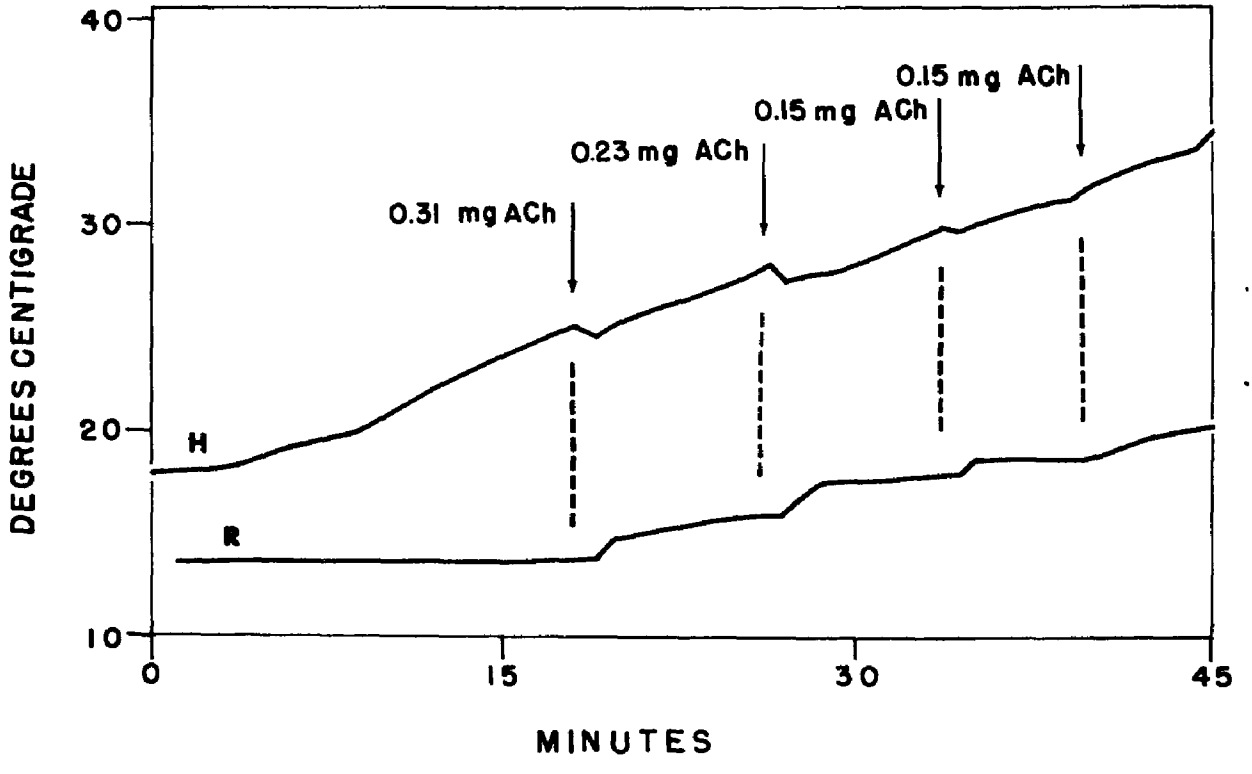

FIGURE 5

Effect of acetylcholine on arousing hibernator while heart temperature is rising and rectal temperature is not. H = heart; R = rectal.

The vasoconstrictor action of norepinephrine was tested during the last stages of arousal when the heart temperature had reached 32° to 38° C and the rectal temperature was rising rapidly. Rapid infusion of a very large dose (0.04 mg/kg) stopped the increase in rectal temperature for two or three minutes, but continuous infusion could not maintain this condition. If infusions were given serially several minutes apart, the rectal temperature could be made to rise in a stepwise manner (Figure 6). Norepinephrine would not augment the increase in heart rate once arousal was under way nor did heavy atropinization increase the heart rate for any given temperature.

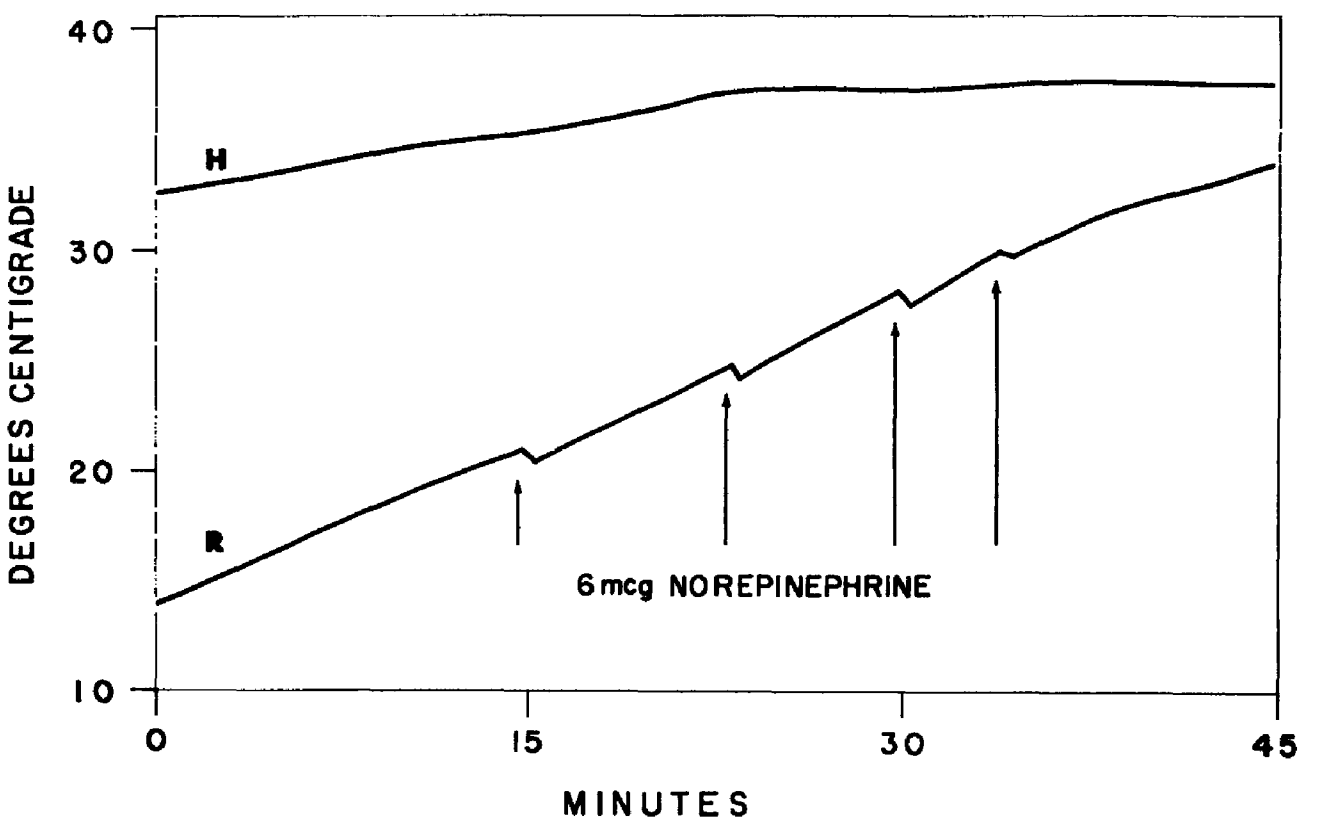

FIGURE 6

Effect of norepinephrine on animal in last stages of arousal when rectal temperature is rising rapidly.
H = heart; R = rectal.

Presumably the differential vasoconstriction during arousal could be caused by a lower threshold to norepinephrine in the posterior part of the body. A change in the level of norepinephrine, or the high blood pressure, might cause vasodilation of the posterior in the last stages of arousal. To test this concept, hibernating ground squirrels were infused with sufficient β-TM10 (9.2 mg/kg) to abolish vasoconstriction. Arousal was induced mechanically 24 hours later and norepinephrine was infused as both the heart and rectal temperature slowly increased. Animals were given a constant infusion of norepinephrine 0.007 mg/kg per minute to which were added rapid infusions of 0.008 to 0.045 mg/kg, so that in one case a ground squirrel received 0.13 mg/kg in six minutes. Even this large and continuing dose did not halt the warming of the posterior though the increase in temperature was not quite as rapid during the period of infusion.

Since β-TM10 does not block the effect of norepinephrine (McLean et al, 1960) at the effector organ, the experiment indicates that the threshold to norepinephrine is the same throughout the body.

SECTION 5. DISCUSSION

Although the autonomic nervous system has been implicated theoretically in the phenomenon of hibernation, direct approaches to the problem are rare. Various drugs have been injected into active animals with the hope of producing the hibernating state, but to our knowledge all such attempts have been unsuccessful. Bilateral removal of the cervical sympathetic ganglia has been thought to hasten the onset and increase the duration of hibernation, but the author does not suggest how this lesion produced the reported result (Arbuzov, 1950). The role of the autonomic system in deep hibernation is similarly unexplored. We have emphasized previously that subcutaneous injections during hibernation cannot be sufficiently controlled to justify conclusions because response to physical stimulation is so variable. It is only during the acute process of arousal that experiments have been carried out which are sufficiently discrete to justify some conclusions (Lyman and Chatfield, 1955).

As far as entrance into hibernation is concerned, observations in the last decade have indicated that this is indeed a controlled process rather than a simple reversion to a primitive poikilothermy. The slowing of metabolic rate (Lyman, 1958), respiratory rate (Landau, 1956), and particularly heart rate (Lyman, 1958; Lyman and O'Brien, 1960) prior to any detectable decline in body temperature strongly suggests that these vital functions are being repressed by something more than temperature alone.

The experiments reported here show that the parasympathetic system plays a part in slowing and regulating the heart as two species of ground squirrels enter hibernation. Skipped beats are an integral part of entrance into hibernation and serve to maintain a very even decrease in heart rate. Under the influence of atropine, skipped beats are abolished for long periods and the even rate is faster for any given temperature. However, something other than parasympathetic action must cause the slowing of the even rate of the heart as the atropinized animal enters hibernation. Obviously, the declining temperature has its effect on the heart rate, but the present investigation does not clarify the fundamental problem of why the temperature starts to drop.

The prolonged periods of asystole which may occur in the heavily atropinized animal are also without clear explanation. It may be that the heart, freed of parasympathetic influence, beats overly fast at each temperature and ceases to beat for long periods when sympathetic influence is withdrawn. Apparently the lack of circulation during these asystoles is sufficiently severe to cause acute anoxemia, as evidenced by obvious respiratory distress, yet this condition can persist for some time before the heart starts to beat again. An alternate, but similar, explanation may be that the rapidly beating heart uses some essential substrate faster than it can be formed at that temperature, or accumulates an inhibiting metabolite faster than it can be removed, and the heart stops until the condition improves. A similar situation is found in the periodic cessations of beat in the denervated heart of the cat under influence of veratramine, and it has been suggested that this drug interferes with the usual rate of formation of an unknown substance which is necessary for the normal activity of the sino-atrial node. When the concentration of this substance falls below a certain level the heart stops, and starts again when the concentration builds up (Kosterlitz et al, 1955). However, some atropinized animals entering hibernation show a continuous rapid heart rate, which makes the theory of withdrawal of sympathetic tone the more logical explanation.

In atropinized animals where asystoles do occur, the periods of asystole can become more frequent as the atropinized animal approaches the deeply hibernating condition, until the heart rate is indistinguishable from that of the unatropinized animal in hibernation (Figure 1d). If the speeding of the heart in atropinized animals is caused by an overbalance of sympathetic activity, then the gradual decrease in the periods of cardio-acceleration may indicate a decline in this sympathetic tone. In the cases where the heart rate remains rapid, it may be postulated that the sympathetic tone does not decline enough to allow the heart to slow.

If this theory is correct, the sympathetic influence on the heart may vary each time the animal enters hibernation, and parasympathetic activity may be essential in bringing the heart rate to a level typical of deep hibernation when sympathetic tone is high. This is emphasized by the fact that atropine causes immediate cardioacceleration when infused into an animal with a heart rate typical of deep hibernation but a body temperature that is still declining. Furthermore, the temperature-heart rate curves of woodchucks (Lyman, 1958) and ground squirrels are not the same during each entrance into hibernation, which may well be due to an interplay between sympathetic and parasympathetic activity.

In spite of its role during entrance into hibernation, the influence of the parasympathetic system on the heart rate once deep hibernation has been reached is minimal, if not completely absent. Atropinization during deep hibernation causes little or no change in heart rate. Electrical or reflex stimulation of the vagus or massive infusions of acetylcholine all fail to depress the slow heart rate of the normally hibernating animal. Furthermore, immediate slowing of the heart occasionally takes place when acetylcholine is forced upstream in animals entering hibernation or during arousal, but this never occurred during the deeply hibernating condition in spite of the fact that the majority of experiments were performed on animals in that state. If the heart is accelerated and beats at a faster rate with the same temperature for some minutes, its threshold to acetylcholine decreases markedly. The actual rate of the heart at the time does not seem to be the factor which determines the sensitivity to parasympathetic influence, for the very slow hearts of animals treated with β-TM10, hexamethonium or curare are slowed by acetylcholine. In every case, however, the heart had been accelerated a short time previously.

A shift in the internal milieu or changes in some component of the heart itself, such as membrane potential, must cause this resensitization. Presumably the rise in threshold to parasympathetic influence takes place during the early part of deep hibernation, and may increase throughout the period of dormancy, though this has not been conclusively demonstrated. It is clear, however, that the heart of an animal which has just entered hibernation recovers its sensitivity to acetylcholine after a shorter period of acceleration than the heart of an animal which has been hibernating for a long time.

Whatever the cause, we have been unable to slow the heart of the ground squirrel in normal deep hibernation, though the rate is easily increased by a variety of drugs. If the parasympathetic system is without influence on the heart during this time and yet the heart beats slowly, it is logical to assume that sympathetic influence on the heart is also at a low ebb, but that any increase in sympathetic influence will produce a maximum effect.

Working with the California ground squirrel (Citellus beecheyi) Strumwasser (1959) found that the heart rate of this species was faster and more uneven when plotted at ten minute intervals during deep hibernation than it was when the animal was entering the hibernating state. He suggested that similar changes in rate had been overlooked in C. tridecemlineatus and the European hedgehog (Erinaceus) by Dawe and Morrison (1955), and that these changes indicated an increase in sympathetic tone once the deeply hibernating condition had been reached. The present investigation confirms the work of the latter authors, for the slowest and most even rates occur during deep hibernation in C. tridecemlineatus and C. lateralis, and these rates may continue for many hours. On the other hand, the rate may increase if the animal is lightly touched or otherwise stimulated, and the faster rate may last for hours even though the animal does not arouse from hibernation. Presumably this acceleration is caused by sympathetic activity rather than lessening of parasympathetic tone in view of the refractoriness of the heart to parasympathetic influence during deep hibernation.

Citellus beecheyi may have higher sympathetic activity during deep hibernation than these other two species of the genus. Alternatively, the environment or the condition of the animals may not have been ideal to produce the deepest possible hibernation. We have noted, particularly during the summer, that some animals are "nervous" hibernators. Their heart rates tend to be fast, in spite of the usual low body temperature, and they are very sensitive to infused liquids – even isotonic saline – so that it is impossible to obtain definitive results from infusion of drugs. Several animals increased their heart rates every time the compressor of the cooling unit started, with the heart gradually slowing when the muffled noise and vibration ceased. Thus, there may be varying levels of sympathetic influence on the heart even in the same species at identical body temperatures.

Alternative explanations for variations in heart rate during deep hibernation are not supported by our observations. Changes in the inherent rhythmicity of the pacemaker probably do not cause the variation, for treatment with veratramine does not slow the heart. On the other hand, one cannot be sure that veratramine affected the heart in hibernation since it failed to produce convulsions until the animal started to arouse. Variations in the condition of the vascular bed might reflexly cause a change in rate, but we have found no absolute correlation between mean blood pressure and heart rate in normal deep hibernation. When the rate is above ten a minute the blood pressure tends to be higher than usual. This indicates that the fast heart is increasing the blood pressure, rather than the high blood pressure causing a decrease in heart rate.

A conspicuous feature of the pulse pressure as the animal enters hibernation is the increase in peripheral resistance. We have previously emphasized that this serves to maintain a reasonably high mean blood pressure in spite of an extremely low heart rate (Lyman and O'Brien, 1960). This contrasts sharply with enforced hypothermia, and may account in part for the long life at low temperatures which occurs in the hibernating mammal alone. Popovic (1960) has shown a positive correlation between high blood pressure and prolonged survival time in hypothermed ground squirrels and rats.

Blood pressure and peripheral resistance may be reduced by infusion of acetylcholine or methacholine, though the blood pressure is always restored by an increase in the heart rate. Ganglionic blockade, adrenergic blockade (Lyman and O'Brien, 1960), or pharmacological sympathectomy causes a marked reduction in peripheral resistance and blood pressure, which is not followed by a compensatory increase in heart rate, and the animal dies in hibernation unless heroic measures are taken. These responses to infused drugs indicate that the peripheral resistance is due in large measure to neurogenic vasoconstriction mediated by the sympathetic nervous system. The fact that drugs can produce such a striking effect with no change in body temperature shows that physical factors such as changes in blood viscosity and elasticity of arteriolar walls at low temperatures cause only a part of the peripheral resistance.

If the blood pressure of hibernating ground squirrels is recorded over long periods, slow changes in the mean blood pressure of 30 mm Hg or more can be noted with no change in heart rate. Though a change in stroke volume could produce this result, it seems more likely that it is caused by changes in peripheral resistance. Strumwasser (1959), using a surface thermistor, has reported fluctuations in the cutaneous dorsal temperature of hibernating C. beecheyi while the brain temperature remained unchanged. He believed that this indicated an alteration in cutaneous blood flow which, in turn, regulated the deep body temperature. Using sufficiently sensitive subcutaneous recording devices, we have not observed these skin temperature changes either in C. tridecemlineatus or in the woodchuck, Marmota monax (Lyman, 1958). A subcutaneous thermocouple should mirror changes in blood flow more accurately than a surface-recording device, which could be easily influenced by the ambient temperature. However, it may be that the hibernating C. beecheyi has a more precise cutaneous vascular control than other species of the same tribe (Marmotini; Simpson, 1945), but that all species alter the resistance in various parts of the vascular bed from time to time, possibly in response to accumulation of metabolites. This concept is reinforced by the observation that the amount of acetylcholine necessary to produce a given result varies greatly from day to day in the same animal with the same body temperature. The effect of the

infused drug must depend on its route through the circulatory system, and this route could well be modified by changes in the vascular tree.

If cardioacceleration during hibernation is sympathetically mediated, it might be expected that infusion of norepinephrine would reproduce the changes which take place during natural arousal. As has been detailed elsewhere (Lyman and O'Brien, 1960), this is not the case, for this drug causes an immediate rise in heart rate and blood pressure. It is the parasympathomimetics which, in the proper doses, can cause the increase in heart rate, drop in peripheral resistance and slow rise in blood pressure which are typical of the disturbed hibernating animal, and the peculiar effect of these drugs may be an important clue in the problem of arousal from hibernation.

In considering the cardioacceleration produced by acetylcholine or methacholine during hibernation, the possibility of either drug producing a direct effect on the heart by release of endogenous catechol amines or by some other means is unlikely because of the time factor. Circulation time from the end of the catheter to the heart is at least 9 to 15 seconds with a heart rate above 20 beats/min. The cardioacceleration can occur 2 to 5 seconds after injection of acetylcholine with the slower heart rate of deep hibernation. Our incidental observation that acetylcholine never increases the rate of isolated ground squirrel hearts at any temperature substantiates this conclusion. The effect could not be caused by direct stimulation of the central nervous system for the time lapse would be even longer. Moreover, the passage of the acetylcholine molecule across the blood-brain barrier must be inhibited due to its size.

It seems fairly certain that the speeding of the heart is produced via the cardioaccelerator fibers of the sympathetic nervous system, for it will not occur with ganglionic blockade or after treatment with the sympatholytic agent β-TM10. Furthermore, the possibility of stimulation of the accelerator fibers at the ganglia is unlikely because the effect can be produced by methacholine, which lacks significant ganglionic effects except in very high doses (Goodman and Gilman, 1955).

When the effects of acetylcholine and methacholine are examined in non-hibernating anesthetized ground squirrels, it becomes apparent that the nicotinic effects of these drugs are very similar to the effects observed in hibernating animals. With animals in either condition, bursts of muscle action potentials occur immediately after infusion of acetylcholine. Both acetylcholine and methacholine produce exaggerated respiratory movements in anesthetized and in hibernating animals, and these movements occur at a time after infusion commensurate with the time required for the drug to circulate to the anterior part of the body. None of these effects can be completely blocked by atropine.

It is in the cardiovascular responses that the anesthetized and hibernating ground squirrels differ. In the anesthetized animals, acetylcholine and methacholine produce typical parasympathomimetic effects, including a reduction in heart rate, and these effects can be readily blocked by atropine. In contrast, the invariable cardioacceleration caused by acetylcholine in the hibernating animal can be only partially blocked by much larger doses of atropine.

The timing of the onset of muscular activity after infusion of these drugs and the onset of cardioacceleration during hibernation seem too precise to be pure chance. Infusion of acetylcholine causes an almost immediate burst of muscle action potentials, and cardioacceleration occurs at this same time in the hibernating animal. Methacholine produces respiratory movements some seconds later in anesthetized or hibernating animals and cardioacceleration occurs at a comparable time in the hibernating animals.

The association of muscular activity with cardioacceleration had been noted some time ago in the hamster during the first stages of arousal (Lyman and Chatfield, 1950). Poking either hamster or ground squirrel gives rise to a burst of muscle action potentials, with no visible muscular movement in the hamster, which continue after removal of the stimulus. Cardioacceleration occurs immediately after stimulation in all species of ground squirrel that have been studied (Dawe and Morrison, 1955; Lyman and O'Brien, 1960) as well as in hamsters (Lyman and Chatfield, 1950), woodchucks (Lyman, 1958), and hedgehogs (Dawe and Morrison, 1955). The cardioacceleration associated with the nicotinic effects of acetylcholine does not occur if the ground squirrel has been previously curarized. Dawe and Landau (1960) have suggested that increased thoracic movements acting against the heart musculature contribute to cardioacceleration in the disturbed hibernating ground squirrel. The acetylcholine-induced cardioacceleration occurs before the respiratory gasp and may occur with no visible muscular movement. Thus, we believe that a more complex reflex must be involved, which may be initiated by muscular activity and must travel via the cardioaccelerator fibers to the heart. This reflex may play an important part in the initiation of normal arousal. In this regard it is of some interest that skeletal muscle from animals in hibernation has been reported to be much more sensitive to acetylcholine than the same muscle in active controls (Wachholder and von Ledebur, 1932).

The role of the autonomic nervous system, once arousal has started, is fairly well documented and the importance of the sympathetic component has been previously emphasized (Lyman and Chatfield, 1955). During arousal it is probable that the heart is driven as fast as its temperature will permit in all hibernators, for cardioacceleration cannot be increased

by injection of sympathomimetic drugs either in the hamster (Chatfield and Lyman, 1950) or the ground squirrel. Furthermore, the parasympathetic system must cause no slowing, for atropinization does not change the rate in the hamster (Chatfield and Lyman, 1950) or ground squirrel, though parasympathomimetic drugs or vagal stimulation are capable of slowing the heart at this time. The same conclusion was reached by Dawe and Landau (1960) who considered that the cardioacceleration after decapitation observed in active ground squirrels was caused by release of vagal influence, while the lack of this acceleration after decapitation of arousing animals indicated its absence. Using this same technique they suggested that vagal influence was also absent during deep hibernation. Although we agree with this conclusion, the reported heart rate is more than 20 beats/min, which indicates that this animal had started arousal.

Cardioacceleration is essential for arousal, for if it is prohibited by a sympatholytic agent or by ganglionic blockade, the ground squirrel cannot arouse without artificial warming and will eventually die. The lethal effects of these procedures serve to emphasize the importance of the sympathetic system in control of heart rate and maintenance of vascular tone in the hibernating and arousing animal. Identical doses of β-TM10 or hexamethonium produce no untoward effects in the active ground squirrel.

The adrenal medulla must play at best a secondary role in the arousal process. Cardioacceleration after stimulation of the hibernating animal usually takes place too rapidly to be caused by blood-borne norepinephrine from the adrenal, though the delayed acceleration sometimes seen after infusion of acetylcholine might be caused in this way. Hibernating animals pretreated with β-TM10 are unable to arouse, yet this drug does not influence the adrenal medulla (McLean et al, 1960). Furthermore, Popovic (1952) has reported normal arousal in adrenalectomized ground squirrels with only a small anterior eye chamber cortical graft, and we have observed the same in hibernating hamsters which were adrenalectomized as quickly as possible (Lyman, unpublished).

The action of the autonomic nervous system in control of the vascular bed at the very start of arousal is puzzling. The ground squirrel in deep hibernation maintains essential vascular tone, and the initial cardioacceleration is usually accompanied by an abrupt drop in peripheral resistance when the animal is first disturbed. The circulation in the anterior part of the body is known to be very rapid in later stages of arousal. Johansen (1961) has shown that circulation in the forelegs is sixteen times greater in the arousing Arctic ground squirrel than in the anesthetized active animal and similar results have been reported for C. tridecemlineatus (Bullard and Funkhouser, 1962). Johansen suggests that an arteriolar shunting

mechanism may be involved. In view of the magnitude and speed of the changes, this idea is attractive, but evidence to date is lacking.

Once arousal is under way, a strong differential vasoconstriction reduces the flow of blood to the post-thoracic part of the body, so that the anterior warms rapidly while the posterior remains cold. Bullard and Funkhouser (1962) have shown that blood flow to the posterior remains unchanged during this period, though the heart rate and blood pressure are rising (Lyman and O'Brien, 1960). This indicates that vasoconstriction in the posterior must increase as arousal progresses.

The vasodilatory action of acetylcholine will abolish the vasoconstriction for a short period so that the posterior is warmed while the anterior is chilled. Contrariwise, if norepinephrine is infused when the heart temperature has reached 37° C and the posterior is warming rapidly, this warming ceases briefly as vasoconstriction is reimposed. The vasoconstriction of natural arousal can be abolished by pharmacologically blocking the sympathetic nervous system, and it cannot be reimposed by infusion of norepinephrine. Therefore, the constriction appears to be caused by discrete action of sympathetic fibers rather than a lower threshold in the posterior to circulating norepinephrine.

We may therefore conclude that the parasympathetic system performs a regulatory, but not essential, function as the ground squirrel enters hibernation. If sympathetic tone is high, the parasympathetic system becomes more important in slowing the heart during entrance into hibernation, but throughout deep hibernation and arousal its effect is minimal and the animal with parasympathetic blockade shows no deficit. On the other hand, sympathetic activity must be reduced to permit the heart to slow as the animal enters hibernation, and the main function of the sympathetic system during this period is to maintain sufficient vascular tone. When in deep hibernation the sympathetic system, though its activity is muted, plays an essential part in the maintenance of circulatory homeostasis, and if the system is blocked the animal dies of circulatory collapse. The sympathetic system remains on guard in the precarious hibernating state to be fired into action for its essential part in the complex coordinated process of arousal. It seems probable that the cardioacceleration, which is an essential part of this process, is produced in evoked arousals when an external stimulus causes a burst of muscle action potentials which result in a reflex speeding of the heart.

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2. Cardiovascular System
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4. Hibernation
5. Rodents
6. Autonomic Agents

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