STUDIES ON THE ROLE OF REGIONAL HETEROTHERMY IN THE ENERGY BALANCE

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MONOGRAPHS IN VETERINARY SCIENCE

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Studies on the Role of Regional Heterothermy in the Energy Balance of Selected Arctic Mammals

by

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1 August 1978
**REPORT DOCUMENTATION PAGE**

1. **REPORT NUMBER**
   2

2. **GOVT ACCESSION NO.**

3. **RECIPIENT'S CATALOG NUMBER**

4. **TITLE (and Subtitle)**
   STUDIES ON THE ROLE OF REGIONAL HETEROTHERMY IN THE ENERGY BALANCE OF SELECTED ARCTIC MAMMALS

5. **TYPE OF REPORT & PERIOD COVERED**
   Technical
   Aug 1, 1977-July 31, 1978

6. **PERFORMING ORG. REPORT NUMBER**

7. **AUTHOR(s)**
   Thomas F. Albert

8. **CONTRACT OR GRANT NUMBER/Grant Numbers**
   N00014-76-C-1059

9. **PERFORMING ORGANIZATION NAME AND ADDRESS**
   Department of Veterinary Science
   University of Maryland
   College Park, Maryland 20742

10. **REPORT DISTRIBUTION STATEMENT**

    Distribution of this report is unlimited.

11. **CONTROLLED OFFICE NAME AND ADDRESS**
    Biophysics Program (Code 444)
    Office of Naval Research
    Arlington, Virginia 22217

12. **DECLASSIFICATION/DOWNGRADING SCHEDULE**

13. **NUMBER OF PAGES**
    26

14. **MONITORING AGENCY NAME AND ADDRESS (IF DIFFERENT FROM CONTROLLING OFFICE)**

15. **SECURITY CLASS. (OF THIS REPORT)**

16. **DISTRIBUTION STATEMENT (OF THIS REPORT)**

17. **DISTRIBUTION STATEMENT (OF THE ABSTRACT ENTERED IN BLOCK 20, IF DIFFERENT FROM REPORT)**

18. **SUPPLEMENTARY NOTES**

19. **KEY WORDS**
    Regional heterothermy, cold exposure, hibernation trigger, marmot, Arctic mammals

20. **ABSTRACT**

    The influence of an infusion of plasma from a hibernating mammal into an awake cold stressed mammal is being investigated at the Animal Research Facility, Naval Arctic Research Laboratory, Barrow, Alaska. Such plasma contains "hibernation trigger" substance which may affect a recipient's thermoregulatory mechanisms. Plasma was collected from hibernating arctic marmots, Marmota brookeri, and infused into woodchucks, Marmota monax. The recipient...
Woodchucks were then subjected to acute cold stress (0°C, 12 hours). Multiple tissue temperatures were monitored by means of implanted thermocouples. Data have not yet been subjected to computerized analysis, however visual inspection of the data reveals no obvious difference between control and experimental groups. During cold exposure heart rate increased from 110-130 beats per minute to the 140-170 range. Regional heterothermy is evident during cold exposure with tissues varying by as much as 6°C within an animal. Of the monitored tissues axillary brown fat is nearly always the warmest followed by anterior muscle, posterior muscle and the subcutaneous areas. Studies are continuing in this area utilizing an increased volume of hibernation plasma.

In line with continuing efforts to establish a self sustaining colony of arctic marmots for use as experimental animals, long term body temperature studies were conducted. Two groups of 4 animals each were allowed to overwinter in artificial dens. Body temperature was monitored by means of implanted temperature sensitive radio transmitters. The animals seem to be communal hibernators, as they enter and arouse from torpor as a group rather than individual animals evidencing different hibernation patterns. Such group behavior causes significant warming of the air inside the dens during arousal from torpor. The monitoring of den air temperature may be useful in monitoring the hibernation pattern of the group within the den, thereby negating the use of implanted transmitters.

Four young were born this year in one of the two dens. During the past two winters a total of three groups have overwintered with young being produced in two of these groups. It would therefore seem that these animals can be successfully bred in captivity, however it remains to be seen whether such reproductive activity is sufficient to establish a self sustaining colony.
Research efforts during the past year (August 1, 1977—July 31, 1978) have been conducted at the Naval Arctic Research Laboratory in Barrow, Alaska. The work has been concerned primarily with six projects which are listed below and then described in detail in subsequent pages of this report.

The overall objective of the studies has been to investigate the role of regional heterothermy in the energy balance of selected arctic mammals. Those mammals utilized include: the arctic marmot (Marmota broweri), the arctic ground squirrel (Spermophilus parryi), and the woodchuck (Marmota monax).

Research Effort August 1, 1977 - July 31, 1978

Project I: Establishment of a self sustaining arctic marmot colony at the Naval Arctic Research Laboratory

Project II: Arctic marmot body temperature and hibernation pattern study

Project III: The influence of season and hibernation upon the blood chemistry of an arctic mammal (arctic ground squirrel)

Project IV: Response to acute cold stress as influenced by plasma from a hibernating animal

Project V: Maintenance and health care of arctic ground squirrels, arctic marmots and woodchucks kept under laboratory conditions.

Project VI: The collection and preservation of tissues from ringed seal (Pusa hispida) for the purposes of preparing a histological study set.
PROJECT I

TITLE
Establishment of a Self Sustaining Arctic Marmot Colony at the Naval Arctic Research Laboratory (NARL)

OBJECTIVE
To establish a self sustaining colony of arctic marmots at NARL in order to provide a ready source of these animals for experimental purposes.

METHODS

Approximately 2 dozen arctic marmots were available by fall of 1977. Seven of these were born at NARL during the spring of 1977. Five marmots trapped by Eskimo hunters each suffered a compound fracture of a leg. The healthy marmots were housed in artificial dens which were connected to large wire cages. Such an arrangement allowed for animal interaction and a reasonable approximation of their natural den structure. The dens were entered through an entrance tunnel from the wire cage. Each den consisted of a "box within a box", both constructed of 1.9 cm thick plywood. The outer box is approximately 127 x 107 x 91 cm deep (outside dimensions). Each has a hinged lid. The outer box contains approximately 8 cm of insulation within its walls. The inner box can be removed from within the outer box to allow for drying out of accumulated urine and moisture condensation. The inner box is also lined with heavy gauge wire mesh to prevent the animals from chewing through the plywood. Each den is provided with thermocouples for the monitoring of den air temperatures.

RESULTS

Two groups, each of four marmots, overwintered in the dens. One adult male was in each group. Four young were
born in one den and are presently growing well. During the past two winters a total of three groups have overwintered with young being produced in two of these groups. It would therefore seem that these animals can be successfully bred in captivity, however it remains to be seen whether such reproductive activity is sufficient to establish a self sustaining colony.

In late May 1978, 15 marmots were placed together in the large central room in building 448 of NARL. This room is 5.8 m long, 4.0 m wide and 2.4 m high. Straw bedding covers the floor and nest boxes (23 cm wide, deep and high) are provided. In addition four wooden "tunnels" (61 cm long, 28 cm wide and high) are provided as the animals seek some form of cover when disturbed. Food consists of Purina Dog Chow and fresh vegetables (cabbage, apples, carrots). The animals have done very well on this diet over the past 2 years that they have been at NARL. It is interesting to note that the marmots will eat the Purina Dog Chow while neglecting the Purina Rat Chow also provided. This is just the opposite of that seen in woodchucks. In order to prevent the animals from chewing through the floor or walls, sheet metal has been applied to the walls extending upward 2 feet from the floor. A two foot wide strip of sheet metal has also been attached to the floor about its periphery.

The marmots have adjusted very well to this housing situation during June and July with there being little evidence of intraspecific fighting. It would seem that group indoor housing of arctic marmots may be possible. This is also quite different from what has been observed with woodchucks, as they are only kept under group indoor housing conditions with great difficulty.
PROJECT II

TITLE

Arctic Marmot Body Temperature and Hibernation Pattern Study

OBJECTIVE

To determine the body temperature throughout the hibernation cycle of arctic marmots housed in artificial dens.

METHODS

Three artificial dens were utilized. They are the same as those described in the section dealing with the establishment of an arctic marmot colony. Into each of two dens were placed 5 arctic marmots on November 1, 1977. Into a third den were placed 4 hoary marmots (Marmota calligata). Each of the 14 animals had earlier received a temperature sensitive radio transmitter placed into its peritoneal cavity. Each den was instrumented with thermocouples in order to monitor den air temperature. From November 1, 1977 through May, 1978, body temperature and den air temperature were monitored twice each day.

RESULTS

Of the 3 groups of animals, 1 group had 3 transmitters malfunction and therefore their Th data are of little value. One group of arctic marmots and the group of hoary marmots have yielded sufficient data to determine that these animals appear to be communal hibernators (several animals hibernate together), that they tolerate den temperatures well below freezing and that they adapt well to the artificial dens. During the first few weeks in the dens the animals would lower their body temperature and then rewarl with no apparent synchronization. By the third week however, a definite synchronization was evident whereby the animals within a den would enter torpor together and reawarm together, acting as a
loosely knit group. During January and February (the third and fourth months in the dens) the animals within a den were acting almost as a unit regarding entrance into and arousal from torpor. The periods of torpor were ranging from four to eight days with body temperatures during torpor ranging from 2-5°C (Figs. 1 and 2). The euthermic periods between bouts of torpor were short being 12-24 hours in length. As the hibernation season drew to a close, the duration of the periods of torpor lessened.

It is apparent that a "staggered" series of arousals from torpor is not utilized by these marmots. Such a "staggering" of arousals would seemingly allow one animal to be awake within the den at any given time and thereby generate heat to keep the den "warm". This is not the case, as the animals act as a "unit", entering and arousing from torpor together (Figs. 1 and 2). By having several days at a time with the den occupants in deep torpor together, it is not surprising that den temperatures drop to values well below freezing (Table 1, Fig. 1).

On December 14, two dens were opened in order to remove an animal from each group whose transmitter had ceased working. Within a den, the five animals were each curled in a "ball" and in close contact with each other with two being above the other three. The air temperature in the nest approximately 6 cm from an animal was 5°C while the thermocouple projecting in from the roof of the nest chamber (approximately 25 cm from the animals) was evidencing -14°C. It would appear that, at the time the den was opened, there was at least a 19°C thermal gradient extending through the straw surrounding the animals. Den air temperature for both the arctic marmot group and the hoary marmot group had a monthly mean value of less than 0°C for November through March (Table 1). As can also be seen in Table 1 the ambient temperature from November through April was well below freezing with monthly mean values of approximately -20°C.

In order to examine the pattern of "group" hibernation in more detail a count of arousals was made from November 1977 through April 1978. A complete arousal was defined as all animals in a den arousing together as determined by T_B telemetry. An incomplete arousal was defined as an instance in which less than the whole group in a den aroused at the same time. The number of animals taking part in an incomplete arousal would therefore be at least 1 less than the total number of
animals in the den.

During this period (November through April) arctic marmots evidenced 15 complete arousals and 4 incomplete arousals, while hoary marmots evidenced 8 complete arousals and 15 incomplete arousals (Table 2). Since the arctic marmot group more often acted as a "unit" as regards entering torpor and arousal, it would indeed seem to be a communal hibernator. The hoary marmot may be less inclined toward communal hibernation as this group evidenced almost twice as many incomplete arousals as complete arousals.

With each arousal from hibernation by an animal or the group, the den air temperature would increase. Arctic marmot complete arousals resulted in large increases in den air temperature (13.0 ± 1.6°C, X ± S.D.) while incomplete arousals resulted in 6.6 ± 2.9°C increase in den air temperature (Fig. 1, Table 2). Hoary marmot complete arousals result in 8.8 ± 0.7°C increase in den air temperature while incomplete arousals result in 4.8 ± 2.6°C increase (Table 2). It seems that den air temperature is a rather accurate reflection of the hibernation status of the animals in the den. However, this method is not completely reliable in differentiating between complete arousals and incomplete arousals.
PROJECT III

TITLE

The Influence of Season and Hibernation Upon the Blood Chemistry of an Arctic Mammal (Arctic Ground Squirrel).

OBJECTIVE

To determine the effect (if any) of season and hibernation on the blood chemistry of a resident arctic mammal (arctic ground squirrel).

METHODS

Beginning in August 1977, a group of arctic ground squirrels was bled at monthly intervals. The group consists of approximately two dozen animals. The animals are housed indoors at room temperature. Approximately one half of the animals were placed into a hibernaculum (7°C) from December through February. Serum samples are frozen until delivery to the Walter Reed Army Institute for Medical Research, Washington, D.C. Clinical chemistry evaluation includes 19 parameter.

RESULTS

Serum has been delivered to Walter Reed Army Institute during January and June of 1978. Unfortunately no examinations have yet been made, as all samples from both deliveries will be examined at one time, hopefully in the near future.

This study has also been hampered by fighting among the squirrels with subsequent injuries and by heater malfunction in the hibernaculum with subsequent freezing of some hibernating animals. Further comments regarding the health care of the animals will be mentioned as part of Project V.
PROJECT IV

TITLE

Response to Acute Cold Stress as Influenced by Plasma
From a Hibernating animal

OBJECTIVE

To determine the influence of plasma from hibernating animals upon the extent of regional heterothermy evidenced by animals subjected to acute cold stress.

METHODS

Plasma was to be collected from summer active and hibernating woodchucks. The plasma was then to be infused into arctic marmots, which had been kept at room temperature. It was essential that the recipient animals not be allowed to become torpid.

Animal procurement problems resulted in not enough healthy marmots being available to act as recipients. The supply of woodchucks was somewhat larger so they were selected to become the plasma recipients with the marmots acting as plasma donors. Unfortunately there were not enough donors (marmots) available to allow for the collection of summer active plasma. Summer active plasma does not contain the "hibernation trigger" substance.

The marmots (plasma donors) were housed in single cages at room temperature, until early November when they were placed into the hibernaculum (70°C).

Collection and Handling of Hibernation Plasma. Blood was collected by cardiac puncture from those marmots which had been in deep hibernation for at least four consecutive days. A torpid marmot was quickly removed from its cage within the hibernaculum and restrained in dorsal recumbancy. Blood was then taken via cardiac puncture. From the time the torpid marmot was touched in its cage until the removal of blood was
stopped was a maximum of 90 seconds. This 90 second time limit is necessary in order to be certain that no "anti-trigger" substance is contained within the extracted blood. A total of 10 marmots were sampled in this manner with blood samples ranging from 5-25 cc. The blood was collected into cold (5°C) heparinized syringes and immediately placed into heparinized vials in a 7°C water bath. The blood filled vials were then centrifuged in a refrigerated centrifuge (7°C). The plasma was then placed into vials (7°C) and then into a freezer (-41°C) until use. Approximately 2 hours prior to its infusion into a recipient marmot, the plasma was thawed in a 7°C water bath. It is felt that the collection of the blood and subsequent handling of the plasma is critical with particular attention being paid to collecting the blood within 90 seconds after disturbing the torpid marmot. Once collected, the blood and the plasma do not exceed 7°C prior to plasma infusion into the recipient marmot.

Housing of Hibernation Plasma Recipients (Woodchucks). The woodchucks that were to be the recipients of the hibernation plasma, as well as those woodchucks to receive only an infusion of heparinized saline, were kept in a large room in building #448 NARL. The objective was to keep these animals at room temperature (to prevent any acclimation to cold) and to prevent them from entering torpor (so that they would not be producing any of their own hibernation trigger substance). Since seasonal hibernators such as woodchucks often show some degree of occasional daily torpor when kept in individual cages even at room temperature, it was decided to house the animals as a group in a single room. Such group indoor housing would likely inhibit any form of daily torpor as the animals would be constantly interacting with each other, rather than being isolated in separate cages.

The room that housed the woodchucks was 5.8 m long, 4.0 m wide, and 2.4 m high and was bisected with a 1 m high septum to provide 2 separate housing areas. Nest boxes (23 cm wide, deep and high) were provided at floor level. Also provided were wooden "tunnels" (61 cm long, 28 cm wide and high). Water and food consisting of Purina Rat Chow were provided ad libitum, with carrots, apples and cabbage 2-3 times per week. Room temperature was 15-20°C. Animals were exposed to a natural photoperiod by means of ample window space. In addition, the room was illuminated by fluorescent
bulbs from approximately 8 AM to 5 PM each day. Bedding material consisted of straw and covered the floor. The number of animals so housed varied but from mid July 1977 to mid March 1978 averaged 25 animals. Such a method of group indoor housing allowed for the feeding and sanitary needs to be provided with a minimum of effort. Unfortunately the woodchucks maintained a rather constant low level of fighting. The injuries which they inflicted upon each other were significant and very time consuming to care for as will be further described under Project V.

Surgical Procedure. Beginning in early March, the woodchucks were utilized in the experiment at the rate of two per week for eight weeks. A woodchuck was anesthetized and a subcutaneous vein on the medial aspect of the left rear leg surgically isolated. A canula was inserted into the vein and 8 cc of hibernation plasma was infused. The vein was then tied off at the site of infusion. The animal was then individually caged at room temperature. Seven days later the animal was again anesthetized and surgically implanted with five thermocouples (copper-constantan, each wire Teflon coated and 0.01 inches in diameter). The five sites for thermocouple implantation were: 1) the left axillary brown fat pad (BP); 2) the longhead of the triceps muscle of the right forelimb (KM); 3) subcutaneous on the lateral aspect of the thorax, approximately 4 cm caudal to the right scapula (ASQ); 4) subcutaneous right flank area, approximately 4 cm cranial to the point of the hip (PSQ); 5) mid lateral aspect of the biceps femoris muscle of the right rear limb (PM).

When placing a thermocouple in the axillary brown fat, an incision is made at the site and the brown fat exposed. Using fine pointed scissors a small (1 mm) hole is made in the brown fat. The thermocouple tip is inserted into the tissue and then the brown fat is further sutured to a loop in the wire. The incision is then closed.

In the other four instances the thermocouple is attached by a strand of suture material to a curved needle. The needle is passed through the skin at the desired site into the selected position. By pulling on the suture the thermocouple is pulled through the skin into position in the selected tissue. The suture is then tied to the skin at that site, thereby holding the thermocouple in position. Each pair of thermocouple wires was passed subcutaneously by means of a large needle and exited
through the skin in the dorsal thoracic region. The animal was again individually caged at room temperature. The preparation of each animal therefore required two separate surgical procedures, one for the plasma infusion and the other for the implantation of the thermocouples.

Acute Cold Exposure. Approximately 48 hours after thermocouple implantation the caged animal was moved to the room containing the controlled temperature box. The thermocouple leads were connected to a recording potentiometer and the five tissue temperatures monitored for the next two hours while the animal was exposed to room temperature in order to obtain baseline data on the noncold stressed animal. At the end of this two hour period the caged animal was quickly placed into the controlled temperature apparatus and exposed to 0°C for the next 12 hours. Following this 12 hour period of cold exposure the caged animal was removed from the controlled temperature apparatus and again exposed to room temperature air for two hours. The monitoring period therefore consisted of 16 hours. The first two hours are at room temperature, then 12 hours at 0°C and the final two hours again exposed to room temperature air. In addition to the continuous monitoring of the five tissue temperatures, heart rate was monitored at 20 minute intervals throughout the 16 hour period. Unfortunately the oxygen analyzer was broken and not available during the experimental period.

At the end of the 16 hour experimental period the animal was anesthetized and the five thermocouples removed. Since each thermocouple was only held in place by a single suture, firm traction on the free end of the thermocouple resulted in its removal.

RESULTS

The 16 hour experimental period was conducted involving 16 animals. Eight were hibernation plasma recipients and eight were heparinized saline recipients. A great deal of data was generated, however its proper analysis must await access to a computer. Data will be tabulated and sent to the University of Maryland for computerized examination. By visual examination of the data there would appear to be no obvious difference between the control and experimental groups.
If there indeed is no difference between the groups in this instance, all that can be said with certainty is that at this "dosage level" of hibernation plasma (8 cc) and at this interval between infusion and cold stress (9 days) there is no detectable effect utilizing the above described methods.

Visual inspection of the data does reveal some consistent physiological responses to the acute cold stress. During the initial 2 hour period at room temperature, the animal moves very little, heart rate is in the 110-130 beats per minute range and all monitored tissues are within 1.5°C of each other in the area of 36-37°C (Fig. 3).

During the 12 hour period of cold exposure, the situation is much different. Upon exposure to cold, the animal slowly but carefully examines its cage for an escape route, heart rate rises to the 140-170 beats per minute range and differentials are noted in the tissue temperatures. As the period of cold exposure continues, the animal spends most of the time curled up with hair erected. Heart rate stays in the 140-170 beats per minute range. The degree of divergence of the tissue temperatures varies, but ranges from 2-6°C within the animal. The warmest tissue is nearly always brown fat (usually in the 35-38°C range) followed by AM, PM, ASQ and PSQ, usually in that order. The animals clearly utilize regional heterothermy during acute cold exposure, however, the extent shows much individual variation. With AM usually higher than PM and ASQ usually higher than PSQ, evidence of an anterior-posterior thermal gradient is rather clear. Since these animals are capable of hibernating, such a finding is less surprising than if the animals were non-hibernators. Shivering was visible for limited periods in nearly all animals, but was much less than anticipated. It appears that under these circumstances, BF is a significant heat source (Fig. 3).

Occasionally the animal would become quite active while attempting to make a nest from the 0.5 m² piece of burlap supplied as bedding material. During these few minute periods heart rates would increase to the 180-210 beats per minute range. During the 2 hour period at room temperature following cold exposure, the animals remain nearly motionless in sternal recumbancy. Heart rate returns to the 110-130 beats per minute range. The extent of regional heterothermy diminishes rapidly as all tissue temperatures are usually within 1.5°C of each other.
Unfortunately, several difficulties were encountered which limited the scope of this study. The limited availability of experimental animals was a major factor in this regard. Continual low level fighting among the group of indoor housed woodchucks resulted in many injuries to the animals. Much time was expended in animal health maintenance. The oxygen analyzer was not functional during most of the winter and spring, therefore no data pertaining to metabolic rate were obtained. The hibernaculum used for the hibernating plasma donors malfunctioned twice during the season, causing much loss in time and the deaths of several animals.
PROJECT V

TITLE

Maintenance and Health Care of Arctic Ground Squirrels, Arctic Marmots and Woodchucks Kept Under Laboratory Conditions.

OBJECTIVE

To provide adequate health care for the arctic ground squirrels, arctic marmots and woodchucks used as laboratory animals mentioned in Projects I-IV.

METHODS

Due to experimental procedural requirements (woodchucks, arctic marmots) and caging limitations (ground squirrels) the species were often kept in groups ranging from two to two dozen individuals. Unfortunately such grouping allowed for fighting and subsequent injury.

Arctic Ground Squirrels The number of ground squirrels varied but ranged from 12-45. Sufficient caging was not available to allow each animal a separate cage. The housing of 2-4 animals in a single cage (0.7 x 1.1 x 0.6 m) resulted in some fighting.

Arctic Marmots During most of the year these animals were either in the outdoor artificial dens or caged separately. Several were also allowed to hibernate in the hibernaculum and served as hibernation plasma donors.

Woodchucks The number of woodchucks present varied with a maximum of 30 animals available. Most of these animals were housed as a group in a single large room in building #448, as has been described under Project IV.

RESULTS

Arctic Ground Squirrels During the past year at least 44
animals were anesthetized for either initial treatment of injuries or purposes of retreatment. Most injuries were penetrating bite wounds of the lumbar and thigh regions. Twenty-six animals died. Of these, 12 died as a result of the heater malfunctioning in the hibernaculum and the animals freezing to death while hibernating. Four animals died as a result of the monthly bleeding by cardiac puncture. Most of the remainder that died died so as a result of infection due to bite wounds.

Arctic Marmots During the summer of 1977, several marmots were obtained from Eskimo hunters. Of these, 5 had suffered broken legs and in each instance the bone had penetrated the skin with the wound being severely infected. During August, September and much of October these animals were anesthetized and retreated at least once a week and often every 2-3 days. This repeated process of anesthetizing each animal, cleaning and flushing the wound, rebandaging the wound, repairing the splint or realigning the splint took a tremendous amount of time. Healing of the broken legs was a slow process but only one animal died during the course of treatment. Four other marmots died as a result of bleeding by cardiac puncture. These four and others were bled while hibernating and served as hibernation plasma donors.

Woodchucks During the last year at least 81 animals were anesthetized for treatment. These included 30 initial treatments for bite wounds totaling 36 separate bites involving 20 separate animals (Table 3). Only rarely did an animal sustain more than 1 bite wound at a time. Bite wounds ranged from simple punctures to extensive lacerations. By virtue of their large incisors the animals can significantly injure each other. Previous experience had shown that rapid therapeutic intervention was necessary in order to prevent extensive bacterial invasion at the wound site. Within a few hours after injury, the hair at the site would become matted with blood or tissue exudate thereby enhancing bacterial proliferation at the site. With the animal anesthetized, the wound site was clipped free of hair and cleansed. Puncture wounds were flushed with an antibacterial solution (nitrofurazone). Lacerations, if fresh, were sutured. Animals tolerated sutures well and only rarely chewed or scratched at them. All animals requiring treat-
ment of sustained injuries were given an injectable antibiotic. Those animals sustaining significant injury were kept in individual cages for most or all of their recovery period.

As can be seen in Table 4, bite wounds more frequently involved the anterior portion of the animal (58%) with the face being the most common site (31%). The face and forearms were the sites of 44% of the bite wounds, while 33% involved the hips, thighs and rear feet.

An erosion of the weight bearing surface of a foot was noted in 9 instances. These nearly always involved the rear feet. Such erosions, if not adequately treated, soon result in injury to the flexor tendons. Treatment included cleaning the wound, topical application of nitrofurazone dressing and bandaging the entire foot. Foot bandages seemed well tolerated and were seldom removed prior to re-treatment.

Conjunctivitis was observed in 4 instances, and in each was secondary to a bite wound involving an eyelid. Treatment of the eyelid injury and use of a suitable ophthalmic ointment resulted in rapid healing.

Two animals were euthanatized. Each had suffered multiple bite wounds, responded poorly to treatment, became rapidly septicemic and subsequently comatose.

On March 8, 1978 a litter of 5 young was born. At this time the adults were segregated as to sex. Another litter of 5 was born on March 20. Previous experience with the colony at the University of Maryland indicates that most births occur during the first 3 weeks of April. These were the only 2 litters born and the reason for them having been much earlier than expected is not clear. In the present instance, females would frequently move the young from one nest site to another, as often as once every other day. This frequent moving of the young and harassment by other females resulted in most of the young dying of either neglect or injury. Only 4 young survived to the stage where they could move independently within the enclosure.

Group indoor housing of woodchucks has both advantages and disadvantages when compared to individual caging of animals. The advantages would include: 1) minimal investment in caging required to house a group of animals; 2) each animal has access to the entire enclosure; 3) animals are free to interact; 4) such interaction lessens the likelihood
of individual hibernators utilizing any form of torpor;
5) labor involved in sanitation and feeding is greatly re-
duced.

Fighting was far greater among woodchucks kept under
group indoor housing conditions than among those housed as
a group outdoors (University of Maryland). A possible ex-
planation for this difference might be the more varied en-
vironment available to those housed outdoors, which may
act to keep intraspecific stresses within acceptable levels.
Animals housed outdoors see vegetation, other animals, etc.,
have the opportunity to lie in the sun or shade and can
climb upon the wire enclosure. Animals that are group
housed indoors have a much less varied environment availa-
ble to them.

The major disadvantage in the present instance of group
indoor housing of woodchucks is the significant commitment
that must be made to animal health maintenance. The commit-
ment is of such a magnitude as to render this housing method
of limited value.
PROJECT VI

TITLE
The Collection and Preservation of Tissues From the Ringed Seal (Pusa hispida) for the Purposes of Preparing a Histological Study Set.

OBJECTIVE
To collect tissue samples from ringed seals and from them prepare a series of histological slides representing the normal animal.

METHODS
During November 1977 and April 1978 a total of 10 ringed seals were examined at autopsy. These animals were killed by gunshot by personnel of the Alaska Department of Fish and Game. Cooperating individuals were John Burns and Thomas Eley. The animals were collected as part of a year round study by the Alaska Department of Fish and Game concerning the natural history and ecology of certain marine mammals.

The dead animals were examined in building #448, NARL. During the above two mentioned periods tissue samples were taken from all major organs and tissues. They were preserved in 10% buffered formalin.

RESULTS
The collected, preserved tissues were carried to Dr. George Migaki, Armed Forces Institute of Pathology, Washington, D.C. in June. The samples were further cut in order to adequately expose the proper structures. A total of 96 micro slides have been prepared and a syllabus describing the slides is in preparation. Such a slide series will be available to interested parties as normal reference material concerning this marine mammal.
CONCLUSIONS

1. The establishment of a self sustaining research colony of arctic marmots at NAHL seems likely as young were born to two of three groups during the past two years.

2. The arctic marmot seems to be a communal hibernator, with animals in a den usually entering and arousing from torpor as a unit rather than on an individual basis.

3. The monitoring of den air temperature may be a reliable index of arctic marmot hibernation pattern within the den.

4. An infusion of plasma (8 cc) from hibernating arctic marmots into non hibernating woodchucks does not seem to enhance the recipient's resistance to acute cold exposure.

5. The group indoor housing of woodchucks is not an adequate method of keeping these animals due to persistent fighting.

6. The group indoor housing of arctic marmots seems to be a suitable housing method and thereby results in much saving of labor.

7. Tissues from the ringed seal have been collected for the preparation of a histological study set.
### Table 1

Temperature ($^\circ$C ± S.D.) of marmot den air and ambient air

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<tr>
<th>MONTH</th>
<th>AMBIENT AIR</th>
<th>HOARY MARMOT DEN AIR</th>
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n = 8, 15

\[ \bar{X} \pm \text{S.D.} 0.8 \pm 0.7, 4.8 \pm 2.6 \]

\[ \bar{X} \pm \text{S.D.} 13.0 \pm 1.6, 6.6 \pm 2.9 \]
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# TABLE 4

Frequency of bite wounds at specific sites on wood-chucks injured while kept under group indoor housing conditions

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<td>Face</td>
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<tr>
<td>Neck</td>
<td>1</td>
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<tr>
<td>Shoulder</td>
<td>2</td>
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<tr>
<td>Forearm</td>
<td>5</td>
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<tr>
<td>Front foot</td>
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<tr>
<td>Back</td>
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</tr>
<tr>
<td>Flank</td>
<td>1</td>
</tr>
<tr>
<td>Thigh, hip</td>
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</tr>
<tr>
<td>Rear foot</td>
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<td>TOTAL</td>
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