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# SOME ASPECTS OF THE PREPARATION FOR HIBERNATION IN THE WOODLAND JUMPING MOUSE, NAPAEOZAPUS INSIGNIS

ROBERT STINE PAWLING

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by

ROBERT STINE PAWLING

B.S., PENNSYLVANIA STATE UNIVERSITY, 1964

M.S., UNIVERSITY OF NEW HAMPSHIRE, 1966

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This thesis has been examined and approved.

Edward N. Francq

Thesis director, Edward N. Francq, Asst. Prof. of Zoology

Philip J. Sawyer

Philip J. Sawyer, Asso. Prof. of Zoology

John J. Sasner

John J. Sasner, Asst. Prof. of Zoology

David P. Olson

David P. Olson, Asso. Prof. of Forest Resources

A. Teeri

Arthur E. Teeri, Prof. of Biochemistry

Samuel C. Smith

Samuel C. Smith, Asso. Prof. of Biochemistry and Poultry Science

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ABSTRACT

SOME ASPECTS OF THE PREPARATION FOR HIBERNATION IN THE WOODLAND JUMPING MOUSE, NAPAEOZAPUS INSIGNIS

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A sample of woodland jumping mice was snap-trapped each month while the animals were in the active condition. Lipid extractions on the entire carcass and gas chromatographic analyses of brown adipose tissue fatty acids were performed on a portion of the sample. The remaining specimens were utilized for the histological and histochemical examination of the adrenal glands, the thyroid gland, and the pancreas.

Lipid indices revealed an annual increase in total body fat preparatory to hibernation in October. The variation among individuals is greatest in September and October when adults low in body fat as well as corpulent adults are present. Although a large amount of unsaturation is maintained in the fatty acids of interscapular and subscapular brown adipose tissue throughout the active months, a marked increase in the percentage composition of linoleic acid occurs before hibernation.

The adrenal cortex of male woodland jumping mice is larger than that of females. The presence of a wider zona reticularis in males seems to coincide with the breeding season. The zona glomerulosa and zona fasciculata are rich in lipid material through the active months until October when the zona fasciculata appears to lose lipid. The zona reticularis gains lipid in October.

The ratio of beta to alpha cells in the islets of Langerhans of

the pancreas of Napaeozapus insignis increases prior to the entrance into hibernation. Cytoplasmic granulation of the beta cells is finer and less pronounced in October.

On the basis of epithelium height and colloid content, the thyroid gland of the woodland jumping mouse showed no evidence of being involved in the preparation for hibernation.

# INTRODUCTION

The family Zapodidae is composed of three living genera: Napaeozapus, the woodland jumping mice; Zapus, the meadow jumping mice; and Sicista, the European birch mice. Two of these genera are found in North America and are represented in New Hampshire by Napaeozapus insignis insignis (Miller) and Zapus hudsonius acadicus (Dawson) (Hall and Kelson, 1959). All of the zapodids are hibernators (Morrison and Ryser, 1962). While Pearson (1960) considers Zapus to be an indifferent homeotherm, i.e., its thermoregulation is poorly developed, Brower and Cade (1966) described the thermal regulatory capabilities of Napaeozapus as being quite precise. According to the definitions of the various types of hibernation discussed by Hoffman (1964) and Folk (1966), Napaeozapus and Zapus should probably be considered seasonal hibernators which have an inherent annual rhythm of physiology and behavior.

Although some studies have dealt with hibernation in jumping mice (Morrison and Ryser, 1962; Neumann and Cade, 1964), the quantity of literature has remained notably sparse in this area. Generally the detailed study of the active state of mammals capable of hibernation has been somewhat neglected in favor of comparative work performed on animals in the hypothermic state or in hibernation. There has been a tendency to underemphasize the importance of the fundamental activities of life such as reproduction, growth, moulting, feeding, and the physiological preparation for the long torporous condition (Kalabukhov, 1960). This study was conducted during the active period of the woodland jumping mouse, Napaeozapus insignis, in an attempt to elucidate specific physiological changes which might contribute to the preparation for hibernation.

According to Preble (1956), Platt (1966), and my own observations over a three year period, Napaeozapus in New England emerges from hibernation early in May and remains active until the first or second week in October. Hamilton, in his contribution toward a comprehensive life-history study of the woodland jumping mouse (1935), noted that mice caught during September and October tended to be fatter than animals from May and June. Blair (1940) and Preble (1956) also found evidence of an autumnal fattening among some members of a population of Napaeozapus.

The periodic deposition of fat reserves is reported quite commonly for a variety of hibernators including woodchucks (Davis, 1967) and numerous ground squirrels (Wade, 1948; Hock, 1960). A unique means of observing this phenomenon has been employed by Jameson and Mead (1964) on several sciurid rodents. The technique consists of performing a lipid extraction on the entire carcass of animals captured throughout the active season. A lipid index is calculated as follows:

$$\frac{\text{weight of body fat}}{\text{weight of fat-free dry carcass (basic weight)}} \times 100$$

The application of this procedure to Napaeozapus would seem to be a logical extension of the aforementioned observations on a seasonal fattening response.

A highly specialized type of adipose tissue, brown adipose tissue, is found in all mammals that hibernate and in some that do not (Johansson, 1960). Recent reviews (Joel, 1965; Smith and Horowitz, 1969) have dealt adequately with brown fat and its thermogenic role

in the hibernator. Brown adipose tissue has been shown to be composed primarily of triglycerides (Spencer et al., 1966). Furthermore, the proposed function of triglycerides as a source of energy for thermogenesis (Hall and Jungas, 1961; Dawkins and Hull, 1964) has been substantiated by Joel (1965). Hence, examination of the specific fatty acid composition of brown fat and its comparison with white adipose tissue has concerned many hibernation physiologists. A complete tabular review of these data has been presented by Smith and Horowitz (1969). The quantity of specific fatty acids in the brown fat of some hibernators, notably the bats, fluctuates annually (Remillard, 1959; Paulsrud and Dryer, 1967), suggesting perhaps a preparation for hibernation. However, in the golden-mantled squirrel Spencer et al. (1966) found that although a high degree of unsaturation appeared to be normal among all animals throughout the year, wide individual variation in lipid level and unsaturation precluded any correlation with season.

In view of the fact that no study has been devoted to the brown fat of Napaeozapus, it was felt that a fatty acid analysis of the brown adipose tissue would be of comparative interest. Likewise, the analysis of adipose tissue removed from animals trapped each month during the active season might reveal an annual pattern in fatty acid levels.

The recent reviews of Popovic (1960), Kayser (1961), Hoffman (1964), and Smith and Horowitz (1969) have dealt with the complex topic of hormonal involvement with hibernation. In an attempt to elucidate possible relationships with season, acclimation to low temperatures, or torpor; most of the endocrine organs have been studied. The adrenal glands, the thyroid gland, and the islets of Langerhans are associated commonly with some aspect of hibernation physiology. With this in mind,

a monthly histochemical and histological examination of these endocrine organs from Napaeozapus was conducted.

# METHODS AND MATERIALS

## Trapping Techniques

Woodland jumping mice were snap-trapped during the animals' active months from 1967 through 1969 in Jefferson Notch, Amonoosuc Ravine, Pinkham Notch, or White Ledge within the White Mountain National Forest of New Hampshire. Snap traps were used and checked often to minimize effects of trapping and tissue changes after death.

The distribution and abundance of Napaeozapus insignis in the White Mountain National Forest were found to be quite erratic. Since the woodland jumping mouse is very trap-prone, a population could conceivably be exterminated by the continual removing of animals from a single area. Hence, when a population of mice was located, only a few individuals were removed at a time to insure a continuous supply. Animals from the different sites within the National Forest were pooled in an attempt to form adequate samples.

## Lipid Extraction

The sex and approximate age of animals was determined; individuals were assigned a number and selected at random for lipid extraction or for histological examination of the endocrine glands. Those mice that were chosen for lipid work were sealed in a plastic bag and immediately frozen in dry ice. Upon return to the laboratory, the frozen animals were transferred to a freezer ($-5^{o}C$) for storage. As needed, the mice were thawed, weighed, paunched, and reweighed. The interscapular and both lobes of the subscapular brown adipose tissue from males were extirpated and weighed. Lipids were extracted by a modified Folch et al. (1957) procedure. A 50 to 100 mg. sample was

homogenized in 20 ml. of a 2:1 chloroform-methanol mixture. All solvents were freshly distilled. The crude extract was mixed with 0.2 its volume of a 0.04% calcium chloride solution and centrifuged at 755 x g for 10 minutes. The final subnatant was taken to dryness on a flash evaporator. Transesterification of lipids was achieved by refluxing with boron trifluoride-methanol (Peterson et al., 1965; McMullin, 1966). Esterified samples were dissolved in methylene chloride to give approximately 30 to 40 ug. per ul. concentrations of the fatty acid methyl esters present. Samples of five ul. were injected into a Barber-Colman model 10 gas chromatograph with a strontium-90 argon ionization detector. The six ft. x 1/4 in. glass column was packed with ethylene-glycol succinate (12% on 80/90 mesh Anakrom A) and maintained at 187°C with 10 p.s.i. inlet pressure. Peak areas were determined by multiplying the height by the width at half-height. The percentage of each fatty acid relative to the total composition of the sample was obtained by multiplying 100 times the area of each peak divided by the total area of all the peaks. This method was subject to approximately two per cent error.

The carcass of an animal (a male or a nonpregnant, nonlactating female from which the stomach contents had been removed) was homogenized in a blender with 2:1 chloroform-methanol at a volume 20-fold that of the carcass to insure an excess of solvents (Folch et al., 1957). Pregnant or lactating females were omitted to insure a greater amount of homogeneity in the samples. The mixture was allowed to stand for eight to ten hours under refrigeration (5°C) before it was filtered into a 500 ml. graduated cylinder with a ground glass stopper. The crude extract was shaken with 0.2 its volume of 0.04% calcium chloride solution. The solid residue was dried and weighed to yield the basic weight of the

animal. When the crude extract and salt solution separated into two phases, the upper phase was siphoned off and discarded. Following three 10 minute washings of the walls of the cylinder with upper phase rinse, the solution was made to one phase by the dropwise addition of methanol. The solution was quantitatively transferred to a one liter round bottom flask and evaporated to dryness on a rotary evaporator. The dry residue was dissolved in hot (60°C) chloroform-methanol and filtered into a 50 ml. volumetric flask. The solution was brought to volume, and two five ml. portions were removed, evaporated and weighed to 0.1 mg. in aluminum foil dishes. From these fractions the total lipid weight was calculated and, in the case of males, was added to the weight of the lipid from the brown fat pads. A lipid index was determined for each jumping mouse. The sum of the basic weight plus the lipid weight was subtracted from the paunched body weight to give the weight of body water. A water index was calculated for each animal (Jameson and Mead, 1964).

## Histological and Histochemical Techniques

The pancreas, thyroid, and adrenal glands were removed from those animals selected for histological examination. Pregnant or lactating females were not examined. Adrenal glands were cleaned of associated adipose tissue and fixed in Baker's calcium formol (Humason, 1962). Each gland was washed in distilled water, placed in embedding medium, and quick frozen with compressed carbon dioxide gas. All sectioning was done on a cryostat microtome at a temperature of -20°C and a thickness of 10 microns. Sections were placed in oil red 0 for six minutes, dipped in 50% isopropyl alcohol, and washed in tap water

for 30 seconds. Nuclei were stained in Ehrlich's hematoxylin for 10 minutes and blued in distilled water. Sections were mounted in Kaiser's glycerol jelly and ringed with permount.

Sections with the largest cross sectional area were chosen for examination. The width of each cortical zone was measured along four randomly chosen axes with an ocular micrometer. Cells were counted along the same axes and the mean cell thickness for each zone determined. Lipid quantities were expressed by assigning the terms high, moderate, or low to the range of color intensities observed. A high rating was given if the cells were completely filled with dyed lipid. If cytoplasmic spaces were observed between lipid droplets, the content was designated as being moderate. Cells low in lipid material were a pale pink color. The distribution of the stained material in each zone of the adrenal cortex was also noted.

Pancreatic tissue was fixed in Bouin's solution and embedded in paraffin. Sections were cut at a thickness of five microns and stained in a modified Gomori's chromium-hematoxylin-phloxine technique (Humason, 1962). Equal parts of 0.5% potassium permanganate and 0.5% sulphuric acid replaced the potassium dichromate oxidizing solution.

Thyroid glands were removed intact with a small portion of the trachea and placed in Bouin's fixative. The tissue was embedded in paraffin, sectioned at eight microns, and stained with hematoxylin and eosin.

## Proposed Live Studies

Proposed objectives dealing with food consumption, behavior, and metabolism were omitted due to difficulties encountered in

transporting and maintaining live jumping mice. Several live-trapped animals were successfully kept in four ft. x four ft. x three ft. enclosures containing wood shavings and a small, dark, nest box. These animals became quite obese on a diet of sunflower seeds. All attempts at breeding the woodland jumping mouse in captivity failed.

# RESULTS

## Lipid Indices

The presentation of data follows the natural sequence of events in the activity of Napaeozapus, beginning with the appearance of individuals after arousal in May and ending with their apparent entrance into hibernation in October. Individual lipid indices are shown chronologically in Table 1. All other data were similarly viewed according to the month of capture.

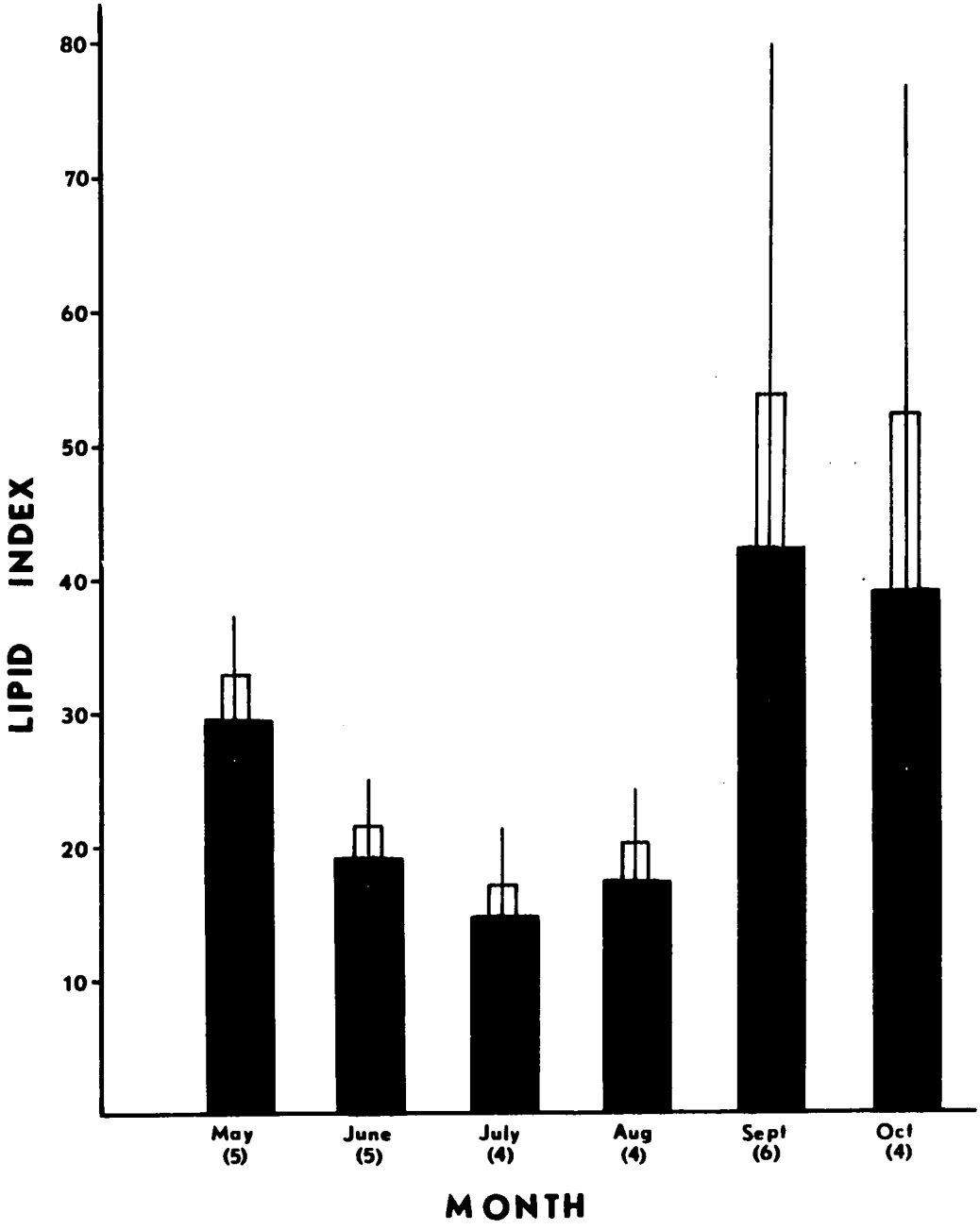
Males comprised most of the jumping mice trapped during the third and fourth weeks of May. Although it was presumed that the population at this time would be composed of adults 12 or more months old and young adults from late litters the previous year, these two groups were not readily distinguishable. Total weights of May animals ranged from 18.7 to 25.5 g. However, the paunched weight (Table 1) was found to be a better measure of the body weight due to the highly variable quantities of food material in the stomach. Throughout this study, an individual with a paunched weight greater than 16.8 g. was classified as an adult.

Following comparatively high lipid indices of about 30 in May, there was a decline to a low of about 15 in July (Figure 1). The gain in fat reserves was slight in August, but by September and October the mean levels increased almost three-fold to 40. However, the variation in lipid index values also increased markedly in September and October. Some members of the natural population attained a lipid index as high as 80 (Table 1). At the same time there were individuals in the population with lipid indices below 20. It is possible that some bias might be inherent in the early autumn samples since animals which had become

Table 1. Weights, lipid and water indices of male and female woodland jumping mice trapped during the season of activity.

| Month | Animal number | Sex | Paunched weight(g.) | Basic weight | Lipid index | Water index |
|---|---|---|---|---|---|---|
| May | 10 | M | 18.5 | 5.1 | 26.8 | 237.7 |
| | 19 | M | 19.1 | 5.0 | 20.7 | 264.7 |
| | 29 | M | 22.6 | 5.7 | 36.3 | 263.0 |
| | 30 | M | 23.7 | 6.3 | 27.3 | 247.3 |
| | 2 | M | 21.8 | 5.7 | 37.5 | 245.0 |
| June | 11 | F | 23.0 | 5.9 | 25.3 | 264.8 |
| | 12 | M | 19.9 | 5.6 | 13.3 | 241.1 |
| | 21 | M | 22.4 | 5.7 | 17.7 | 273.8 |
| | 6 | M | 21.9 | 5.4 | 22.4 | 283.5 |
| | 27 | M | 19.8 | 5.3 | 18.5 | 257.0 |
| July | 8 | M | 23.5 | 6.3 | 21.6 | 250.0 |
| | 17 | M | 21.0 | 5.8 | 11.8 | 250.0 |
| | 18 | M | 22.3 | 6.0 | 12.6 | 261.5 |
| | 20 | M | 19.6 | 5.4 | 13.4 | 247.0 |
| August | 7 | M | 16.9 | 3.7 | 24.4 | 330.5 |
| | 14 | M | 21.0 | 5.6 | 15.0 | 259.0 |
| | 13 | M | 23.6 | 6.4 | 11.4 | 254.9 |
| | 5 | M | 17.2 | 4.2 | 19.3 | 292.9 |
| September | 22 | M | 22.8 | 5.3 | 31.6 | 297.8 |
| | 9 | M | 21.5 | 5.9 | 31.0 | 235.9 |
| | 15 | M | 19.2 | 5.5 | 16.9 | 229.8 |
| | 16 | M | 17.1 | 4.0 | 19.5 | 263.5 |
| | 31 | F | 24.2 | 5.4 | 76.1 | 270.9 |
| | 3 | M | 26.4 | 5.8 | 80.0 | 275.9 |
| October | 23 | M | 19.0 | 5.1 | 18.3 | 252.0 |
| | 24 | M | 23.3 | 5.8 | 36.8 | 265.9 |
| | 25 | M | 24.0 | 5.6 | 24.7 | 302.1 |
| | 26 | M | 26.1 | 5.9 | 77.1 | 263.3 |
| | 28 | F | 17.5 | 4.7 | 24.0 | 248.9 |

Figure 1. Monthly change in lipid indices of adult male and female woodland jumping mice. Solid bar shows mean value, open rectangle encompasses ±1 S.E., vertical line shows range of actual values. Numbers of animals comprising samples are indicated in parentheses.


LIPID INDEX
80
70
60
50
40
30
20
10
May (5)
June (5)
July (4)
Aug (4)
Sept (6)
Oct (4)
MONTH

fattened earlier might have entered hibernation earlier and removed themselves from the active population.

A Cochran's Test for homogeneity of variance (Bartlett, 1937) revealed a lack of homogeneity in the fall lipid index data. The data from September and October were particularly heterogeneous (Table 1). This failure to satisfy one of the basic assumptions of an analysis of variance precluded further statistical examination of this data.

The basic weight (fat and water-free carcass) of adult woodland jumping mice is comparatively stable through the months of activity (Table 1). Water indices showed a greater amount of variability, but no seasonal trend was apparent. A linear regression analysis of the data from Table 1 indicated that no relationship exists between water index and paunched weight or water index and lipid index. However, there is a significant linear relationship between paunched weight and lipid index ($b=4.82$). As one might expect, heavier animals tend to have higher lipid indices ($P<0.01$).

## Brown Adipose Tissue

Although Napaeozapus possesses dorsal cervical, suprasternal, and superior mediastinal complements of brown adipose tissue, the fatty acid components of only the subscapular-axillary and the interscapular brown fat deposits which comprise the largest mass, were examined in this study. Combined wet weights of brown fat from subscapular and interscapular depots of males ranged from 20 to nearly 200 mg. with the heaviest coming from the animals taken in September and October (Table 2). A t test on the data from Table 2 revealed that the combined wet weights of the interscapular and subscapular brown fat depots of the pooled September and October animals were significantly heavier than the

Table 2. Combined wet weights (mg.) of the interscapular and subscapular brown fat depots of male woodland jumping mice.

May

| Animal number | Tissue wt. |
|---|---|
| 4 | 33.2 |
| 2 | 40.0 |
| 10 | 93.4 |
| 19 | 35.4 |

June

| Animal number | Tissue wt. |
|---|---|
| 6 | 83.5 |
| 12 | 82.7 |
| 21 | 97.7 |
| 27 | 40.0 |

July

| Animal number | Tissue wt. |
|---|---|
| 17 | 33.0 |
| 18 | 56.6 |
| 20 | 40.0 |
| 8 | 23.8 |

August

| Animal number | Tissue wt. |
|---|---|
| 5 | 68.8 |
| 7 | 35.7 |
| 13 | 66.8 |
| 14 | 83.9 |

September

| Animal number | Tissue wt. |
|---|---|
| 3 | 198.2 |
| 9 | 43.1 |
| 15 | 94.4 |
| 16 | 68.0 |
| 22 | 104.5 |

October

| Animal number | Tissue wt. |
|---|---|
| 23 | 110.7 |
| 24 | 166.8 |
| 25 | 90.8 |
| 26 | 190.0 |

same deposits of the pooled animals for each month, May through August ($P < 0.05$).

The interscapular brown fat pad showed some particularly dramatic changes in color and texture. This tissue in May, presumably soon after arousal, was brilliant red and rather thin and flaccid (Figure 2), while from September on the pad was pale brown, thicker, somewhat bilobed, and of a more rigid consistency (Figure 3).

The results of the fatty acid analysis of the brown adipose tissue from each animal are presented in the appendix. Due to the large amount of individual variation in these samples, comparison of the mean relative percentages is amenable only to the observation of general trends (Table 3). Of the acids constituting greater than 50% of the total, linoleic acid showed the most pronounced change (Figure 4). This fatty acid comprised 40% or more of the total fatty acid composition within the subscapular and interscapular brown adipose tissue of Napaeozapus from August until the time of hibernation in October. A linear regression analysis revealed a positive ($b=1.17$) linear relationship between high lipid indices and high amounts of linoleic acid in the brown fat. Analysis of variance of the monthly linoleic acid composition of brown fat (Appendix Tables 2-7) yielded sufficient evidence to reject the hypothesis of equal monthly means. A Duncan's Multiple Range Test (Duncan, 1955) of the same linoleic acid data revealed that the linoleic acid content of brown fat from August, September, or October males is significantly different from May, June, or July animals ($P < 0.05$). The mean relative per cent for the dietary essential linoleic acid was lowest in July. All of the other fatty acids excepting stearic and oleic peaked at this time. Although the changes certainly were not large, the

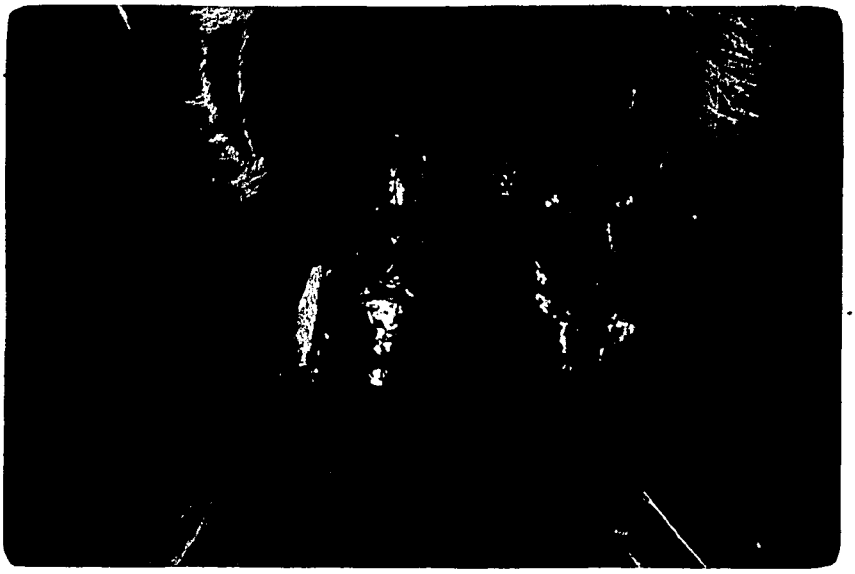


Figure 2. Interscapular Brown Adipose Tissue of a male Woodland Jumping Mouse trapped in May. (Tissue is at the point of the scissors.)

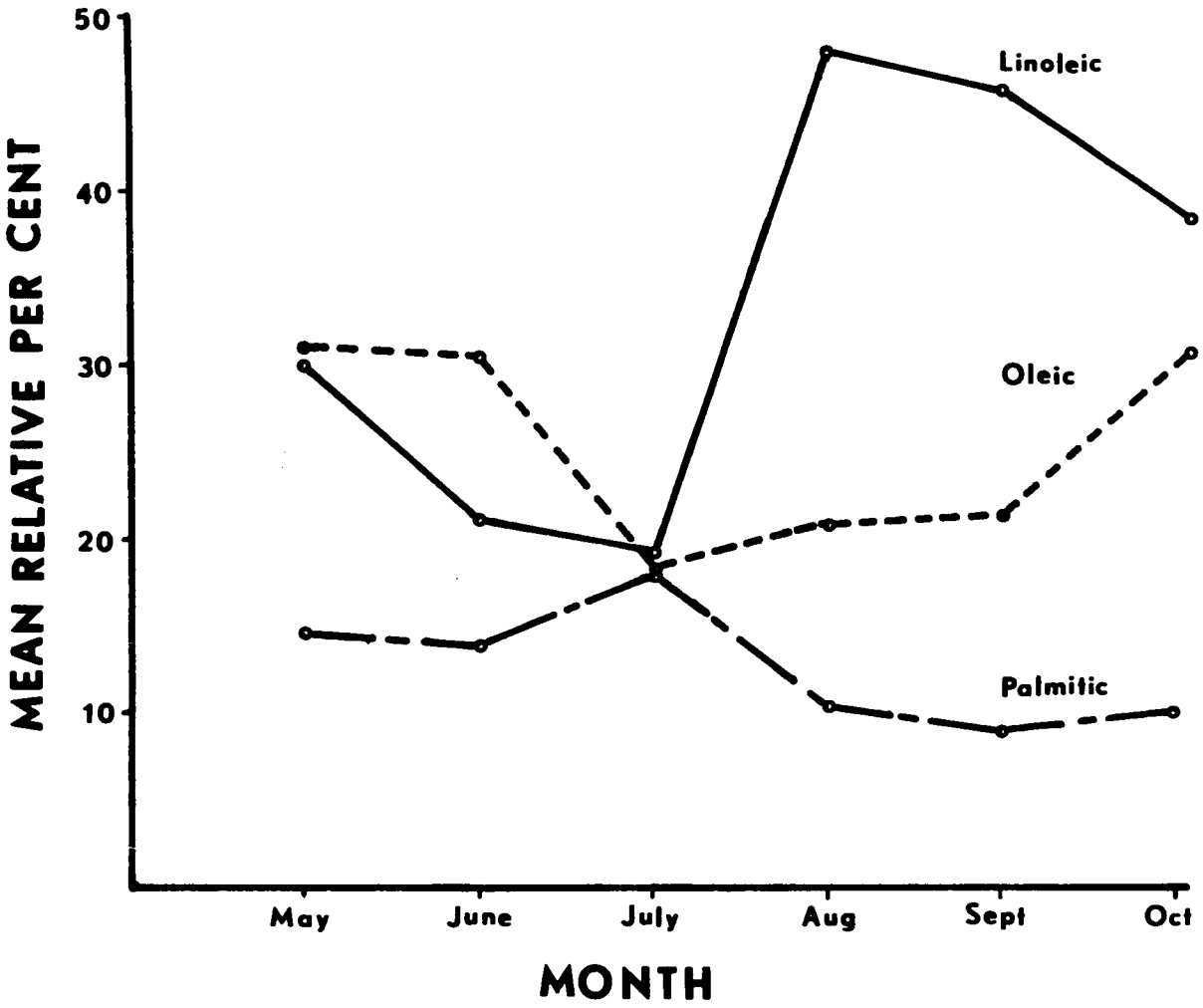
Figure 3. Interscapular Brown Adipose Tissue of a male Woodland Jumping Mouse trapped in September. (Tissue is at the point of the scissors.)

Table 3. Monthly mean relative per cent fatty acid composition of the brown fat of male Napaeozapus insignis.*

| Fatty Acid | Month | | | | | |
|---|---|---|---|---|---|---|
| | May | June | July | August | September | October |
| 14:0 | 0.79 ±0.45 | 1.44 ±0.67 | 1.80 ±0.94 | 1.04 ±0.41 | 0.99 ±0.32 | 0.18 ±0.06 |
| 15:0Br | ---------- | ---------- | ---------- | 2.04 | 0.92 | ---------- |
| 15:0 | 0.27 ±0.14 | 0.52 | 0.62 | 0.56 ±0.41 | 0.30 | 0.36 |
| 16:0 | 14.69 ±1.84 | 14.06 ±1.49 | 18.15 ±1.54 | 10.64 ±2.03 | 9.17 ±1.14 | 10.21 ±1.76 |
| 16:1 | 3.72 ±1.38 | 3.58 ±0.54 | 10.85 ±2.99 | 4.12 ±0.70 | 4.97 ±1.19 | 5.20 ±3.08 |
| 16:2 | ---------- | ---------- | ---------- | ---------- | 0.30 | 0.07 ±0.02 |
| 18:0 | 7.94 ±1.28 | 12.11 ±2.34 | 10.19 ±3.37 | 7.68 ±0.85 | 6.19 ±1.44 | 4.61 ±0.47 |
| 18:1 | 31.10 ±3.35 | 30.69 ±2.44 | 18.33 ±0.50 | 21.03 ±5.37 | 21.54 ±4.06 | 30.95 ±3.97 |
| 18:2 | 30.09 ±4.41 | 21.28 ±1.00 | 19.31 ±5.72 | 48.23 ±8.00 | 46.08 ±8.27 | 38.73 ±0.57 |
| 18:3 | 8.59 ±1.76 | 11.23 ±1.95 | 12.62 ±2.19 | 3.76 ±1.07 | 6.64 ±2.64 | 4.88 ±0.93 |
| 20:0 | ---------- | ---------- | ---------- | ---------- | 2.17 | 1.17 ±0.51 |
| 20:1 | ---------- | ---------- | 2.80 | ---------- | ---------- | 0.16 |
| 20:2 | ---------- | 3.51 ±1.00 | ---------- | ---------- | 1.30 ±0.65 | 0.44 ±0.14 |
| 20:4 | 2.38 ±0.55 | 2.07 ±1.00 | 4.27 ±1.40 | 1.67 ±0.69 | 1.31 ±0.29 | 3.78 ±1.91 |

*Figures are mean values ±1 S.E. expressed as per cent of fatty acids analyzed from several animals trapped during specified months. See appendix tables 2-7 for composition from individual specimens.

Figure 4. Seasonal change in the mean relative per cent of the predominant fatty acids from the brown fat of male Napaeozapus insignis.


MEAN RELATIVE PER CENT
50
40
30
20
10
Linoleic
Oleic
Palmitic
May
June
July
Aug
Sept
Oct
MONTH

upward trend in terms of per cent of total composition reversed in August for palmitic, palmitoleic, linoleic, and arachidonic acids (Table 3). Oleic acid began a slow increase in August (Figure 4). Analysis of variance of the monthly polyunsaturation levels (Table 4) suggests that the conclusion on different monthly means is not supported beyond the 10 per cent confidence level.

Histology and Histochemistry of Selected Endocrine Glands

Data on the width of the adrenal cortex zones (Tables 5 and 6) were subjected to a multiple regression analysis. Neither the total cortical widths nor the mean zone widths showed any significant monthly change. There was sufficient evidence to suggest that the mean widths of the total cortex as well as the mean zona reticularis widths showed significant sex differences, i.e., larger in males than in females ($P<0.05$). Several males in May and June possessed adrenals in which greater than 40% of the total cortical width was zona reticularis (Table 5). These same reticular zones contained three times the usual number of cells (Figure 5). On the other hand, the zona reticularis of nonpregnant, nonlactating females never exceeded 25% of the total cortical width (Table 6). In terms of morphology and mean width, the zona reticularis showed the greatest amount of variability. Fluctuations in the width or the mean number of cells in the zona reticularis did not appear to be related to changes in the zona fasciculata (Figures 5 and 6). Lipids were either absent or were present in very low quantities in the zona reticularis from May until September (Figure 5), whereas the zona reticularis of October males and females contained a moderate amount of lipid material (Figure 6).

The histology and lipid histochemistry of the zona glomerulosa and the zona fasciculata were generally constant through the active months.

Table 4. Monthly mean relative percentages of saturated, monounsaturated, and polyunsaturated fatty acids from the subscapular and interscapular brown fat of male Napaeozapus insignis.

| | Percentage of Total Composition | | | | | |
|---|---|---|---|---|---|---|
| Fatty acids | May | June | July | August | Sept. | Oct. |
| Saturated | 23.6 | 28.0 | 31.6 | 20.7 | 18.1 | 16.2 |
| Monounsaturated | 34.8 | 34.3 | 31.1 | 25.2 | 26.5 | 36.2 |
| Polyunsaturated | 41.6 | 37.9 | 37.4 | 54.3 | 55.4 | 47.9 |
| Total unsaturation | 76.4 | 72.2 | 68.5 | 79.5 | 81.9 | 84.1 |

Table 5. Adrenal cortex of males. A monthly tabulation of the total cortex width and the percentage that each zone contributes to the total cortical width in the woodland jumping mouse.

Males

| Month | Animal number | Adrenal cortex width (u) | Per cent of total width | | |
|---|---|---|---|---|---|
| | | | Zona glomerulosa | Zona fasciculata | Zona reticularis |
| May | 1 | 3.93 | 4.6 | 77.4 | 18.0 |
| | 2 | 3.86 | 3.3 | 75.1 | 21.6 |
| | 14 | 6.35 | 4.3 | 57.0 | 38.7 |
| | 15 | 4.38 | 4.1 | 47.9 | 47.9 |
| June | 20 | 6.35 | 3.1 | 52.4 | 44.4 |
| | 19 | 3.95 | 6.4 | 75.2 | 18.3 |
| July | 25 | 5.90 | 1.8 | 63.8 | 34.4 |
| August | 4 | 6.32 | 3.4 | 68.8 | 27.8 |
| | 5 | 4.56 | 7.9 | 79.4 | 12.7 |
| September | 7 | 3.47 | 10.9 | 83.3 | 5.7 |
| | 8 | 4.07 | 7.1 | 83.5 | 9.3 |
| | 12 | 4.11 | 11.0 | 82.8 | 6.2 |
| October | 27 | 5.32 | 4.8 | 82.9 | 12.2 |
| | 28 | 4.80 | 7.9 | 70.9 | 21.1 |

Table 6. Adrenal cortex of females. A monthly tabulation of the total cortex width and the percentage that each zone contributes to the total cortical width in the woodland jumping mouse.

| | Females | | | | |
|---|---|---|---|---|---|
| | | | Per cent of total width | | |
| Month | Animal number | Adrenal cortex width (u) | Zona glomerulosa | Zona fasciculata | Zona reticularis |
| May | 16 | 4.13 | 7.0 | 71.9 | 21.1 |
| June | 18 | 2.57 | 9.9 | 76.1 | 14.1 |
| | 21 | 3.98 | 5.5 | 72.7 | 21.8 |
| | 22 | 2.52 | 9.4 | 74.8 | 15.8 |
| July | 24 | 3.46 | 9.1 | 71.0 | 19.8 |
| August | 3 | 3.96 | 6.4 | 71.2 | 22.4 |
| | 9 | 3.86 | 6.6 | 82.6 | 10.8 |
| | 11 | 4.78 | 7.2 | 81.8 | 11.0 |
| September | 6 | 4.42 | 6.2 | 86.8 | 6.9 |
| October | 29 | 3.18 | 11.4 | 68.4 | 20.2 |
| | 30 | 4.47 | 5.7 | 89.1 | 5.3 |

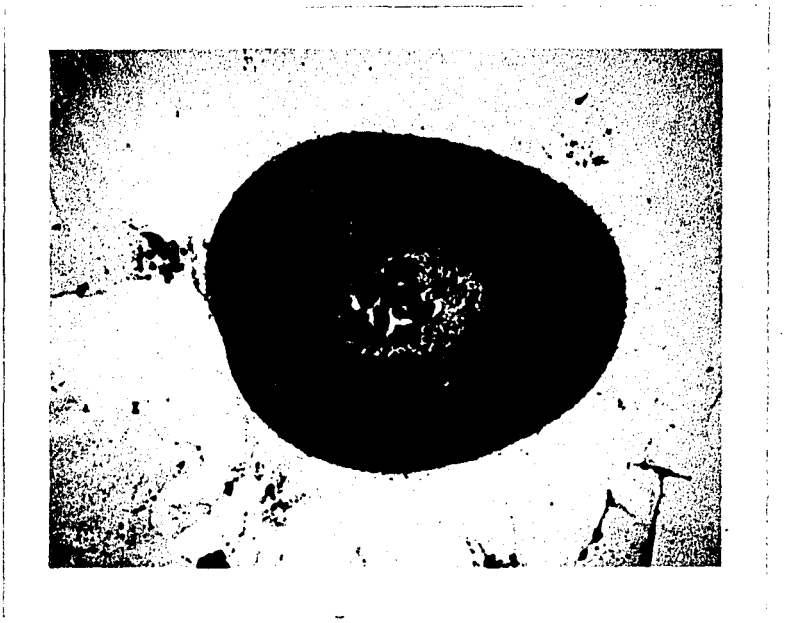


Figure 5. Adrenal gland of a male woodland jumping mouse that was trapped in May. Note the large size of the comparatively lipid-free zona reticularis, the innermost layer of the adrenal cortex. 44X

Figure 6. Adrenal gland of a female woodland jumping mouse that was trapped in October. Note the narrow width of the zona reticularis which now contains some lipid material. 44X

As early as May 22, these zones showed a high lipid content. The cells of the lipid rich zona glomerulosa were arranged in clumps three to five cells wide. These cells were consistently packed full of lipid droplets (Figures 5 and 6). Lipid in the zona fasciculata diminished progressively toward the inner end of the one cell thick fascicles. As a rule, the outer 1/3 to 1/2 contained a high to moderate amount, whereas the inner 1/2 to 2/3 was low in lipid. A slight diminution in lipid content was observed in the zona fasciculata of October animals (Figure 6).

The histology of the thyroid gland of Napaeozapus showed little variation throughout the months of activity. The follicular epithelium varied in height between 0.03 to 0.09 microns (Appendix Table 8). A moderate to large amount of colloid was common with the larger follicles usually occupying the periphery of the gland. Thyroid glands in both the "active" and "resting" condition were noted in all months of the active season determined on the basis of cell height and colloid content (Appendix Table 8).

The ratio of beta to alpha cells in the pancreatic islets of Langerhans tissue was approximately three to one from May to September (Table 7). Beta cell granules were large and dark staining (Figure 7). In October animals, the granulation was less pronounced, lighter, and more diffuse (Figure 8). The ratio of beta to alpha cells appeared generally to increase from three to four or five to one (Table 7).

Table 7. The ratio of beta to alpha cells and the granulation of the beta cells of the islet of Langerhans tissue from Napaeozapus insignis trapped during the active season.

| Month | Animal number | Sex | Beta/Alpha | Granulation Dark | Granulation Light |
|---|---|---|---|---|---|
| May | 1 | M | 2:1 | X | |
| | 2 | M | 3:1 | X | |
| | 13 | M | 3:1 | X | |
| | 14 | M | 3:1 | X | |
| | 15 | M | 2:1 | X | |
| | 16 | F | 3:1 | X | |
| June | 17 | F | 2:1 | X | |
| | 18 | F | 3:1 | X | |
| | 19 | M | 3:1 | X | |
| | 20 | M | 2:1 | X | |
| | 21 | F | 3:1 | X | |
| | 22 | F | 3:1 | X | |
| July | 23 | F | 2:1 | X | |
| | 24 | F | 3:1 | X | |
| | 25 | M | 3:1 | X | |
| | 26 | F | 3:1 | X | |
| August | 3 | F | 3:1 | X | |
| | 4 | M | 3:1 | X | |
| | 5 | M | 3:1 | X | |
| | 9 | F | 3:1 | X | |
| | 11 | F | 3:1 | X | |
| September | 6 | F | 3:1 | X | |
| | 7 | M | 3:1 | X | |
| | 8 | M | 4:1 | X | |
| | 12 | M | 3:1 | X | |
| October | 27 | M | 5:1 | | X |
| | 28 | M | 4:1 | | X |
| | 29 | F | 4:1 | | X |
| | 30 | F | 5:1 | | X |

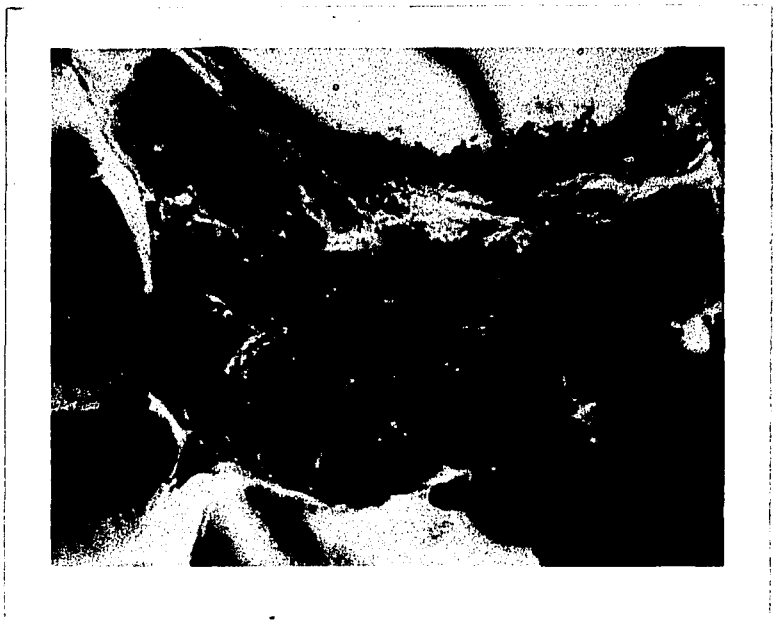


Figure 7. Islet of Langerhans tissue of Napaeozapus insignis trapped in June. Note the large, dark blue granules of the beta cells. 1125X

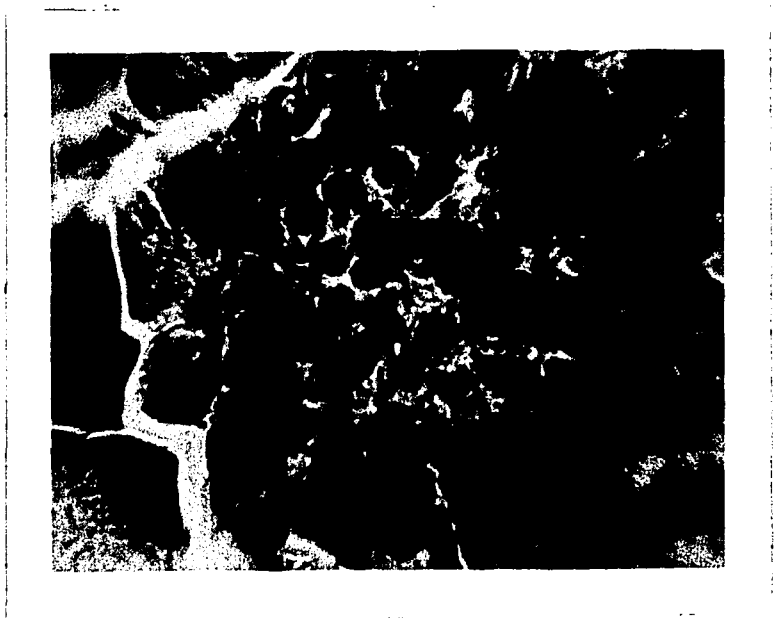


Figure 8. Islet of Langerhans tissue of Napaeozapus insignis trapped in October. Note that the granulation in most beta cells is lighter and less pronounced. 1125X

# DISCUSSION

## General

Equally as remarkable as the complex phenomenon of hibernation are the apparent anticipatory physiological and behavioral events which prepare the seasonal hibernator for its bouts in the torporous condition. The survival of the species must necessarily be dependent not only upon its ability to hibernate, but also upon certain characteristic adaptations which insure that a portion of the population will be able to hibernate successfully and arouse to breed the next season. Thus, in accord with the thesis of Kalabukhov (1960), a knowledge of the physiology and ecology of mammals which are capable of hibernation is essential to the understanding of hibernation itself.

## Interpretation of Trapping Observations

According to Brower and Cade (1966) the density of woodland jumping mice is related to the density of ground cover, that is, the largest populations are often found in very dense cover. The erratic distribution of dense cover habitats in the White Mountain National Forest area greatly influenced the trapping pattern in this study. Platt (1966) noted in what was apparently an ideal habitat, that a population of Napaeozapus showed seasonal and annual fluctuations. These two factors plus the possibility of seasonal movements (Blair, 1940) were sufficient to render most habitats unpredictable in terms of the presence of large numbers of jumping mice.

Traps set late in April or October in sites known to contain Napaeozapus failed to produce animals. This supports the findings of Preble (1956) and Platt (1966) who suggested that these animals were active

from early May to October. A paucity of fat in immature animals late in September tends also to support Preble's proposal that these animals might enter hibernation later than some of the adults. Sheldon (1938) noted that young jumping mice were able to hibernate at the age of 10 weeks. If this is the case, then the date of hibernation for litters born the last part of August would be early November. Entrance dates as late as November have been reported for jumping mice in the State of New York (Hamilton, 1935), but seem improbable in northern New Hampshire.

## The Deposition of Fat Reserves

As a seasonal hibernator, Napaeozapus has evolved a means of insuring survival through the winter months which might otherwise constitute a severe metabolic threat. The animals deposit extensive fat reserves (Hamilton, 1935), retreat below the ground to a nest (Sheldon, 1934) where temperature variation is least severe, and lower their metabolic rate (Hoffman, 1964). The results of this study indicated that the deposition of fat reserves began slowly in August and proceeded in some individuals at an accelerated rate until hibernation. The fattening response is believed to be affected by both shortening photoperiod (Neumann and Cade, 1964) and decreasing environmental temperatures (Patkin and Masoro, 1961). The fact that the range of values increased greatly in September and October (Figure 1) suggests that the fattening process occurs at varying rates which differ on an individual basis. That is, some individuals were very fat in September, when others had not yet begun to gain weight. This large amount of variation in lipid indices observed among individuals trapped during September and early October is difficult to explain without a knowledge of the role that factors such as age, sex, environmental conditions before and after hibernation, and previous

selection for success in fattening play in the preparation of the population for hibernation. Whether the heterogeneity is a result of small sample size or, as Jameson and Mead (1964) have suggested, a characteristic of the biology of the particular species; is not known. Variation may itself be of adaptive advantage to the population when the chances of early or late catastrophic weather conditions are considered.

Blair (1941) has suggested that females become fat earlier than males. Lipid index values of eight and nine observed for juveniles in September attest to the fact that immature animals are slow in preparing for hibernation, and therefore may suffer a disproportionate winter mortality. Several corpulent adults from September and October had lipid indices between 70 and 80 (Table 1). One animal with a lipid index of 80 had a total proportion of body fat of 17 per cent. This was the highest value observed in the natural population. However, in captivity a 41.4 g. male that had been kept on a constant photoperiod of 16L-8D and fed sunflower seeds ad. lib. attained a lipid index of 322. This animal was 49 per cent fat. Morrison (1960) noted that although wild animals usually carry lower amounts of fat, values of 50 per cent of total body weight have been observed for many species.

The mean lipid index of 29.7 and the narrow range of values in May (Table 1) suggests that jumping mice emerge with a portion of their fat deposits remaining. This might supply additional reserves in case inclement conditions should arise before natural foods become available and abundant. The fact that lipid index values below 20 were not found may indicate either that all animals do ultimately become fattened and emerge with a surplus, or that the individuals that are poorly prepared do not survive to show their presence in the spring.

The low lipid indices observed for adult jumping mice in the period June through August (Figure 1) provide convincing evidence that large amounts of insulative and reserve energy lipids are less advantageous at this time. In animals during these months fat formed only three to six per cent of the total body composition. This plateau in the lipid index values in spite of the greater abundance of food, is probably a result of the greater metabolic demands of increased activity and reproduction during the summer months.

If the fall lipid index data were biased by the fatter animals entering hibernation early (Neumann and Cade, 1964; Hamilton, 1935), the bias was more toward lower values since lighter animals would tend to remain active longer. Thus, the data contained herein may be a modest estimate of the amount of lipid material that is deposited in September and October. However, the findings of this study were consistent, relative to lipid content, with those of Jameson and Mead (1964) for the golden-mantled squirrel. An increase in lipid content may not account for all the autumnal gain in body weight so well described in Napaeozapus, but it certainly contributes a large portion. In terms of efficiency as a reserve material, fat supplies more chemical energy in a smaller volume than carbohydrate or protein (Masoro, 1962).

## The Gross Morphology of Brown Adipose Tissue

The gross differences observed in the appearance of the interscapular brown adipose tissue of Napaeozapus insignis (Figures 2 and 3) have been noted previously and are discussed in a review by Smith and Horowitz (1969). In other mammals the appearance of brown fat is a function of season, nutrition, and environmental conditions. The thin flaccid nature of the brown fat in the spring was found to be due to conditions

of inanition. The brilliant red color was a result of the vascularity which became apparent at this time (Grodums et al., 1966). Jumping mice from September possessed brown fat which was typical, in color and density, of the prehibernation condition. The light brown color is imparted by hemoglobin, heme porphyrins, flavin compounds, and the presence of many triglycerides (Smith and Horowitz, 1969). Remillard (1959) observed an increase in the size of brown adipose cells with the approach of hibernation. This could account for an increase in the rigidity of the tissue.

## Fatty Acid Patterns of Brown Adipose Tissue

Since the animals from which brown adipose tissue was excised for fatty acid analysis were removed directly from their natural surroundings, it was reasonable to presume that a large amount of variation would be observed as a consequence of varying diets, environmental temperatures, and genetic compositions. This study included also the temporal variations which may or may not have been consistent for the entire sample of animals. Spencer et al. (1966) conducted an investigation on a laboratory colony of golden-mantled squirrels maintained under controlled conditions, and concluded that individual squirrels varied so much that significance could not be associated with any particular fatty acid. Vagaries of fat deposition under captive conditions have already been alluded to by Hilditch and Williams (1964).

Several significant trends were observed in this study. Linoleic acid made up the largest relative percentage of the brown fat fatty acids from August to October (Table 3). As the paunched weight of the woodland jumping mouse increases, the lipid index increases. With the higher lipid indices comes an increase in the percentage composition of linoleic acid as a constituent of the brown adipose tissue. This is probably a reflection

of the diet (Turchetto, et al., 1963) since seeds, especially those ripening in a cooler climate, are high in linoleic acid (Shorland, 1962). Dietary linoleate is incorporated readily into depot fat (Mohrhauer and Holman, 1963), and diet does determine the percentage of fatty acids in the brown fat (Wells et al., 1964). The fact that the change in trend observed for linoleic acid (Figure 4) and most other fatty acids (Table 3) occurred in August might have been due to a change in the turnover rate, i.e., fatty acids are preferentially released from the cells at varying rates. Mussacchia and Wilber (1952) have noted a rapid turning over of fat preparatory to hibernation in the Arctic ground squirrel. A high turnover of endogenously synthesized lipids in brown fat has been proposed (Angel, 1969) to account for the low lipid contents which are sometimes seen in analyses (Chalvardjian, 1964). Future research should be directed toward possible factors affecting the turnover rate of specific fatty acids in brown fat.

The complete fatty acid composition of the brown fat observed in this study is in general accord with the findings for the golden-mantled squirrel (Spencer, et al., 1966), the house mouse (Spencer and Dempster, 1962), the woodchuck (Davis and McCarthy, 1965), the little brown bat (Wells, et al., 1964), the big brown bat (Paulsrud and Dryer, 1967) and rabbits (Dawkins and Stevens, 1966; Clement and Meara, 1951). Palmitic, oleic, and linoleic acids comprise consistently the largest percentage of brown fat fatty acids. However, the results of this study showed linoleic acid to be the predominant component in the fall (Figure 4). In many of the other mammals studied, oleic acid was the major component. Oleic was higher in the spring animals of this study, indicating perhaps that linoleic acid had been utilized preferentially either as a source of energy for

arousal or as a precursor for the synthesis of arachidonic acid.

Like the golden-mantled squirrel (Spencer, et al., 1966), the woodland jumping mouse maintains a high level of unsaturation in its brown fat through the active season. More than 70 per cent of the fatty acids of the interscapular and subscapular brown fat pads were unsaturated acids (Table 4). Thus, there would be no apparent need to increase the level of unsaturation to prepare for hibernation. Storage of a comparatively larger amount of linoleic acid in the fall, however, does increase the level of unsaturation. The advantages of unsaturates to the hibernator were suggested by Fawcett and Lyman (1954).

Prehibernation Changes in Selected Endocrine Glands

During the active period the adrenal cortex of male woodland jumping mice was larger than that of females (Tables 4 and 5). The same condition has been noted in another hibernator, the hamster (Deane and Lyman, 1954). Observations in this study indicated that sex differences were largely a result of a hyperplasia of the zona reticularis (Figure 5). Although this study did not include the collection of reproductive data, the reticular hyperplasia coincides well with heightened reproductive activity which occurs in the spring (Hamilton, 1935). Zalesky and Wells (1940) observed similar changes in the ground squirrel, Citellus tridecemlineatus.

There has been some disagreement in the literature regarding whether high lipid content in the cortical zones is an indicator of high or low metabolic activity (Kayser, 1965; Christian, 1962). Throughout this study, high lipid was interpreted as indicating large amounts of stored material. Oil red O is most readily taken up by various cholesterol esters (Schjeide, et al., 1963), and since cholesterol is a precursor of adrenal

steroids, the cell layers containing positively staining lipid droplets may be producing hormone at a rate that is slower than their supply of precursor material (Moses, et al., 1964). A high lipid content in the zona glomerulosa and outer portions of the zona fasciculata of the adrenal cortex of Napaeozapus was the same as conditions reported for the active woodchucks (Christian, 1962) and Arctic ground squirrels (Mayer and Bernick, 1959). According to the latter authors, the zona glomerulosa and zona fasciculata regain lipid within 24 hours after arousal. The zona fasciculata assumes greater importance in the fall, since cortisone and cortisol may influence the fattening response both directly and indirectly by increasing the volume and weight of brown adipose tissue (Aronson, et al., 1954) and by stimulating the release of triglycerides and glucose by the liver (Klausner and Heimberg, 1967). An increased output at this time could account for the lower lipid content observed in the zona fasciculata of October animals (Figure 6).

The histological condition of the thyroid gland of active woodland jumping mice gave no evidence of any seasonal involvement in the preparation for hibernation. Morphologically, the gland was similar to that described for active Arctic ground squirrels (Mayer and Bernick, 1959) and thirteen-lined ground squirrels (Zalesky and Wells, 1940). Contrary to the initial findings of Deane and Lyman (1954), Knigge (1957, 1963) has reported morphological and functional changes in the thyroid gland of cold-exposed hamsters.

Both alpha and beta cells were distinguishable in the islet of Langerhans tissue of the active woodland jumping mouse. An increase in the relative number of beta cells just before hibernation in October (Table 6), and a decrease in the granulation within the beta cells (Figures 7 and 8)

coincides well with the preparation for hibernation. If these histological changes are accepted as indicators of increased beta cell activity, the secretion of insulin could also be increasing concomitantly. The involvement of insulin in the lipogenic activity of brown and white adipose tissue has been well established (Lyman, 1968; Smith and Horowitz, 1969).

The preparation for hibernation in jumping mice is characterized by the annual deposition of fat reserves. The quantity deposited is highly variable. The fatty acid composition of the interscapular and the subscapular brown adipose tissue shows a distinct seasonal pattern which is to some extent a reflection of the animal's diet. The fattening process is most likely a result of a hyperphagic response to decreasing photoperiod and temperature (Mrosovsky, 1968). As a possible consequence, the endocrine glands, and likewise, the ensuing metabolism of the adipose cells could be responding to increased fatty acids and glucose levels in the blood stream. Further investigation of these interrelationships might provide additional insight into the preparation of mammals for hibernation.

## SUMMARY

Lipid extractions were performed on the carcasses of Napaeozapus insignis trapped just after emergence in the spring until the animals hibernated in the fall. Results indicated an annual rhythm of fat deposition, the extent of which varied among individuals within the population. Highest lipid indices were observed to occur before hibernation in September and October. Gas chromatography was employed for the identification of the fatty acids of brown adipose tissue removed from monthly samples of active males. The fatty acid showing the greatest increase in the percentage composition was linoleic which rose from a mean of 19 per cent in August to 48 per cent in October. High amounts of linoleic acid appeared to be directly related to the lipid index of the particular animal. The woodland jumping mouse maintains a high level of unsaturation in its brown fat deposits throughout the active season. Histological examinations of the thyroid gland, the pancreatic islets of Langerhans, and the adrenal glands were conducted. The ratio of beta to alpha cells increased and the granulation of the beta cells decreased in the islets of Langerhans from May to October. These observations may indicate a higher insulin output in the fall. The adrenal cortex of male jumping mice was wider than that of females. A hyperplasia of the zona reticularis accounted for the largest part of this difference. This hyperplasia may be related to increased hormonal production during the breeding season. An increase in the lipid content of the zona reticularis was observed from September to October; a time when storage of steroid precursors might be expected to occur. The thyroid gland showed no morphological signs of involvement in the preparation for hibernation.

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# APPENDIX

## Explanation of Appendix Tables

1. Throughout this appendix each fatty acid methyl ester is referred to by a shorthand designation consisting of two numbers separated by a colon. The first number indicates the number of carbon atoms in the fatty acid chain; the second denotes the amount of unsaturation, i.e., the number of double bonds within this chain. (See appendix Table 1.)

2. Males only were examined in an attempt to eliminate some of the variation observed in pregnant or lactating females.

3. Values are presented as percentages of individual fatty acids relative to the total fatty acids analyzed.

Table 1. A list of the common fatty acids encountered in the analysis of brown adipose tissue.

| Fatty Acid | Common Name | Systematic Name |
|---|---|---|
| 14:0 | Myristic | tetradecanoic |
| 16:0 | Palmitic | hexadecanoic |
| 16:1 | Palmitoleic | 9-hexadecenoic |
| 18:0 | Stearic | octadecanoic |
| 18:1 | Oleic | 9-octadecenoic |
| 18:2 | Linoleic | 9, 12-octadecadienoic |
| 18:3 | Linolenic | 9, 12, 15-octadecatrienoic |
| 20:0 | Arachidic | eicosanoic |
| 20:4 | Arachidonic | 5, 8, 11, 14-eicosatetraenoic |

Table 2. Fatty acid composition of brown fat removed from male Napaeozapus insignis trapped in May.

| Fatty Acid | Animal Number 19 | 10 | 2 | 4 |
|---|---|---|---|---|
| 14:0 | 0.21 | 0.49 | 1.67 | ----- |
| 15:0Br | ----- | 0.69 | ----- | ----- |
| 15:0 | 0.21 | 0.06 | 0.54 | ----- |
| 16:0 | 11.84 | 12.44 | 14.54 | 19.92 |
| 16:1 | 3.28 | 1.44 | 2.44 | 7.70 |
| 18:0 | 5.50 | 8.66 | 6.38 | 11.21 |
| 18:1 | 40.47 | 24.61 | 29.49 | 29.81 |
| 18:2 | 23.28 | 38.81 | 36.52 | 21.74 |
| 18:3 | 12.99 | 4.86 | 6.90 | 9.62 |
| 20:2 | ----- | 4.56 | ----- | ----- |
| 20:4 | 2.22 | 3.40 | 1.52 | ----- |
| Total | 100.00 | 100.02 | 100.00 | 100.00 |

Table 3. Fatty acid composition of brown fat removed from male Napaeozapus insignis trapped in June.

| Fatty Acid | Animal Number 6 | 12 | 21 | 27 |
|---|---|---|---|---|
| 14:0 | 0.57 | 3.41 | 1.14 | 0.66 |
| 15:0Br | 0.91 | ----- | ----- | ----- |
| 15:0 | 0.53 | ----- | 0.52 | ----- |
| 16:0 | 12.62 | 14.33 | 18.09 | 11.19 |
| 16:1 | 4.91 | 2.48 | 3.94 | 2.98 |
| 16:2 | ----- | ----- | 0.26 | ----- |
| 18:0 | 7.07 | 18.00 | 9.08 | 13.67 |
| 18:1 | 34.70 | 26.28 | 35.11 | 26.65 |
| 18:2 | 24.64 | 22.59 | 19.22 | 18.67 |
| 18:3 | 9.07 | 8.07 | 10.97 | 16.79 |
| 20:2 | 4.69 | ----- | 0.69 | 5.16 |
| 20:4 | 0.28 | 4.84 | 1.00 | 2.15 |
| 22:2 | ----- | ----- | ----- | 2.07 |
| Total | 99.99 | 100.00 | 100.02 | 99.99 |

Table 4. Fatty acid composition of brown fat removed from male *Napaeozapus insignis* trapped in July.

| Fatty Acid | Animal Number 8 | 17 | 20 |
|---|---|---|---|
| 14:0 | 2.08 | 3.28 | 0.05 |
| 15:0Br | 2.08 | ----- | ----- |
| 15:0 | 1.12 | ----- | 0.11 |
| 16:0 | 15.64 | 20.96 | 17.86 |
| 16:1 | 8.37 | 7.39 | 16.80 |
| 18:0 | 9.31 | 16.42 | 4.84 |
| 18:1 | 18.54 | 17.38 | 19.07 |
| 18:2 | 30.75 | 13.52 | 13.65 |
| 18:3 | 9.90 | 11.01 | 16.95 |
| 20:0 | ----- | ----- | 0.91 |
| 20:1 | ----- | 3.10 | 2.50 |
| 20:3 | ----- | ----- | 0.91 |
| 20:4 | 2.23 | 6.95 | 3.63 |
| 22:2 | ----- | ----- | 2.72 |
| Total | 100.02 | 100.01 | 100.00 |

Table 5. Fatty acid composition of brown fat removed from male *Napaeozapus insignis* trapped in August.

| Fatty Acid | Animal Number 13 | 5 | 14 | 7 |
|---|---|---|---|---|
| 14:0 | ----- | 1.50 | 0.21 | 1.40 |
| 15:0Br | ----- | 1.50 | ----- | 2.57 |
| 15:0 | 0.51 | 0.52 | 0.28 | 0.94 |
| 16:0 | 16.49 | 7.76 | 10.30 | 7.99 |
| 16:1 | 5.01 | 3.86 | 5.34 | 2.25 |
| 16:2 | ----- | 0.79 | ----- | ----- |
| 18:0 | 9.46 | 8.66 | 6.87 | 5.71 |
| 18:1 | 36.90 | 13.13 | 16.88 | 17.20 |
| 18:2 | 24.49 | 53.09 | 56.40 | 58.95 |
| 18:3 | 6.53 | 4.20 | 2.77 | 1.52 |
| 20:2 | ----- | 1.42 | ----- | ----- |
| 20:4 | 0.61 | 3.67 | 0.95 | 1.46 |
| Total | 100.01 | 100.10 | 100.00 | 99.99 |

Table 6. Fatty acid composition of brown fat removed from male *Napaeozapus insignis* trapped in September.

| Fatty Acid | Animal Number 15 | 16 | 9 | 22 | 3 |
|---|---|---|---|---|---|
| 14:0 | 0.18 | 1.09 | 0.96 | ----- | 1.74 |
| 15:0Br | ----- | 0.67 | 1.17 | ----- | ----- |
| 15:0 | 0.10 | 0.14 | 0.43 | ----- | 0.52 |
| 16:0 | 5.39 | 11.48 | 11.07 | 7.76 | 10.13 |
| 16:1 | 2.12 | 5.76 | 5.12 | 2.94 | 8.91 |
| 16:2 | 0.24 | 0.35 | ----- | ----- | ----- |
| 18:0 | 4.11 | 6.91 | 7.01 | 10.67 | 2.24 |
| 18:1 | 9.72 | 16.29 | 23.15 | 24.83 | 33.73 |
| 18:2 | 76.01 | 47.04 | 28.39 | 45.40 | 33.54 |
| 18:3 | 1.33 | 5.53 | 15.93 | 2.06 | 8.35 |
| 19:0 | ----- | 0.57 | ----- | 1.76 | ----- |
| 20:0 | ----- | 1.16 | ----- | 3.17 | ----- |
| 20:2 | ----- | 0.21 | 2.36 | 1.41 | ----- |
| 20:3 | ----- | 0.71 | ----- | ----- | ----- |
| 20:4 | 0.82 | 1.95 | 1.65 | ----- | 0.83 |
| 20:5 | ----- | 0.24 | ----- | ----- | ----- |
| 22:2 | ----- | ----- | 2.74 | ----- | ----- |
| Total | 100.02 | 100.01 | 99.98 | 100.00 | 99.99 |

Table 7. Fatty acid composition of brown fat removed from male *Napaeozapus insignis* trapped in October.

| Fatty Acid | Animal Number 23 | 24 | 25 | 26 |
|---|---|---|---|---|
| 14:0 | 0.23 | 0.09 | 0.07 | 0.34 |
| 15:0 | 0.62 | ----- | ----- | 0.10 |
| 16:0 | 14.56 | 10.35 | 5.96 | 9.96 |
| 16:1 | 14.18 | 1.50 | 0.87 | 4.23 |
| 16:2 | ----- | 0.03 | 0.08 | 0.10 |
| 18:0 | 4.65 | 5.77 | 4.55 | 3.46 |
| 18:1 | 19.89 | 37.93 | 30.80 | 35.16 |
| 18:2 | 37.56 | 38.19 | 38.94 | 40.22 |
| 18:3 | 3.52 | 3.90 | 7.60 | 4.48 |
| 20:0 | 2.16 | ----- | 0.88 | 0.48 |
| 20:1 | 0.21 | ----- | ----- | 0.01 |
| 20:2 | 0.51 | 0.17 | 0.80 | 0.28 |
| 20:4 | 2.47 | 2.08 | 9.45 | 1.10 |
| Total | 100.47 | 100.01 | 100.00 | 99.92 |

Table 8. Epithelium height and colloidal content of the thyroid follicles of Napaeozapus insignis trapped during the active months.

| Month | Animal number | Sex | Epithelium height (u) | Colloid content Moderate | High |
|---|---|---|---|---|---|
| May | 1 | M | 0.07-0.09 | | X |
| | 2 | M | 0.07-0.09 | X | |
| | 14 | M | 0.03-0.07 | | X |
| | 15 | M | 0.07-0.09 | | X |
| | 16 | F | 0.07-0.09 | | X |
| June | 18 | F | 0.07-0.09 | | X |
| | 19 | M | 0.07-0.09 | | X |
| | 20 | M | 0.03-0.07 | X | |
| | 21 | F | 0.03-0.05 | | X |
| | 22 | F | 0.07-0.09 | X | |
| July | 23 | F | 0.07-0.09 | | X |
| | 24 | F | 0.05-0.07 | | X |
| | 25 | M | 0.03-0.07 | | X |
| | 26 | F | 0.07-0.09 | X | |
| August | 3 | F | 0.07-0.09 | | X |
| | 4 | M | 0.05-0.07 | | X |
| | 5 | M | 0.07-0.09 | X | |
| | 9 | F | 0.03-0.07 | | X |
| | 11 | F | 0.07-0.09 | | X |
| September | 6 | F | 0.07-0.09 | X | |
| | 7 | M | 0.07-0.09 | | X |
| | 8 | M | 0.03-0.07 | | X |
| | 12 | M | 0.07-0.09 | | X |
| October | 27 | M | 0.07-0.09 | | X |
| | 28 | M | 0.07-0.09 | | X |
| | 29 | F | 0.03-0.07 | | X |
| | 30 | F | 0.07-0.09 | | X |