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A STUDY OF HOMING IN THE INDIANA BAT *Myotis sodalis*

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Introduction

Homing occurs in many species of bats. Griffin (1940), Davis and Hall (1952), Smith and Hale (1953) and Hitchcock and Reynolds (1942) have reported homing in *Myotis sodalis, Eptesicus fuscus and Myotis lucifugus* respectively.

It was felt that if large numbers of *M. sodalis* were released at varying distances from the point of capture more information concerning homing in this species might be obtained.

Acknowledgments

For their advice and encouragements I am indebted to Dr. Wayne H. Davis and Dr. Roger W. Barbour. Mr. Michael Harvey and Mr. Charles M. Rippy accompanied me on trips to Bat Cave. Special recognition is due Mr. Stewart Peck for his assistance in collecting and distributing the bats. A portion of the transportation was furnished by the Department of Zoology of the University of Kentucky.

Materials and Methods

The bats were obtained from Bat Cave at Carter Caves State Park, Carter County, Kentucky. They were selected from the wintering population of approximately 100,000 *Myotis sodalis.* Seven hundred were banded with number 2 aluminum bird bands supplied by the United States Fish and Wildlife Service. The bands were painted red with dilute fingernail polish. This was to aid in distinguishing the bats used in this study from the many banded bats already in the cave.

All of the bats were released within eleven hours of the time they were collected. They were released at twelve locations, each twelve air miles farther west from Bat Cave than the preceding. Although it was desirable to keep all release sites on a single radius, the convenience of highway release sites necessitated deviations off the radius.

Larger numbers of bats were released as the distance from Bat Cave increased (see Table I). The first were released at 6:00 p.m. October 20, 1962; the last were released at 3:45 a.m. the following day. Approximately 45 minutes elapsed between each release point. The bats were banded immediately prior to their release, and at this time sex, band number, release site and time of release were recorded.

The first return trip to Bat Cave was made on the weekend of October 26-27. At this time an attempt was made to observe all bats

Table I.— Number of *Myotis sodalis* Released

Release site	Miles from Bat Cave	Number released		
		Males	Females	Total
1	12	10	15	25
2	24	9	16	25
3	36	10	15	25
4	48	11	14	25
5	60	27	24	51
6	72	24	25	49
7	84	24	26	50
8	96	38	37	75
9	108	21	57	77
10	120	39	59	98
11	132	29	71	100
12	144	39	61	100
Total		281	419	700

in the cave. Band number and date of recapture were recorded for all red-banded bats observed. Subsequent trips either by the author or Dr. Wayne Davis were made to Bat Cave throughout the winter.

Results and Discussion

Fifty-six red-banded *Myotis sodalis* were found in Bat Cave during the first return trip (see Table II). Of the 56 bats recaptured 25 were males. Thus 8.9% of the males and 7.4% of the females had returned within six nights. Those bats released at the eleventh station were released in the early morning hours of October 21st. Therefore, the one recaptured from this point had completed the return trip in a maximum of five nights.

To date 119 or 17% of the red-banded bats have been recaptured at Bat Cave. This should not imply that more have not returned. Only 48 of the 119 bats were found in the cave more than once. One can thus assume that there were bats which returned to the caves but were not observed.

As can be seen in Table II, the total number of recaptured *M. sodalis* gradually decreased as the distance from Bat Cave increased. Although the percentages shown represent the present tally, approximately the same relationship existed after each of the first three return trips to the cave. The decrease in percentage of return may possibly be accounted for by several factors. First, as Hall (1962) suggests, *M. sodalis* may return to the caves by using familiar landmarks. If

such is the case, it may be assumed that those bats which did not return were removed from their normal range and could not find familiar landmarks upon which to orinet. Secondly, those bats released west of Lexington were just as close to suitable caves in central Kentucky and southern Indiana, and they may have gonue to these. Finally, those which did not return were unable to sustain flight of that length due either to lack of energy or adverse weather conditions.

There was considerable difference in the return percentages of males and females from several release points. However, the total returns from these points were in normal relationship to the others. No significant difference was found to exist in the percentage return of males and females when all recaptures were considered.

One of the red-banded bats was found near Somerset, Kentucky, on December 13, 1962. The bat was found dead in a non-heated room of a house. There is no indication as to how long the bat may have been there. Somerset is approximately mid-way between Bat Cave and Louisville, the release point of this bat. However, it is about 100 miles south of a direct line between the two.

No additional information has been gathered at this time to help determine the fate of the remaining bats which did not return to Bat Cave. It is hoped that additional band numbers will be reported to the Fish and Wildlife Service by other workers or interested parties. Until this happens or until other suitable caves are personally visited, the only avenue open is speculation.

Table II.— Number and Percentage of *Myotis sodalis* Recaptured

Release site	Number recaptured		Percentage recaptured		
	Oct. 26-27	Total	Males	Females	Total
1	11	17	80.0	60.0	68.0
2	7	16	77.8	56.2	64.0
3	7	14	70.0	46.7	56.0
4	6	9	9.1	57.1	36.0
5	7	16	25.9	37.5	31.4
6	6	8	25.0	8.0	16.3
7	3	9	25.0	11.5	18.0
8	3	9	7.9	16.2	12.0
9	5	6	9.5	7.2	7.8
10	0	5	5.1	5.1	5.1
11	1	6	3.4	7.0	6.0
12	0	4	5.1	3.1	4.0
Total	56	119			

Summary

A study of homing in *Myotis sodalis* has been conducted. Homing ability was found to decrease as the distance from the capture point increased. No significant difference was found to exist in the homing ability of males and females.

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COMPARATIVE BEHAVIORAL CHARACTERISTICS OF SIX GENERA OF MICE

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There have been various studies of behavioral relationships between different genera, species, and subspecies of mice. Most were concerned with a comparison of only two taxa. King (1957: 355) studied intra—and interspecific conflict of *Mus* and *Peromyscus*. Catlett and Shellhammer (1962: 133) compared the behavioral and biological characteristics of *Mus* and *Reithrodontomys*. Wirtz and Pearsen (1960: 131) observed aggressive behavior in *Microtus pennsylvanicus* and *Peromyscus leucopus*. Getz (1962: 351) observed aggressive behavior in *Microtus pennsylvanicus* and *Microtus ochrogaster*. Foster (1959: 496) studied behavioral differences in *Peromyscus maniculatus bairdi* and *Peromyscus maniculatus gracilis*.

This study compared behavior of six genera of mice when caged together.

Twenty adult and sub-adult mice representing six genera and six species were used in the experiment. Four *Peromyscus leucopus leucopus* (2 males; 2 females) and three *Peromyscus leucopus noveboracensis* (1 male; 2 females), one golden mouse, *Ochrotomys nuttali aureolis* (male), two house mice, *Mus musculus* ssp. (1 male; 1 female), six prairie voles, *Microtus ochrogaster ochrogaster* (4 males; 2 females), three pine voles, *Pitymys pinetorum auricularis* (2 males; 1 female), and one southern bog lemming, *Synaptomys cooperi kentucki* (female). All of these mice were captured in the wild with the exception of the house mice which were reared in captivity.

The golden mouse was captured two miles south of Beulah, Hickman Co., Kentucky. The prairie voles, pine voles, bog lemming, and four of the white-footed mice (*P. l leucopus*) were captured five miles south of Lexington, Fayette Co., Kentucky. Two of the white-footed mice (*P. l. noveboracensis*) were taken at Indianapolis, Marion Co., Indiana. One white-footed mouse (*P. l noveboracensis*) was taken two miles north of Paris, Edgar Co., Illinois. This particular combination of mice was used because of availability.

The mice were confined in a cage 58 inches long, 24 inches wide, and 14 inches high, having four glass sides and a wooden floor. Commercial laboratory litter covered the floor. Food consisted of various seeds, rolled oats and greens. No feeding device was used. Food was placed on the floor of the cage. Water was furnished by a single

bottle fitted with a one hole stopper and a length of glass tubing. This was suspended in one corner of the cage.

Observations were made of interspecific and individual behavioral patterns both when food and water were readily available and when food and water supplies were replaced after 24 hour periods of deprivation. Ten daytime and ten nighttime observations were made during a period of 60 days. Each observation lasted from 30 to 90 minutes.

When observations were made at night, illumination was furnished by a single 25 watt red bulb suspended above the cage. Finley (1959: 591) used ordinary red light to observe wood rats (*Neotoma floridana*) at night and found that red light, for all practical purposes, closely simulated nighttime conditions. This proved to be a useful device. The mice were much more active under red light than under ordinary lighting. During the daylight hours sunlight provided illumination in the laboratory. Little activity was noted during the daytime.

Prior to their introduction into the experimental cage the mice were kept in smaller cages and separated by genus. All of the animals, with the exception of the two house mice, were placed in the cage at the same time. The house mice were added eight days later.

Initial behavior consisted of random wandering and exploration, sniffing unfamiliar individuals, and a few brief tussles. The prairie voles, pine voles, and bog lemming displayed a higher degree of aggressiveness than the white-footed and golden mice.

Brief fighting occurred between pine voles and prairie voles; pine voles and the bog lemming; and prairie voles and the bog lemming. On one occasion a prairie vole bit the nose of a pine vole drawing blood. Immediately thereafter the injured animal ran along the side of the cage, smearing blood on the glass wall. When prairie voles encountered this blood during their explorations they licked it from the glass.

Pine voles were also observed fighting with one another as were prairie voles, although these same mice were compatible while caged together prior to their introduction into the larger cage.

When next observed, after having been confined together for 24 hours, all of the mice except the bog lemming were clumped together in one corner of the cage. Throughout the study, this clumping behavior was observed during periods of inactivity.

Although apparently socially accepted, the bog lemming usually isolated itself in a separate corner of the cage and rarely was it observed in a large aggregation.

When the two house mice were introduced into the cage they

showed the same initial behavior as did the others: random wandering, exploration, and sniffing unfamiliar individuals. The house mice were docile, but some of the other mice, particularly the pine voles and prairie voles, displayed aggressiveness by snipping at the house mice. In one instance a pine vole grasped a house mouse by the base of the tail and dragged it across the cage. After being in the cage for a few hours, however, the house mice joined the aggregation.

Half of the nighttime observations were devoted to food and water deprivation experiments. In these experiments the mice were deprived of food and water for 24 hour periods. Food and water were then replaced and behavioral responses were observed. Little conflict over food occurred when there was a sufficient amount available for each mouse. When an insufficient amount of food was added, two or three grains of corn for example, much fighting occurred. Many squeaks and guttural noises were emitted during these conflicts. Mice with corn were pursued by deprived individuals who attempted to obtain the food for themselves. Fighting also occurred when water was replaced after periods of deprivation, especially since only one individual at a time could drink from the glass tubing. This fighting, accompanied by squeaks and guttural noises, consisted primarily of pushing in which each individual tried to gain access to the water. Several mice were usually involved. The pine voles and prairie voles displayed more aggressiveness and dominated the water bottle. The house mice, white-footed mice, and golden mouse attempted to drink, but were usually pushed away and forced to wait until the pine voles and prairie voles had finished. Occasionally the golden mouse would climb onto the glass tubing and hang on, head downward, by grasping the tubing with feet and tail. In this manner it could secure water at intervals when other mice were fighting to drink. On these occasions, and at other times, when climbing on the wire support of the water bottle, the golden mouse exhibited apparent prehensility of the tail. The bog lemming did not attempt to drink until the other mice had finished.

Summary

1. When caged together, the prairie voles, pine voles, and bog lemming were more aggressive than the white-footed mice, house mice, and the golden mouse.

2. Initial aggressiveness lasted less than 24 hours.

3. The bog lemming, although apparently socially accepted, usually isolated itself from the other mice.

4. All other mice exhibited a clumping behavior during periods of inactivity.

5. Little conflict over food occurred except when there was an insufficient amount available.

6. Much fighting occurred when water was replaced after periods of deprivation, especially since only one individual at a time could drink.

7. The golden mouse exhibited apparent prehensility of the tail when climbing.

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THE ROLE OF GEOLOGY IN CONSERVATION IN KENTUCKY

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Who of you has ever looked around his home, office, or car, and considered the importance of the Earth's mineral resources in your daily affairs? What sort of living standard would we have if these raw materials were not exploited? How would we operate our businesses? Clays and shales provide the raw materials for brick and structural tile; gypsum and limestone are used in the manufacture of plaster and cement; pure sands and sandstones are the source of silica for light bulbs and tubes, window glass, and mirrors; iron and aluminum ores are processed for structural steel, metal trim, plumbing, hardware, heating appliances, automobile bodies and motors, and cooking utensils; copper is an essential component of any electrical system; most of the electricity consumed in this area comes from coal-burning generating plants; and natural gas and petroleum provide heat for buildings and homes, and fuel and lubricants for transportation. As impressive as this list might appear to be, we still haven't touched on such fields as ceramics, food containers, plastics, chemicals, fertilizers, aggregates, structural stone, mineral fibers, mineral pigments, mineral fillers, alloys, gem stones, precious metals, and numerous others. A list would range from asbestos to zinc, or literally from "A" to "Z."

These are part of the Earth's abundant natural resources. Yet in one very significant aspect they differ from other natural resources—*they are one-crop resources.* Rains replenish water supplies in lakes, streams, and underground reservoirs. New forests will grow where timber crops have been harvested and, with time, soils will naturally regain a certain degree of fertility. But when a barrel of oil is pumped from the ground or a ton of iron ore is scooped from a mountain side, that portion of the particular mineral resource has been depleted.

An adequate, dependable, and continuing supply of raw materials is indispensable to the United States and its industries in meeting the needs of an expanding population, a rising standard of living, and national defense. The demand upon our mineral resources is heavy and can be expected to increase in the future. Geologists and engineers are charged with the responsibility of their exploration and development. Although the exhaustion of mineral supplies is not imminent, man's quest for adequate food and clothing and a wide range

of material comforts, cultural advantages, education, and recreation are producing constantly increasing demands.

Since mineral resources are "one-crop" or non-renewable resources, the geologist's concept of conservation frequently deviates from the orthodox definition which includes preserving, guarding, or keeping in a safe place. There is probably no safer place for keeping our petroleum and natural gas resources than in the porous reservoir rocks thousands of feet underground, but can one visualize our civilization if the supply of our mineral fuels and lucricants were cut off? The writer's concept of the conservation of mineral resources includes wise and proper use, and development along constructive lines incorporating economical and efficient use of mineral supplies.

Voskuil (1955) has suggested steps necessary to maintain a steady flow of minerals, two of which directly concern the conservationist and the third, although not augmenting or conserving the original resource itself, has a saving consequence by increasing the efficiency of resource utilization. His suggestions are:

1. The reduction of loss and waste in present mining practices.
2. A program of ore discovery, including discovery and measurement of "sub-ore."
3. A program of technological improvement thru research for greater efficiency of resource utilization.

Kentucky is rich in a variety of mineral resources. While it is generally not considered a mineral state, an income of approximately 400 million dollars annually from mineral raw materials is evidence of the importance on the economy of the Commonwealth. According to the U. S. Bureau of Mines (1961), Kentucky, in 1960, ranked 15th in the United States in value of minerals produced, exceeding such states as Alaska, Colorado, Montana, New York, and all of our bordering states except West Virginia and Illinois; among the fifty states, Kentucky ranked second in ball clay, bituminous coal, and fluorspar production. (Available figures for 1961 indicate that Kentucky ranks third in bituminous coal production.) Only two states east of the Mississippi River (Illinois and Mississippi) produced more oil (U. S. Bureau of Mines, 1961).

Changing processes and new industries have created new and varied demands. Within our generation we have seen principal coal markets change from fuel consumption by home and railroad to electric generating plants. For maximum recovery, economy, and use, surface and underground coal-mining methods have become highly mechanized. The same has applied to the clay industry. Yet these very attempts to reduce loss and waste have caused these industries to be targets for criticism by some conservationists.

Research and technology will increase our estimates of ultimate resources beyond the rather limited concept of short-term reserves. An example of conservation through technological research is strikingly illustrated in the recovery of petroleum by new and improved techniques resulting from geological and engineering studies. The injection of fluids to supplement natural sources of energy in the reservoir rock results in a 50- to 100-percent increase in oil recovery from fields using such methods. Several million barrels of oil are produced in Kentucky each year by water-flooding, much of this from fields that would have been abandoned had not secondary recovery procedures been applied. Studies of such pertinent data as down-hole records, permeability and porosity tests, production figures, and subsurface geologic maps aid in determining the method to be used in securing the greatest ultimate recovery. The Kentucky Geological Survey has in its files, and available for reference by interested parties, more than 70,000 logs and 6,500 sets of rock cuttings from wells drilled for oil and gas in the Commonwealth. These materials are referred to daily by those interested in developing known gas and oil reserves and those searching for new production.

Attempts at crude oil recovery from Kentucky's natural rock asphalt deposits by new and improved technological research is being watched with considerable interest. Success here could rejuvenate a currently economically depressed region.

Kentucky's statewide areal geologic mapping program, conducted cooperatively by the State and Federal governments through the Kentucky Geological Survey and the United States Geological Survey, is providing essential knowledge for locating and developing our mineral wealth. The project, if carried to completion 8 to 10 years hence, will make Kentucky a national leader in this field. Mineral resource companies and exploration geologists, relating known deposits of asphalt, clay, coal, fluorspar, limestone, natural gas, and petroleum to the character and structure of the rocks in which they occur, can develop sound exploration programs for hidden new minerals in areas where favorable rocks and structures have been mapped. Little-known industrial minerals such as vein deposits, evaporites, absorbent and catalyst clays, and high-silica sands may be disclosed also. These maps will create new interest in mineral exploration in Kentucky which, in turn, will result in new mineral wealth and industrial development.

Water, although not generally considered a mineral by the layman, is as dependent upon geological factors as are petroleum and other hydrocarbons. Adequacy of supply, replenishment of underground reservoirs, and water quality are all related to the geological

environment. A program of water conservation should include a knowledge of the local geological relationships.

Geologic maps also provide information which enables engineers to more effectively select sites for the construction of dams, and plan flood-control and navigation structures, river development, water-supply and sewage facilities, and other pipeline networks. Geologic maps are the basic tool in the preparation of water availability maps. Cases could be cited where ground water investigations were delayed until adequate geologic maps could be assembled. And there is a growing trend in the use of geologic data in urban and industrial planning.

Since soil classifications are in large part based on the character of rock formations at or near the surface of the ground, foresters, soil scientists, and agriculturalists obtain valuable information from geologic maps in planning reforestation, land utilization, and soil conservation programs. The ability of a farm pond to retain water is due in a large measure to the underlying soil and rock conditions.

Conservation of our mineral resources can be accomplished by an orderly development and maximum recovery of our mineral commodities. Cooperation of all parties is requisite or regulatory agencies for enforcing conservation practices will be established.

Time will bring changes in our knowledge of the amounts and nature of the nonrenewable resources, and the grade of material that can be worked profitably. Dr. Thomas B. Nolan, Director of the U. S. Geological Survey, has recently pointed out (1962) that changes in adequate grade result not only from improvement in technology but also from the geologic accomplishment of establishing adequate and economic deposits of hitherto unutilized substances.

Although the fields dealing with renewable resources receive more publicity than those concerned with nonrenewable resources, they are equally important. Geologists can assist in our State and National planning in both areas, and aid our people to enjoy an increasingly higher standard of living and in building a better Kentucky.

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RELATIONSHIP BETWEEN CHOLESTEROL AND LECITHIN INTAKE TO LEVELS OF LECITHIN AND FREE CHOLESTEROL IN THE BLOOD SERUM OF RATS*

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It is only recently that the qualitative, as well as the quantitative, aspects of fat ingestion have been appreciated. The practical importance of these with regard to atherosclerosis is not clear as yet. However, in experimental animals the formation of arterial plaques has been shown to be affected by the fat type as well as its abundance. It has been found that in association with these plaques is a high concentration of serum cholesterol, and in association with the high serum cholesterol level is a diet high in saturated fats. It has also been postulated that atherosclerosis results from the chylomicrons which follow meals. (Gofman, et. al., 1950)

The present study had more than one objective. Since it was a controversial issue as to whether rats show a response to increased lipid intake, as other experimental animals have been found to do, one of the objectives was to see what response, if any, these animals would show when two lipids, cholesterol and lecithin, were added to their diet.

Before these objectives could be fulfilled, the experimental techniques of previous investigators had to be analyzed. From these investigations the experimental approach for this study was developed, with the aim of making it as closely correlated with previous studies as possible.

Studies of atherosclerosis in various parts of the world, on humans and experimental animals, have revealed that climate, locale, strain and racial characteristics are not the greatest factors producing atheromatous lesions, but diet is now believed to be one of the most important etiologic factors. (Kreglow, 1958)

The quantitative aspect of dietary fat has become of prime importance in the past few years, since the possible pathogenicity of an excessive amount of fat in the body was recognized. Some rather interesting and pertinent observations have been made in the

* Aided by a grant from the Justin Potter Memorial Fund, Nashville, Tennessee.

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past. First, it has been noted that not all animals are susceptible to hypercholesterolemic response after overloading the diet with cholesterol, and second, in animals which are susceptible it was observed that after a period of time they seemed to increase their capacity to handle this load. One may suspect on the basis of these observations that there exists in the animal's organism a homeostatic mechanism which controls cholesterol metabolism and regulates blood cholesterol levels. This mechanism apparently can be stimulated under forced conditions of dietary overload. (Boyd, 1952) It has also been observed that serum cholesterol levels are seemingly influenced by alteration in the type of lipid intake.

Since these observations were made much work has been done in connection with overloadnig the diet with a particular fat and observing the levels of this, and also other fats in the blood and tissues of the animal.

Patil and Majar (1959) found that in tissues and serum, the level of phospholipids was significantly lowered in cholesterol-fed rats. The same investigators also found that the ratio of cholesterol to phospholipids was higher in cholesterol-fed groups, and that the cholesterol and phospholipid contents of tissues and serum of animals from the control groups did not show wide variations. In disagreement with this, others (Schwenk, et. al., 1959) found that the phospholipid values and the ratio of serum cholesterol to phospholipid was closely parallel to serum cholesterol levels, for rats receiving daily an increased intake of cholesterol. The serum cholesterol value was observed to reach a peak in 9-11 weeks and then decline, even when daily intake was continued, which would support the hypothesis of a homeostatic mechanism being in operation for the control of cholesterol level in the blood. The same investigators found similar response in another study in which it was noted that serum cholesterol of cholesterol-fed rats rose to a peak in 2-4 weeks, then declined during the remainder of the experiment even though diets were kept constant. Serum phospholipid curves resembled cholesterol curves with initial rise and subsequent decline. In other studies (Hegsted, 1957, 1959) it was found that in no instance did the addition of dietary cholesterol raise the phospholipid level above that of the control group, but it did raise serum cholesterol levels. By using various diets it was concluded that there is no great tendency for serum cholesterol to rise unless dietary cholesterol is added, and that this elevating effect is most pronounced if the cholesterol supplement is given in combination with a highly saturated fat-containing diet. Apparently homeostatic factors are involved, but they are least effective in the presence of highly-saturated lipids.

In disagreement with these findings another group of investigators (Okey, et. al., 1951, 1959) found that after injecting cholesterol into rats for seven weeks the serum cholesterol values remained comparatively constant, while the liver cholesterol esters were greatly increased, indicating that the dietary cholesterol was being held as an ester in the liver. They found that the concentration of free cholesterol in the livers was very small. Patil and Majar (1959) observed the slight increase in cholesterol content of serum and large increase in liver content for animals receiving cholesterol.

It is believed by some that a large part of the circulating cholesterol is in combination in giant molecules, with protein, phospholipids, and fatty acids. If this is true then there is assumed to be a connection between the level of circulating cholesterol and phospholipids.

Materials and Methods

In the present study animals were fed two different lipid supplements, one of them being cholesterol which is the most common lipid found in the deposits in blood vessels. Having in mind that possibly blood serum cholesterol could be lowered with another lipid, lecithin was the other supplement.

For this study forty-five young adult male rats (210gm-265gm) of a closely inbred albino strain were used.

Normal serum lecithin and free cholesterol were determined for each animal and the determinations then averaged for final figures. These values were in close agreement with lipid values obtained by several oxidative micromethods. (Boyd, 1942) Blood was withdrawn via heart puncture from ether anesthetized animals. No anticoagulant was used. The blood was allowed to clot and cenrtifuged at 2500 rpm for 20 minutes. A period of at least two weeks was allowed to elapse before blood was taken from the same animal again. All animals were kept at an even temperature and fed standard Purina lab chow plus Cod-liver oil, milk and cracked wheat bread.

For the determination of free cholesterol one ml of serum was added to five ml of a 50-50 mixture of absolute ethyl alcohol and acetone, and the mixture then centrifuged. The filtrate was added to three ml of digitonin solution, which contained one drop of 10% acetic acid, and allowed to precipitate over night at room temperature in a closed preserving jar. The digitonin solution was prepared by dissolving 400 mg of digitonin in 100 ml of distilled water. Color was developed for the final determination by dissolving the digitonide in six ml of glacial acetic acid (Searcy and Berquist, 1950) saturated with ferrous sulfate, and then two ml of reagent grade

concentrated sulfuric acid was added. The solution was diluted to ten ml with acetone-alcohol, and ten minutes were allowed for color development. Optical densities were measured on a Bausch and Lomb colorimeter at a wave length of 490 millimicrons, against a blank prepared with acetone-alcohol.

Lecithin was determined by a modification of the Youngburg method in which inorganic phosphate was measuredfl One ml of serum was added to ten ml of mixture containing three parts absolute ethyl alcohol to one part anhydrous ether, and the resulting mixture brought to boiling in a water bath. The solution was filtered through filter paper which had been previously moistened with alcohol-ether. Five ml of the filtrate was evaporated to dryness and the residue then digested with 5 N sulfuric acid and 30% hydrogen peroxide. One ml of 2.5% ammonium molybdate solution, and 0.4 ml of aminonaphtholsulfonic acid was added to the solution, which was then diluted to ten ml with distilled water and allowed to stand five minutes for color development. Optical densities were measured on a Bausch and Lomb colorimeter at a wave length of 800 millimicrons, against a blank prepared with 10% trichloroacetic acid.

The 2.5% ammonium molybdate solutoin was prepared by dissolving 2.5 grams of ammonium molybdate in distilled water and diluting to 100 ml. The aminonaphtholsulfonic acid reagent was prepared as follows: 0.5 grams of 1, 2, 4-aminonaphtholsulfonic acid was added to 195 ml of 15% sodium bisulfate solution. Five ml of 20% sodium sulfite solution was added and this was mixed thoroughly. Sodium sulfite was then added, one ml at a time, until the powder dissolved. This reagent was prepared fresh every two weeks.

For experimental purposes the animals were equally divided into three groups. Group I received 125 mg cholesterol each, dissolved in corn oil; Group II received 125 mg lecithin each, dissolved in normal saline; and Group III received 125 mg cholesterol and 125 mg lecithin each, daily. All animals were fed the lipids orally. The animals were force fed with a pipette at first, but after a short time they readily ate the lipid supplements. The dosage was calculated on the basis of 0.5 gram per kilogram of body weight.

Free cholesterol and lecithin determinations were made on blood from animals in each group at 3, 10, 19 and 26 days after the beginning of the experiment, according to the methods previously described.

Results

In contrast to the results obtained by some previous investigators these animals showed a response to increased lipid intake. Regardless

of the type of lipid being given, with every increase in lecithin content, there was a decrease in free cholesterol content. This was also observed in the group receiving cholesterol and lecithin except for the determination made at 19 days in which the cholesterol content had dropped below normal, but rose on the 26th day. This latter rise cannot now be explained. (Chart I)

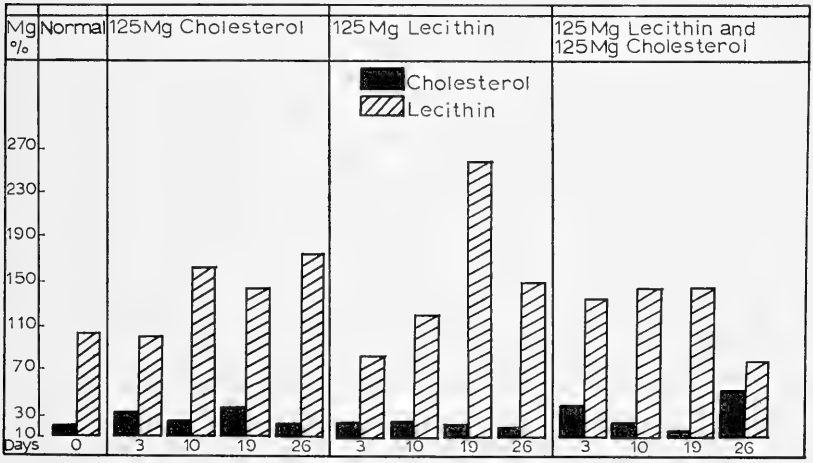

CHART 1

BLOOD SERUM CONTENT OF LIPIDS IN RATS

Discussion

This study, in agreement with some and disagreement with other previous studies, indicates that rats do show a difference in serum levels with increased lipid intake. Factors such as water and food intake, and individual difference, should be considered as possible explanation of the deviations.

It is now rather well established that the intake of unsaturated dietary fats reduce serum cholesterol. (Lewis, et. al., 1961) This decrease appears to be due to accelerated catabolism of cholesterol to bile acids, and to increased excretion of neutral sterols. The mechanism of action of unsaturated fat is probably to produce qualitative changes in circulating lipoproteins, which facilitates their catabolism and the elimination of the contained cholesterol, either as bile acids and neutral sterol, or possibly cholesterol handling at the cellular level.

From the results obtained in this study several questions are proposed. Apparently there exists some antagonistic action between lecithin and cholesterol. Is there a definite correlation between the amount of free cholesterol and lecithin, as some have believed? If so, could hypercholesterolemia be prevented by an increased lecithin intake, or would the organism increase its capacity to handle this lipid too, after which it might not have any effect? The possibility of a homeostatic mechanism, which others have suggested, involving lecithin should also be considered. The latter seems likely since intake of lecithin was observed to decrease cholesterol content. Also with increased cholesterol intake, there was observed an increase in lecithin content. Could there be a feed-back mechanism in which the amount of cholesterol is regulated by the amount of lecithin in the organism? In cases of hypercholesterolemia could there have been a prior inefficiency in the synthesis of lecithin? It is also quite possible that high concentrations of lecithin are causng a change in the lipoproteins, which speed up their catabolism, and cholesterol is subsequently eliminated.

Another point to consider is the belief by some workers that the main role of lecithin in the body is to aid in the transport of lipids, since lecithin is soluble in the body fluids. On this basis lecithin may decrease serum cholesterol by "carrying" it into the tissues, rather than having any effect on its metabolism.

Summary

Forty-five young adult albino rats of closely-inbred strain were divided into three equal groups. One group received orally 125 mg of cholesterol daily, the second 125 mg of lecithin daily, and the third group 125 mg of both lecithin and cholesterol. At weekly intervals and prior to feedings, determinations of the level of serum lecithin and free cholesterol were made on all animals. Animals receiving lecithin and those receiving both lipids showed a consistent reduction in cholesterol levels with the exception of the final rise already noted. Increased water intake also decreased cholesterol level. Techniques used and a discussion of the possible explanations are included.

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ON THE ACTIVITY OF PLETHODON GLUTINOSUS AS INFLUENCED BY LIGHT

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Anyone who has done extensive collecting of salamanders could scarcely have failed to note the existence of a well-marked periodicity of many species. It is my purpose to here record a brief study of this periodism in one species, and the effects of various light conditions on this pattern of daily activity.

In April and May, 1961, I determinued the times of activity of a series of 10 large, robust, and apparently healthy slimy salamanders, *Plethodon glutinosus glutinosus* Green, while exposing them to varying light conditions.

Essentially, the experiment consisted in releasing the animals in a 23 by 3 inch circular stainless steel tray, the bottom of which had been covered by dirt, dead leaves, and pieces of tree bark to simulate a forest floor. At hourly intervals for a period of 23 consecutive days the tray and its contents were photographed, using a Praktina FX 35 mm. camera with a motor drive and a Braun SP2 electronic flash unit. Included in the photographs were also a Numechron Tymeter GMT clock and a Minneapolis Honeywell thermometer with its bulb and capillary tube inserted in the leaf mold within the tray. Two 100 watt bulbs in goose-neck lamps supplied the only light other than that of the flash unit. The bulbs were placed at opposite sides of the tray, two feet above the rim, and pointing toward the center of the tray. The lights were controlled by an Intermatic Time-all clock; the camera was activated hourly by means of an Eagle Signal Co. Cycl-Flex timer.

Examination of the negatives disclosed the number of salamanders on the surface of the leaf mold, the temperature within the container, and the time the photograph was taken. Animals were considered "active" if as much as the anterior third of the body was visible; otherwise they were judged "inactive." Incidentally, in nearly every case the animal was either completely exposed or completely hidden.

The experiment was divided into three parts; the termination of one part was followed immediately by the onset of the next.

Part 1, Normal day. Lights were turned on at 6 a.m. and off at 6:00 p.m. Ten animals were introduced into the tray but one died after two days, a second died after five days. No additional animals were lost during the experiment. Part 1 ran for seven days, a total of 65 animal/days. (One animal for one day equals one animal/day.)

Part 2, Continuous dark. All lights continuously off, the only light being that of the electronic flash unit firing hourly, each flash with a duration of approximately 1/1000 second. Part 2 ran for five days, a total of 40 animal/days.

Part 3, Continuous light. Lights on continuously; neither of the bulbs burned out, so the light was essentially constant for four days, a total of 32 animal/days.

Part 4, Reverse day. The lights were turned off at 6:00 a.m. and on at 6:00 p.m., thus shifting the normal day-night rhythm 12 hours out of phase. This was continued for seven days, a total of 56 animal/days.

All data from the same hour in each part as outlined above were averaged. For example, all photographs taken at 9:00 a.m. during the five days of continuous dark were considered together, and the average number of individuals visible at this time was calculated. This was expressed as a percentage of the total number it would have been possible to see were all active, (in this case, 40 animals) and the results plotted on a graph. (Fig. 1)

Although this experiment has barely scratched the surface, there are certain conclusions that I feel may be safely drawn.

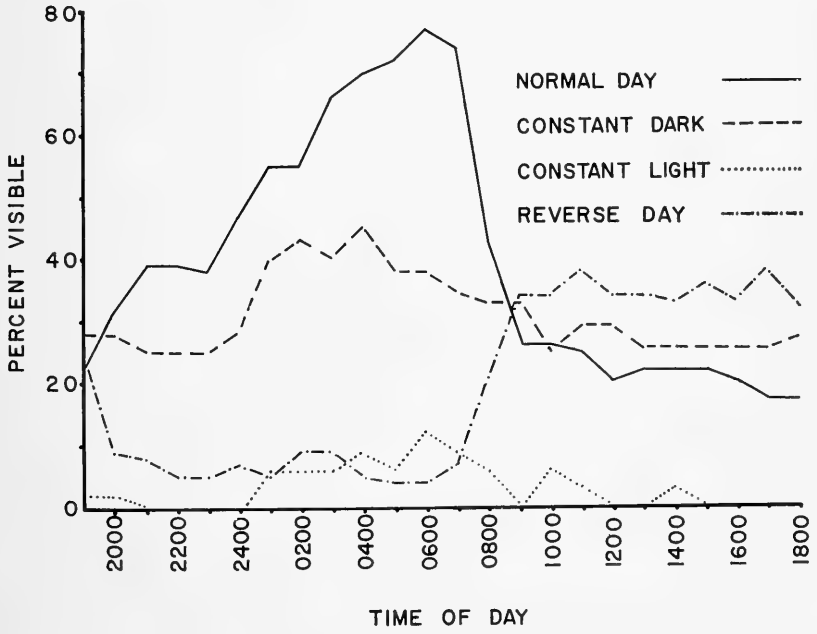

Fig. 1.— Activity of *Plethodon glutinosus* under varying light conditions

1. *Plethodon g. glutinosus* exhibits a well-marked circadian rhythm, being more active in darkness than in light. The number of active animals increased as the dark period progressed. Number of active animals fell off sharply with the onset of the lighted period.

2. Conditions of constant dark tend to inhibit activity during the normally dark hours, and to increase activity during the normally light hours. However, the normal pattern of activity remains well-marked.

3. Conditions of constant light exert a great deal of influence in suppressing the normal rhythm, but it was still apparent under the conditions herein imposed.

4. Temperature fluctuations on the order of 10 degrees Fahrenheit or less have little or no effect on the normal rhythm of *Plethodon g. glutinosus.*

5. Under conditions herein imposed, it is possible to shift the daily rhythm of *Plethodon g. glutinosus* some 12 hours out of phase. In other words, by manipulation of light alone, individuals can be induced to become active when they would normally be quiescent, and vice versa.

AN ANNOTATED CHECKLIST OF FISHES FROM DIX RIVER AND TRIBUTARIES (EXCLUSIVE OF HERRINGTON RESERVOIR)

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Introduction

A study in the fish population dynamics of Herrington Reservoir by Whitney (1962) instigated a study of the fish fauna of the Dix River and its tributaries. Twenty-seven collecting stations were randomly selected throughout the system and collections were conducted during the summer months (June, July, August and September) of 1962.

The checklist includes the fishes taken with seines (4, 6 and 15 feet in length) and with gill nets (100'x6' with 1½" mesh). The classification by families, genera, and species is according to Moore in Blair et al. (1957). The common names are those suggested by the American Fisheries Society (1960).

Dix River and Tributaries

The Dix River, a branch of the Kentucky River, is located in the southern portion of the Bluegrass region of Central Kentucky. It extends a distance of 78 miles through Mercer, Boyle, Garrard, Lincoln, and Rockcastle counties. An area of 449 square miles composes the watershed.

Even though the Dix River system is relatively small, it offers three distinct ecological habitats; a shallow river system, a deep impoundment, and a cold tailrace. The shallow river system, the concern of this article, consists of two primary, complexly-branched systems: the Dix River proper and the Hanging Fork Creek system. These two systems join at the extreme head of Herrington Reservoir.

The water depth ranges from a few inches in the head waters to four feet at the reservoir junction. The relatively steep gradient (about 5.9 feet per mile) combined with this water shallowness produces riffles and areas of swift current. The flow is interrupted during periods of drought and periodical pools result ranging in dept from two inches to two feet. This is typical of the summer months. Flash floods are common during the spring and fall rainy seasons.

Overburden of the basin is composed of thin, rocky materials on the hilltops; the slopes of the more rugged sections are of rich limestone soils. In the source region (the edge of the Knobs), tight and clayey materials are found in the lowlands (Whitney, 1962).

Results of Collections

Clupeidae

Dorosoma cepedianum (LeSueur). Twenty-one specimens of the gizzard shad, an important forage fish, were collected from the main trunk stream. Two individuals were taken from Hanging Fork Creek.

Esocidae

Esox vermiculatus Le Sueur. No specimens of the grass pickerel were collected but a single individual, taken from the extreme headend of the Dix River proper, exists in the collections of the Department of Zoology, University of Kentucky. It was taken in October, 1960.

Catostomidae

Carpiodes cyprinus (LeSueur). Three young quillbacks were collected at the junction of the river and the impoundment.

Catostomus commersoni (Lacépède). One specimen was obtained from a shallow headwater stream, but the greatest abundance of the white sucker appeared to be in the deeper portions of the main trunk and in the main stream of Hanging Fork Creek.

Hypentelium nigricans (LeSueur). Five hog suckers were collected from the deeper pools of the shallow streams and eleven individuals were taken at various points along the main trunk.

Moxostoma breviceps (Cope). The shorthead redhorse was the typical sucker of the system. Twelve specimens were taken from headwater streams. Their relative abundance increased as the streams became larger.

Moxostoma erythrurum (Rafinesque). The golden redhorse was obtained from intermediate-size streams but its greatest abundance was found in the Dix River proper. Thirty-six individuals were captured.

Cyprinidae

Campostoma anomalum (Rafinesque). A total of six hundred and thirty-seven stonerollers were collected throughout the entire system. Their relative abundance was greater in headwater and intermediate-size streams.

Chrosomus erythrogaster Rafinesque. The southern redbelly dace was collected from only one headwater stream. Two specimens were obtained.

Ericymba buccata Cope. One hundred and fifty-four individuals were collected from intermediate-size streams. An occasional silverjaw minnow was collected from the main trunk stream.

Hybopsis amblops (Rafinesque). The deeper water of the Dix River proper yielded seven specimens of the bigeye chub.

Notropis ardens (Cope). Two hundred and seventeen rosefin shiners were taken from the deeper pools of the intermediate-size streams and from the trunk stream of Hanging Fork Creek.

Notropis atherinoides Rafinesque. A moderate abundance of the emerald shiner characterized the pools of the main trunk stream and the trunk of the Hanging Fork Creek.

Notropis boops Gilbert. The bigeye shiner was collected from all portions of the main trunk stream and the lower portions of the larger tributaries. One hundred and twenty-five specimens were obtained.

Notropis cornutus (Mitchill). The common shiner, like the bigeye shiner, was found in all portions of the main trunk stream and the lower portions of the larger tributaries. One hundred and thirty-one specimens were collected.

Notropis rubellus (Agassiz). Thirteen rosyface shiners were obtained from a deeper section of Hanging Fork Creek.

Notropis spilopterus (Cope). Twenty spotfin shiners were collected from the lower end of both the main truck stream and Hanging Fork Creek.

Notropis whipplei (Girard). A moderate abundance of the steel-color shiner was obtained from the deeper waters of the river. Fifty-seven individuals were collected.

Pimephales notatus (Rafinesque). The bluntnose minnow was collected throughout the entire river system, but its greatest abundance was in the intermediate-size streams. Very few specimens were taken from Hanging Fork branch.

Pimephales promelas Rafinesque. Only three fathead minnows were collected. They appeared in a pool of a single headwater branch.

Semotilus atromaculatus (Mitchill). The creek chub, the characteristic minnow-form of the river, was collected from all stations. Hundreds were captured but only five hundred and fifteen were retained.

Ictaluridae

Ictalurus melas (Rafinesque). A single specimen of the black bullhead, expected to be restricted to the deeper waters, was netted in a deep pool of a larger headwater stream. Several spcimens were collected from the main trunk stream.

Ictalurus natalis (LeSueur). Three yellow bullheads were obtained from the Dix River proper.

Ictalurus punctatus (Rafinesque). A single channel catfish was recorded at the junction of the river and the reservoir.

Noturus miurus Jordan. A single brindled madtom was collected about midway in the main trunk stream.

Atherinidae

Labidesthes sicculus (Cope). Collections from Hanging Fork Creek and the main trunk stream revealed nine brook silversides.

Serranidae

Roccus chrysops (Rafinesque). Only two young specimens were netted in the river. According to Tompkins and Peters (1951), the river becomes the site for the annual spawn runs of the white bass.

Centrarchidae

Ambloplites rupestris (Rafinesque). A total of five rock bass were collected from four stations located on the main trunk stream and the Hanging Fork Creek.

Lepomis cyanellus Rafinesque. The green sunfish extended into the extreme headwaters and its greatest abundance was in the intermediate-size streams. A total of twenty-six specimens were netted.

Lepomis macrochirus Rafinesque. Thirty-three bluegill individuals were obtained from the system. They were found in the lower, pooled portions of the headwaters and extended to the lake junction.

Lepomis megalotis (Rafinesque). The longear sunfish was collected in the intermediate-size streams buts its greatest abundance appeared to be in the deeper waters. Twenty-one specimens were netted.

Micropterus dolomieui (Lacépède). Two young specimens of the smallmouth bass were collected in a deep pool in Hanging Fork Creek.

Micropterus punctulatus (Rafinesque). Single specimens were collected from each of four stations. The spotted bass was taken only in the deeper waters.

Micropterus salmoides (Lacépède). Five largemouth bass were netted in the deeper waters. On one occasion, a single specimen was captured in an intermediate stream.

Pomoxis annularis Rafinesque. Eight specimens of the white crappie were obtained in the main trunk stream and one specimen from Hanging Fork Creek.

Pomoxis nigromaculatus (LeSueur). The black crappie is rare in the Dix River. Only one specimen was collected in Hanging Fork Creek.

Percidae

Etheostoma blennioides Rafinesque. Thirty-three greenside darters were obtained from swift riffle areas of the Dix River. Two specimens were collected in riffles of the lower Hanging Fork Creek.

Etheostoma caeruleum Storer. The rainbow darter appeared to have an even distribution throughout the main stream and the lower ends of tributaries. Twenty-four specimens were recorded.

Estheostoma flabellare Rafinesque. The fantail darter appeared to be the most stream diversified species. It was collected from every station. Thousands were captured but only one hundred and ninety-five were retained.

Etheostoma nigrum Rafinesque. The johnny darter was collected from riffles in the main trunk and in the intermediate-size streams. Its greatest abundance was in the latter.

Etheostoma spectabile (Agassiz). Six orangethroat darters were captured in lower headwater riffles and two in a main trunk riffle. This strange separation might indicate overlooked individuals in between.

Percina caprodes (Rafinesque). The greatest abundance of the log perch was in the deeper portions of the main trunk, but it extended to the lower margins of intermediate-size streams. A total of seventeen specimens were collected.

Percina macrocephala (Cope). The longhead darter is very rare in the system. A single specimen was obtained at the junction of the river and the lake.

Sciaenidae

Aplodinotus grunniens Rafineque. Two freshwater drums were collected from the deeper portion of the main trunk.

Discussion

The fishes collected from twenty-seven stations in the Dix River system represented forty-four species from ten families. The specific distributions were quite uniform and distinct patterns were revealed.

The suckers were the predominant rough fish and were collected throughout the enitre system. Their greatest abundance was in the deeper portions of the Dix River and Hanging Fork Creek.

The intermediate-size streams were characterized by the abundance of minnows. The genus, *Notropis,* appeared to be restricted to these waters where seven species were netted. The creek chub, the bluntnose minnow, and the stoneroller were the only family representatives extending into the headwaters; but, even here, they were not characteristic.

The deeper waters at the lower end of the river proved to be a favored habitat for all the collected species of catfish. The Centrarchidae (as well as the freshwater drum, the brook silversides, and the crappie) were also primary inhabitants of this area.

The most pronounced distribution pattern was exhibited by the sunfishes of the genus *Lepomis*. The green sunfish was collected throughout the entire river system. The bluegill was collected from all streams except those of the extreme headwaters. The longear sunfish made its first appearance in the intermediate-size streams and its relative abundance increased as the water depth increased.

Darters were collected from practically all riffled areas but members of the genus *Etheostoma* had their greatest numbers in the headwaters and the intermediate-size streams. The log perch was distinctive of the deeper waters.

Summary

1. Following a fish population study of Herrington Reservoir (Whitney, 1962), a study was undertaken to determine the fish fauna of the Dix River and tributaries.

2. Twenty-seven collecting stations were established and collections were made during a four month period in 1962.

3. The findings are discussed with regards to the family representatives and relative distributions.

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AGING BATS IN WINTER

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A method for separating young bats from old ones in winter has long been desired by bat banders studying survival and longevity. Recent observations on *Pipistrellus subflavus* have suggested that some young of the year can be recognized in hibernating populations in certain areas by examining epiphyses and pelt.

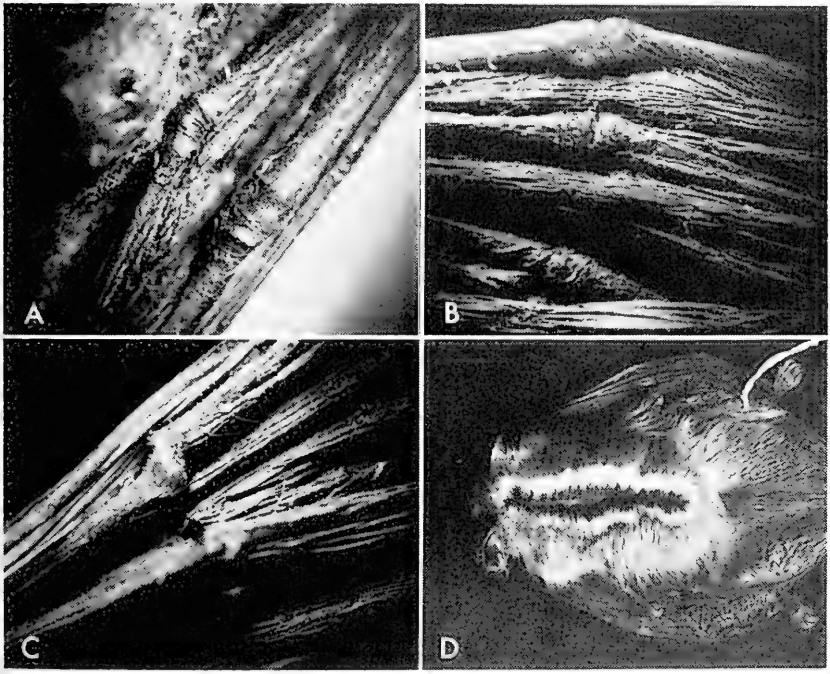
In those populations of *Pipistrellus subflavus* which winter in Vermont, Quebec and eastern Ontario young of the year can easily be separated from the older bats. The epiphyses of the young have not closed by the time they enter the caves for hibernation. Apparently no growth of the epiphyses takes place after the bats enter the caves, for young are as readily distinguished in April and May as in October. This is based upon examination of 33 study skins from the above areas. Farther south, in the caves of West Virginia and Kentucky, most young have closed epiphyses by the time they enter hibernation. A few individuals, however, are recognizable (Plate I B). This is also true of those wintering in southern Wisconsin and Minnesota.

Juvenile pipistrelles in West Virginia molt in the autumn. In the northeastern part of their range young pipistrelles go into hibernation before molting. Some of those taken from the caves of Vermont in the early winter have a juvenile pelage which is no different from that of young shot in West Virginia in August and September (e.g. Natl. Mus. nos. 296717 & 296718, from East Dorset, Bennington County, Vermont, Dec. 12, 1948). The juvenile pelage is recognizable in that it is darker, particularly the subterminal band of the hair, and the banding is not as distinctive as on the adults.

Pipistrelles wintering in Vermont are probably born in early July, and may be little over two months old when entering the caves for hibernation. At the other extreme are those pipistrelles which hibernate in the caves at Florida Caverns State Park in Jackson County, Florida. Among 14 skins examined from this population, young of the year could not be distinguished. The epiphyses are closed and the first molt has been completed in all individuals. These bats probably do not go into hibernation until late in the autumn.

Since the molt has not begun when the young pipistrelles of Vermont go into hibernation, and is completed before hibernation by

those in Florida, there should be an intermediate area in which some young have completed molting and some have not when taken from the caves in autumn. A series of 43 pipistrelles taken from the caves in Carter Caves State Park, Carter County, Kentucky, during the autumn and winter months was examined for signs of molt. Three individuals were found which apparently were in the process of molting when they entered the caves for hibernation. All of these were young of the year. One is of particular interest in that most of the juvenile pelage is still apparent, and the new fur can be seen coming in at the base of the old in the dorsoposterior region (Plate I D). The pelt in this region is unprime. That the bat was quite young when entering hibernation is evident from the epiphyses (Plate I B).



Photos by Luis de la Torre

PLATE I *Pipistrellus subflavus*

A.—Individual very young at the time of entering hibernation (Amer. Mus. No. 100161, from a cave at Rutland, Chittenden Co., Vt., Nov. 8, 1931).

B.—Young bat, somewhat older than A at the time of entering hibernation (W. G. Frum No. 1479, from Carter Cave, Carter Co., Ky., Nov. 12, 1950).

C.—Adult (W. G. Frum No. 2308, from Cave Mt. Cave, Pendleton Co., W. Va., Dec. 31, 1954).

D.—Same bat as B, showing juvenile molt in posterior region. Juvenile pelage on head and shoulders.

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