A Quantitative Study of Enzymatic Porphyrin Synthesis in Some Tissues of the Hibernating Bat.* (28659)

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It is well established that porphyrins have a widespread distribution and play a major role in metabolic processes in the animal and the vegetable kingdoms(1,2). Since previous workers(3,4) found that certain cellular enzymes have the ability to synthesize porphyrin from delta aminolevulinic acid, the presence and activity of porphyrin-producing enzymes have been determined for some tissues and organs of many animals(5). From these studies it has become apparent that the porphyrin-producing enzyme activity varies markedly from species to species and between tissues in animals of the same species. Also, there seems to be a relationship of the level of this activity with age differences, hormonal variations and other characteristics which represent striking metabolic fluctuations. It seemed logical, therefore, to initiate a study of the porphyrin-producing enzyme activity of certain tissues from an animal that normally elicits extremes in metabolic activity throughout its yearly cycle. Consequently, the hibernating bat, *Myotis lucifugus lucifugus,* was selected for this investigation. Since this animal does not feed and its metabolic rate is greatly reduced during the hibernating period(6,7,8), this study was undertaken to establish: first, the presence of porphyrin-producing enzyme and, second, the levels of this enzymatic activity in certain tissues of the bat in hibernation.

Materials and methods. This study is based on the biochemical determination of the presence of enzymes capable of producing porphyrins in muscle, liver, kidney, brain, parathyroid and thyroid tissues of 59 mature female bats *(Myotis lucifugus lucifugus)* of the family Vespertilionidae. These animals were collected in April and May from a cave in New Jersey and were maintained in hibernation in a 12″ × 14″ × 20″ dark cage at 5° ± 2°C with high humidity for periods not exceeding one month.

All animals were anesthetized with chloroform and weighed. Immediately after each animal was skinned, the tissues were removed, blotted briefly on filter paper and, where possible, 100 mg of each tissue was weighed for the analysis. All tissues were removed from each animal in the following order: *first,* the left, and part of the right, pectoralis major muscle was taken; *second,* due to the small size of the thyroid and parathyroid glands, they were removed and analyzed as one sample; *third,* the left lobe of the liver was excised; *fourth,* both kidneys were removed and the perirenal fat was stripped off; *finally,* the entire brain rostral to the inferior colliculi was taken for this study. Although the desired 100 mg of tissue was obtained for muscle, liver and brain from each animal, the thyroid plus the parathyroid glands weighed between 15-22 mg and both kidneys weighed between 75-100 mg.

Each of the above tissues, in turn, was placed in a mortar with a small amount of coarse grinding sand. To this was added 2.5 cc of 50:50 buffer-saline solution. The mixture was homogenized, rinsed from the mortar with 2.5 cc of the buffer-saline solution and centrifuged at 2400 rpm for 5 minutes. The supernatant was then utilized as the enzyme solution and 1 cc of substrate was added which consisted of a 0.01 M delta aminolevulinic acid hydrochloride buffer-saline solution. Tissues from control animals had 1.00 cc of buffered saline added in place of the delta aminolevulinic acid substrate. The buffer-saline solution was comprised of equal volumes of isotonic sodium chloride solution and a pH 7.2 buffer of sodium and potassium phosphate standardized at 20°C in 1% solution.

From each of the resulting enzyme-substrate and control solutions, 0.8 cc was

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TABLE I. Porphyrin Produced by Aqueous Extracts of Tissue Homogenates Prepared from the Little Brown Bat (*Myotis lucifugus lucifugus*).

Tissues	Tissue wt used, mg/animal	No. of animals	Avg μg of porphyrin/9.5 cc of total mixture* Hours of incubation 24	48	72
Liver	100	59	.232 ± .07	.207 ± .07	.194 ± .10
Muscle	100	58	.176 ± .04	.177 ± .04	.179 ± .04
Kidney	75–100	58	.102† ± .09	.0875† ± .03	.0926† ± .02
Brain	100	58	.0485 ± .02	.0344 ± .05	.0511 ± .03
Thyroid-parathyroid	15– 22	59	.0392† ± .05	.00547† ± .02	.0544† ± .07
Wt of animals, g*	—	59	5.84 ± .55	5.90 ± .40	5.69 ± .47

* Mean ± S.D. † Calculated on basis of 100 mg of tissue/animal.

pipetted into colorimetric tubes and the volume was brought to 3 cc by adding buffer-saline solution. Each tube was then stoppered with cellophane covered corks and incubated at 37°C for either 24, 48 or 72 hours. All 5 tissue samples from each of the 20 animals were incubated for 24 hours, tissues from 19 animals underwent incubation for 48 hours, and tissues from 20 animals were incubated for 72 hours. At the end of each of these periods, 6.5 cc of concentrated HCl were added to each tube to stop the reaction and to clear the solution. The tubes were then allowed to stand overnight at room temperature. After this period of time, the absorbance of each of the enzyme-substrate and control solutions was measured with a Beckman colorimeter. The control readings were subtracted from the enzyme-substrate readings and the resultant values were converted to the "amounts of porphyrin formed" by use of a standard curve which was based on various concentrations of hematoporphyrin hydrochloride in 25% HCl.

Results. The amount of porphyrin formed by the enzymes of each tissue is summarized in Table I. From this table, it may be seen that after 24 hours of incubation, the degree of activity in the tubes containing the prepared enzyme extracts of liver was greater than that of the other 4 tissues examined. A successive decrease in amount of enzyme activity appeared in muscle, kidney, brain and parathyroid-thyroid tissue.

For those experiments where incubation time was increased to 48 and 72 hours, the same relationship in porphyrin-producing activity existed between the tissues as in those incubated only 24 hours with the exception that during the 72 hour period the thyroid-parathyroid enzyme activity was slightly higher than for that of brain.

The variations noted in amount of porphyrin produced during the different incubation times were not statistically significant.

Discussion. The presence of porphyrin-producing enzymes in the liver, kidney, brain, muscle and thyroid-parathyroid tissues of the hibernating bat is not surprising when one considers that evidence of certain enzymatic activity has been shown for other species during the hibernating period(9,10), and that porphyrin complexes mediate many vital cellular processes in the animal kingdom(1). The greater enzymatic activity found for the liver, as compared to that for the other tissues studied, is consistent with the results of previous work done on non-hibernating rodents(5,11). The amounts of porphyrin produced by the liver extracts of the hibernating bat, however, were much lower than the constant levels reported for non-hibernating animals(5). No conclusions can be drawn concerning these similarities and differences since too many variables are present. It cannot be determined whether species difference, hibernation and/or fasting are the factors responsible for the observation that tissue extracts of the hibernating bat display an apparent lower capacity to convert delta aminolevulinic acid to porphyrin than do the extracts of non-hibernating animals.

Although a low activity of porphyrinogenic enzymes from brain extracts has been reported consistently for non-hibernators(5), the low enzymatic activity of the brain and thyroid-parathyroid tissues of the hibernating bat may be due, in part, to several factors

associated with the process of hibernation. It is generally agreed that most endocrine organs are involuted during the hibernating period and the thyroid gland, especially, demonstrates many regressive histological changes during this period(12). Thus, the same factors which lead to a general decrease in the activity of the thyroid gland during hibernation(13) may have directly, or indirectly, effected the low porphyrinogenic enzyme activity observed in the bat. Also, it has been shown that there is a 90% reduction in the amplitude of the general activity of the brain in other animals in deep hibernation(14). If this great reduction in activity holds true for the bat, then it is possible that very low porphyrinogenic enzyme activity is also related to the hibernating state.

The effects of chloroform-killing of bats should be considered, however, since this method usually initiates the arousal process immediately before the animal dies. This probably leads to a slight increase in temperature and activity in the brain since Lyman(15) has shown in other hibernators that the anterior portion of the animal warms more rapidly and has a greater increase in blood circulation than the posterior region during the process of arousal. It is questionable therefore, if the values of porphyrinogenic enzymatic activity of bat brain tissues are true values for the deep hibernating state.

The results of this study emphasize the need for further investigation of porphyrin production in the active bat as compared with that found in the hibernating bat.

Summary and conclusions. 1. A quantitative study was undertaken to determine if porphyrin producing enzymes were present in the liver, muscle, kidney, brain and thyroid and parathyroid glands of the hibernating bat, *Myotis lucifugus lucifugus*. 2. Porphyrin producing enzymes were found to be present in the liver, muscle, kidney, brain and thyroid-parathyroid glands of the hibernating bat, *Myotis lucifugus lucifugus*. 3. The degree of enzymatic porphyrin activity was greatest in the liver with a successive decrease in activity found in muscle, kidney, brain and thyroid-parathyroid tissue. 4. The variations noted in the amount of porphyrin produced during different incubation times were not statistically significant. 5. A comparison was made between the amounts of porphyrin production by tissues of the hibernating bat and by that produced in non-hibernating animals. No conclusion was made concerning the significance of this comparison.

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