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Abstract approved: Katherine N. Smalley

A study of seasonal changes in metabolic activity and energy reserves in the frog, Rana pipiens, was conducted. Values for oxygen consumption, and carbon dioxide production were collected and used to indicate changes in the metabolism of the frogs' energy reserves. In contrast to the results of other investigators, respiratory quotients indicating a change to carbohydrate metabolism for winter and spring animals were never obtained in this study.

One of the purposes of this study was to investigate the possibility of pentose shunt activity during carbohydrate metabolism. Animals were injected with glucose-1-C^{14} and glucose-6-C^{14} to test for pentose shunt activity in February, April, May and June. An increase in pentose shunt activity was found only during the month of April. It was concluded that the increased pentose shunt activity was probably caused by a combination of fasting and the onset of the reproductive activity of the animals during March and April. The failure to obtain values indicative of carbohydrate metabolism for O_2 consumption, CO_2 production, and R.Q.'s was possibly due to the maintenance of the animals within the laboratory, the juvenile age at which the animals were obtained, and because Tetracycline-HCl was used for the treatment of diseased frogs.

Seasonal Metabolic Changes in the Frog, *Rana pipiens*

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INTRODUCTION

The Russian physiologist Bykov stated, "Physiology of man and animals is the study of function of human and animal organisms in their interaction with the environment". An understanding of function brings with it the realization that life is a dynamic system which involves continual interchanges between the organism and its environment.

Many organisms adjust their "functions" according to environmental changes. These functional changes may be in behavioral actions or in the physiology of the organism. There are many examples of such functional adaptations in the animal world, each being unique to a particular situation. The common leopard frog, *Rana pipiens*, has been observed to make various adaptations according to season.

Holzapfel (1937) studied the effects of temperature, food and moisture on the behavior of *Rana pipiens*. She found that winter-hibernating frogs were in a state of torpor. The animals usually buried themselves in the mud and existed in a lethargic condition. Jones (1968) also found that only winter *R. pipiens* would bury themselves in the mud during low temperature acclimation in the laboratory. It was found (Holzapfel, 1937) that frogs only became torpid in the cold between October and April. Cold temperatures during the remainder of the year caused the frogs to become sluggish but not completely torpid.

Food did not change the observed behavior (Holzapfel, 1937). Frogs kept in the cold during the winter months refused to eat. Autopsies indicated the stomach contents of control frogs maintained at room temperature were negligible between the months of October and February, even when the frogs had access to live food. Holzapfel (1937) also observed that moisture did not influence emergence from hibernation in *R. pipiens*.

Mizell (1965) conducted a study on energy reserves in *R. pipiens*. Changes in blood glucose levels, liver glycogen content and fat body weights were observed for one year. From this study, Mizell concluded that metabolic changes occur in the frog throughout the year. He made this conclusion from the cycling pattern observed in the energy stores.

Blood sugar levels were found to vary seasonally. They were found to be highest in the summer (June through August) and fall (September through November), when the frogs were stated to be "active". Winter (December through February) and spring (March through May) frogs showed low blood sugar levels (Mizell, 1965). After a minimum in May, the blood sugar levels increased rapidly until maximizing in July. Blood sugar levels remained high until late fall when they began to decrease gradually through the year, reaching their minimum again in May.

Blood glucose levels in frogs have been reported to vary from species to species (Farrar and Frye, 1979). Also, within a certain species, they fluctuate according to season (Smith, 1950; Mizell, 1965; Byrne and White, 1975). Recent work by Farrar and Frye (1979) indicates that some of the wide variation in blood glucose levels obtained in previous studies may have been attributed to handling and measuring techniques. However, they agreed that differences occur with season in blood glucose as well as other energy reserves in the frog.

Mizell (1965) observed that liver glycogen levels were lowest in May and remained low through the summer. A rapid increase in liver glycogen content was noted in September. Liver glycogen reached its highest level in November, then gradually decreased until spring, reaching its lowest value again in May. Mizell found liver glycogen levels for each season to be significantly different from each other.

Fat body weights observed by Mizell (1965) were at their minimum in June. A rapid increase was found during July, maximizing in August. During the months of September and October, a rapid decrease in fat body weights occurred. A gradual decrease in fat body weight continued from November through the winter and spring. Fat body weights were found to be significantly different from one another according to season.

Smith (1950) made a similar study on energy reserves in *Rana temporaria*. However, Smith included changes in liver and gonadal weights. The same seasonal cyclic changes in energy reserves noted by Mizell (1965) were observed by Smith. Smith found that as liver weights increased or decreased, liver glycogen levels also increased or decreased in a parallel fashion.

The decrease in blood sugar and liver glycogen content described by Mizell (1965) and Smith (1950) appeared during the winter months. This is a period of inactivity for the frog, and the animals do not feed during this time. In order to live, therefore, the frog must utilize energy stores for metabolism. Glycogen is the most readily available source. The continued decrease in liver glycogen stores along with the decrease in blood sugar during the winter months, both becoming minimal in May, would indicate the animals are utilizing glycogen at this time. Liver glycogen and blood glucose both increase during the active summer and fall months when the frogs feed, thus replenishing glycogen levels in the liver before the onset of hibernation.

Fromm and Johnson (1955) measured oxygen consumption, carbon dioxide production, and respiratory quotients in *Rana pipiens* throughout the

year. They concluded from the respiratory quotients that frogs metabolize carbohydrates during the winter, but change to fat-based metabolism for the summer.

Jungreis and Hooper (1970) studied the effects of starvation and temperature acclimation on glucose regulation and nitrogen anabolism in winter *R. pipiens*. Frogs were starved for up to 27 days at temperatures of 4, 20 and 25 C. The concentrations of blood glucose and serum amino acids were measured and found to remain relatively constant. However, it was observed that the concentrations of serum amino acids and blood glucose were related in such a manner that an increase in one resulted in an increase in the other. The input of amino acids into the serum occurs from the turnover of proteins, which at a constant temperature and in the presence of large glucose pools, should occur at a fairly uniform rate. They suggested that in the presence of large glycogen stores in the winter, as found by Smith (1950) and Mizell (1965), the rate of gluconeogenesis from amino acids should be minimal. Therefore, Jungreis and Hooper (1970) theorized that the high degree of "consistency" in blood glucose indicated metabolism of carbohydrates by winter frogs. They suggested that the frogs might exhibit high pentose phosphate shunt activity during this time of the year. It was thought that the pentose shunt would give the frogs large amounts of reducing power in the form of nicotinamide adenine dinucleotide phosphate (NADPH) which could be used for biosynthetic processes such as triglyceride synthesis.

The frogs might try to maintain or increase fat reserves during the winter with the biosynthetic ability of the pentose shunt. Brenner (1969) stated that the fat body plays an important role in amphibian metabolism

upon emergence from hibernation and during the spring breeding season. Athanasiu and Dragoiu (1910, cited by Seymour, 1973) found that winter frogs had a greater amount of fat in their skeletal muscles than summer frogs. This suggests that the animals are storing triglycerides to some extent while in hibernation. Also, the ribose molecules produced by the pentose shunt would be important for the synthesis of nucleic acids. Therefore, the biosynthetic ability of the pentose phosphate shunt is probably more important than high energy yields during the winter since the animal is torpid.

Jungreis (1970) conducted a study similar to that of Jungreis and Hooper (1970), but for summer *R. pipiens*. Frogs were starved for 14 and 8 days at 4 and 25 C, respectively. These animals showed excellent regulation of serum glucose and serum amino acid concentrations. Changes in blood glucose levels were independent of changes observed in serum amino acids. Jungreis (1970) stated that this was the expected result for summer animals since the concentration of amino acids in the serum would be dependent on the rate of gluconeogenesis and the rate of fatty acid synthesis.

Urea output, a measurement of urea biosynthesis and protein catabolism, was inversely correlated with blood glucose levels. This suggested to Jungreis (1970) that amino acids were being converted to blood glucose at times when reduced glycogen to glucose conversion occurred.

Jungreis (1970) used his observations of summer *R. pipiens* along with those of other workers (ie., Smith, 1950; Mizell, 1965) to suggest that frogs metabolize triglycerides primarily during the summer. This was thought to be true since glycogen stores are minimal and fat stores maximal in the summer.

MATERIALS AND METHODS

Animals

Source and General Maintenance. Sixty, young Northern Leopard Frogs, *Rana pipiens*, weighing an average of 8.4 g were obtained on July 28, 1978 from William A. Lemberger Associates, Germantown, Wisconsin. An additional twenty-two animals, with an average weight of 11.3 g, were collected locally from a small pond near Hartford, Kansas, on August 30, 1978. The frogs were kept in large covered tanks supplied with running tap water at 20 ± 2 C. Tanks were illuminated by fluorescent lights on a 14:10 L:D photoperiod. Frogs were maintained on a diet of earthworms, mealworms, and crickets.

Records of each frog's weight and treatment were maintained throughout the study by using the system described by Nace *et al*., 1973. Experimental results were collected without regard to sex.

Health. Frogs which had become ill with "red leg", as well as animals within the same confinement, were treated with Tetracycline-HCl prepared to a concentration of 25 mg ml^{-1}. Animals were administered 0.2 ml of this solution by stomach tube twice daily for five to seven days (Gibbs, 1963).

Experimental Design

The frogs were divided into experimental groups, Group I and Group II, on December 22, 1978. Animals within these groups were tested weekly for any changes in overall metabolism as noted by Fromm and Johnson (1955). Both groups were fed weekly. Animals acclimated to the cold (7 ± 2 C) were allowed to feed at room temperature.

Group I

Frogs were maintained in holding tanks in the laboratory.

Temperature was kept at 20$\pm$2 C (warm) throughout the study. Daylengths of 14:10 L:D (long day) were provided by fluorescent lights.

Group II

Frogs were placed in covered glass aquaria. One end of each aquarium was filled with sand which provided the frogs with a terrestrial habitat. The aquaria were placed in Sherer environmental chambers at 7$\pm$2 C. Daylength was maintained at 14:10 L:D with fluorescent lights.

From March 4, 1979 to May 26, 1979, frogs were maintained in the following groups (Groups III, IV, V, VI) in which photoperiod was manipulated as well as temperature. Since animals maintained in the cold refused to eat, all groups were not fed during this period.

Group III

Frogs were maintained in holding tanks in the laboratory at 20$\pm$2 C and on 14:10 L:D photoperiods.

Group IV

Frogs were placed in covered glass aquaria as explained for Group II. These animals were maintained at 7$\pm$2 C and on photoperiods of 14:10 L:D.

Group V

These animals were placed in covered glass aquaria as explained for Group II, except they were maintained at 20$\pm$2 C and on 8:16 L:D (short day) photoperiods.

Group VI

Frogs were placed in covered glass aquaria as explained for Group II, except they were maintained at 7$\pm$2 C and 8:16 L:D photoperiods.

From May 25 to June 22, frogs were allowed to resume feeding, and

kept in the following group.

Group VII

All frogs were placed in holding tanks in the laboratory and maintained at 20$\pm$2 C and on 14:10 L:D photoperiods.

Table I summarizes the conditions for each group.

Metabolism

In vivo Measurement of Glucose. In order to investigate the possibility of increased pentose shunt activity, frogs were injected with C^{14} glucose labeled in either the one or six position. The labeled glucose was obtained from New England Nuclear. If the pentose shunt was being used at an increased rate, more $^{14}CO_2$ would be obtained from animals injected with glucose-1-C^{14} than animals injected with glucose-6-C^{14}. The labeled glucose molecules were made up in volumes containing 0.5 microcuries per milliliter (µCi/ml) with a specific activity of 0.17 µCi/µmole glucose. Unlabeled glucose was added to make the total concentration of glucose in solution to be 50 mg per cent. The unlabeled glucose was added to make the solution have a concentration similar to that maintained in the animals blood throughout the year (Mizell, 1965).

Preliminary experiments were conducted to determine the amount of labeled solution to be injected per animal. It was determined that by injecting 100 µl/15 g body weight into the dorsal lymph sac, enough counts could be obtained to permit analysis of the data.

Animals maintained in the cold were allowed to sit at room temperature over night before being injected the following morning. Following injection, frogs were placed in metabolism chambers and allowed to acclimate for 90 minutes before samples were collected. Each chamber

TABLE I. Experimental Grouping of Animals

Group	Period of Acclimation	Habitat	Photo-period (L:D)	Temperature (C)	Diet
I	12/22/78 to 3/ 4/79	Holding Tank[1]	14:10	20 ± 2	Fed Weekly
II	12/22/78 to 3/ 4/79	Chamber[2]	14:10	7 ± 2	Fed Weekly
III	3/ 4/79 to 5/26/79	Holding Tank	14:10	20 ± 2	Not Fed
IV	3/ 4/79 to 5/26/79	Chamber	14:10	7 ± 2	Not Fed
V	3/ 4/79 to 5/26/79	Chamber	8:16	20 ± 2	Not Fed
VI	3/ 4/79 to 5/26/79	Chamber	8:16	7 ± 2	Not Fed
VII	5/26/79 to 7/12/79	Holding Tank	14:10	20 ± 2	Fed Weekly

1 Holding Tank - animals were maintained in a large covered stock tank.

2 Chamber - animals were maintained in glass aquaria placed in an environmental chamber.

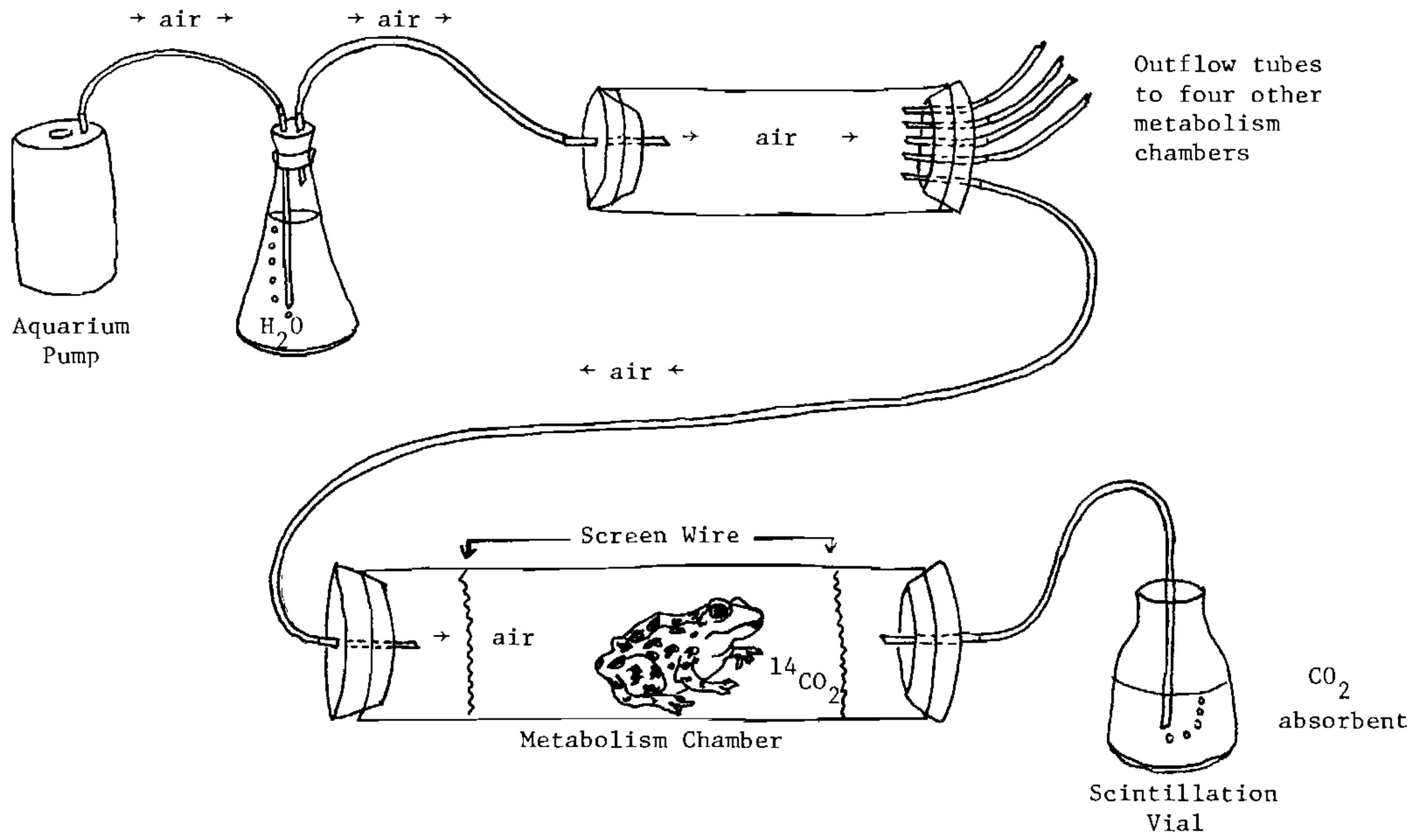
contained a moist piece of paper towel. This helped to keep the animal moist throughout the experiment.

The metabolism chambers were prepared from one and one-half inch diameter polyvinyl chloride tubes, seven inches in length (Figure 1). The tubes were closed at both ends with rubber stoppers. Each stopper was provided with a tube which ran through it. This system allowed air to flow through the metabolism chamber. Moist air was passed through a manifold connected to five metabolism chambers. The air passed through the chamber, over the frog, and out the outflow tube where it was collected in a plastic scintillation vial containing six ml of CO_2 absorbent (80:20; methanol:ethanolamine; v:v). During the experimental runs, the metabolism chambers were placed in a 21 ± 2 C waterbath.

After collection, approximately 10 ml of toluene based scintillation fluid (1 gallon toluene, 16g POP, 200 mg POPOP) was added to the six ml of CO_2 absorbent. Methanol was added as needed to make a one phase solution. The samples were analyzed in a Nuclear-Chicago Unilux II Spectrometer. All samples were allowed to sit in the spectrometer overnight before counting. Each sample was counted for 20 minutes. Then, an internal standard (C^{14} toluene) was added to each vial, and the samples were recounted for 20 minutes. This was done to standardize the data and correct for quenching.

In vivo Measurement of Palmitic Acid. In March and May, frogs were injected with C^{14} palmitic acid obtained from New England Nuclear to determine the effect of temperature on lipid metabolism. Frogs were injected in the dorsal lymph sac with 100 µl/15 g body weight of a stock palmitic acid solution containing 1µCi/ml with a specific activity of 10 µCi/µmole.

Figure 1. Apparatus used to collect $^{14}CO_2$ from animals injected with labeled substrates.


→ air →
→ air →
Outflow tubes to four other metabolism chambers
→ air →
Aquarium Pump
H_2O
← air ←
Screen Wire
→ air
$^{14}CO_2$
CO_2 absorbent
Metabolism Chamber
Scintillation Vial

The palmitic acid was made up in a solution of one per cent bovine serum albumen. Data were collected using the same methods as for glucose metabolism.

Data Analysis. After the samples of $^{14}CO_2$ were standardized by adding a known amount of C^{14} toluene, the counts obtained for each sample were converted to disintegrations per minute. Using the specific activity of the injected material, the amount of labeled glucose or palmitic acid metabolized could be calculated (see Appendix I). These results were then calculated as μmoles substrate metabolized hr^{-1} g^{-1}. Each animal was tested for three 90 minute intervals for a total of 270 minutes. The results were pooled and averaged. Prior to the injection, a collection was taken to determine the amount of C^{14} carbon dioxide remaining in the frog from previous experiments. The residual amounts of C^{14} carbon dioxide were subtracted from the results. A Student's t-test (p=0.05) was used to determine significance of the results.

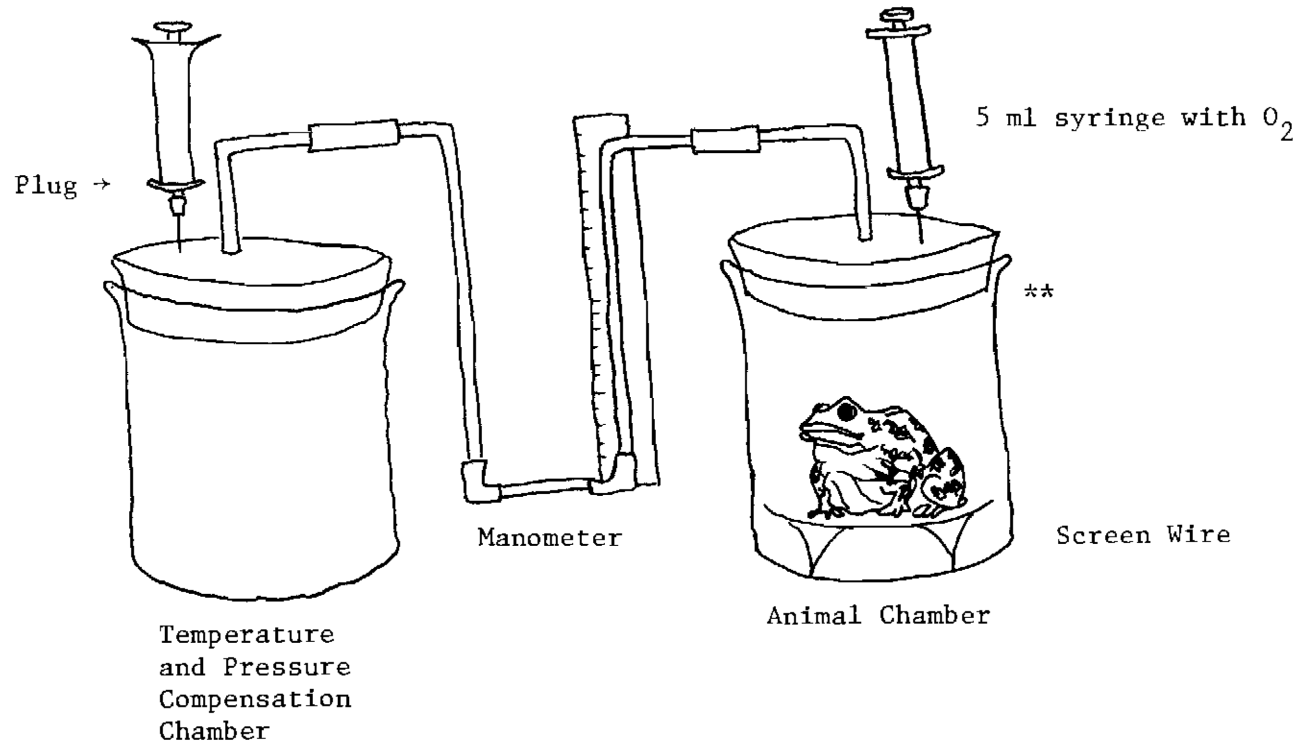
Respiratory Quotients

Respiratory quotients (RQ) were measured by using closed-system respirometers (Jones, 1970). The respirometer chambers (Figure 2) were covered with dark plastic to prevent disturbance of the frogs. All respirometers were placed in a 21 ± 2 C waterbath. A moistened piece of filter paper was placed in the bottom of the animal chambers to maintain humidity. Animals were placed in the respirometers for at least one hour before collecting data. Frogs maintained in the cold were allowed to stay at room temperature over night before taking measurements.

Respiratory quotients were calculated by measuring carbon dioxide production and oxygen consumption. Carbon dioxide production was

Figure 2. Respirometers used to measure oxygen consumption and CO_2 production of the frogs.

**The animal chambers were covered with dark plastic, and the whole apparatus was placed in a water bath.



5 ml syringe with O_2
Plug →
**
Manometer
Screen Wire
Animal Chamber
Temperature and Pressure Compensation Chamber

determined before oxygen consumption was measured on each animal. CO_2 production and O_2 consumption were each measured separately for 15 minutes.

Measurement of Oxygen Consumption. Carbon dioxide absorbant (3 ml of 30% KOH, w:v) was placed in the bottom of each animal chamber. The chambers were then sealed and pressure changes within the system were noted on the manometer (Figure 2) using Sudan Black in kerosene as the manometer fluid. Oxygen was added to the chambers in three 5 minute intervals to replace the O_2 consumed by the frog. The volume of oxygen added each interval was measured by using a 5 ml syringe.

Measurement of Carbon Dioxide Production. Carbon dioxide production was measured by performing the same experiment as for measurement of oxygen consumption, except CO_2 absorbant was not placed in the chambers. The amount of oxygen added to the chambers during this experiment was subtracted from the amount of oxygen added with absorbant in the chamber. The difference in these two volumes gave the amount of carbon dioxide produced.

Data Analysis. All values of CO_2 production and O_2 consumption were calculated as ml hr^{-1} g^{-1} at STP. A Student's t-test ($p=0.05$) was used to test significance.

Organ Weights

Throughout the course of this experiment, autopsies were performed on all animals lost due to experimental procedures and illness, but no animals were purposely sacrificed to obtain these data. Livers, gastrocnemius muscles, gonads, and fat bodies were all removed from the frogs. These organs were weighed on a Sartorius analytical balance. Records were maintained on the weights of the organs, date of autopsy and cause of death.

RESULTS

In vivo Metabolism of Glucose

Between February 22 and March 2, 1979, 31 frogs were tested to determine the effect of temperature on pentose shunt activity by injecting the frogs with labeled glucose. The results from this experiment are summarized in Table II. Statistical analyses were determined on the utilization of glucose-1-C^{14} between animals acclimated at warm (20 ± 2 C) and cold (7 ± 2 C) temperatures. The same analyses were determined for frogs injected with glucose-6-C^{14} and acclimated at warm and cold temperatures. Analyses also were made between the utilization of glucose-1-C^{14} and glucose-6-C^{14} in animals acclimated at the different temperatures. No significant differences were found to occur.

Identical experiments were conducted using 21 frogs between April 17 and April 21, 1979. These experiments were designed to test the effects of temperature and photoperiod on pentose shunt activity. The results are listed in Table III. Neither temperature nor photoperiod caused a significant difference in utilization of C-1 or C-6 glucose among the different groups of animals. The animals were then grouped according to whether they received either the C-1 or C-6 labeled glucose. The amount of glucose utilized (mean$\pm$S.E.M.) for animals injected with ^{14}C-1 glucose was 4.23 ± 0.34 µmole hr^{-1} $g^{-1} x10^{-4}$. This value is significantly different ($p \leq 0.05$) than the mean for animals injected with ^{14}C-6 glucose (2.71 ± 0.33 µmole hr^{-1} $g^{-1} x10^{-4}$), indicating that more C-1 labeled glucose was being used. This suggested that perhaps higher pentose phosphate shunt activity was taking place in glucose metabolism during April than during February.

TABLE II. *In vivo* Utilization of C-1 and C-6 labeled Glucose during the Month of February by Frogs Acclimated to Temperature

Group	Temperature (C)	Photo-period (L:D)	# of Animals	Glucose Isotope	μ Moles Glucose[1] Utilized hr^{-1} g^{-1} X 10^{-4}
I	20 ± 2	14:10	8	C-1	2.43 ± 0.34
I	20 ± 2	14:10	6	C-6	1.69 ± 0.26
II	7 ± 2	14:10	8	C-1	2.12 ± 0.49
II	7 ± 2	14:10	7	C-6	2.15 ± 0.30

1 Values represent the mean ± standard error of the mean.

TABLE III. <u>In vivo</u> Utilization of C-1 and C-6 labeled Glucsoe during the Month of April by Frogs Acclimated to Temperature and Photoperiod

Group	Temperature (C)	Photo-period (L:D)	# of Animals	Glucose Isotope	μ Moles Glucose[1] Utilized hr^{-1} g^{-1} X 10^{-4}
III	20±2	14:10	4	C-1	4.12±0.80
III	20±2	14:10	4	C-6	3.07±0.18
V	20±2	8:16	5	C-1	4.45±0.65
V	20±2	8:16	4	C-6	2.43±0.52
IV	7±2	14:10	3	C-1	3.69±0.62
IV	7±2	14:10	1	C-6	1.55
VI	7±2	8:16	2	C-1	4.74±0.70
VI	7±2	8:16	5	C-6	2.87±0.84

1 Values represent the mean ± standard error of the mean.

The effect of temperature and photoperiod on metabolism was tested again between May 4 and May 11, 1979. Table IV summarizes the results from this experiment. Due to a substantial loss of animals, it was not possible to statistically analyze the data in all conditions. There was no significant difference in the utilization of C-1 and C-6 labeled glucose between the groups containing more than one animal. Also, when the animals were grouped as to those receiving C-1 and C-6 labeled glucose, regardless of acclimation, no significant difference in the utilization of these glucose isotopes was found.

Feeding was resumed on May 25 and the animals were tested again between June 28 and July 9, 1979. Since a change to carbohydrate metabolism was not demonstrated by temperature or photoperiod during the months of February and previous months of study, the animals were all acclimated at 20 ± 2 C and placed on 14:10 L:D photoperiods. The results from this study are shown in Table V. There was no significant difference in the utilization of C-1 versus C-6 labeled glucose in the animals.

Since temperature and photoperiod did not produce an effect on carbohydrate metabolism during February, April, and May, temperature and photoperiod were ignored, and the animals were grouped into those receiving C-1 and those receiving C-6 labeled glucose according to the month of study. The results are shown in Figure 3. April was the only month that the frogs demonstrated a significantly greater utilization of C-1 labeled glucose than C-6 labeled glucose. There also was significantly more C-1 glucose utilized in April than in either February or May.

Measurement of Metabolism by Respiratory Quotient

Oxygen consumption and carbon dioxide production of the frogs were

TABLE IV. *In vivo* Utilization of C-1 and C-6 labeled Glucose during the Month of May by Frogs Acclimated to Temperature and Photoperiod.

Group	Temperature (C)	Photo-period (L:D)	# of Animals	Glucose Isotope	μ Moles Glucose[1] Utilized hr^{-1} g^{-1} X 10^{-4}
III	20$\pm$2	14:10	4	C-1	1.13$\pm$0.39
III	20$\pm$2	14:10	4	C-6	2.15$\pm$0.22
V	20$\pm$2	8:16	1	C-1	3.04
V	20$\pm$2	8:16	3	C-6	2.64$\pm$0.78
IV	7$\pm$2	14:10	1	C-1	4.78
IV	7$\pm$2	14:10	1	C-6	1.21
VI	7$\pm$2	8:16	4	C-1	2.80$\pm$0.92
VI	7$\pm$2	8:16	3	C-6	4.23$\pm$0.72

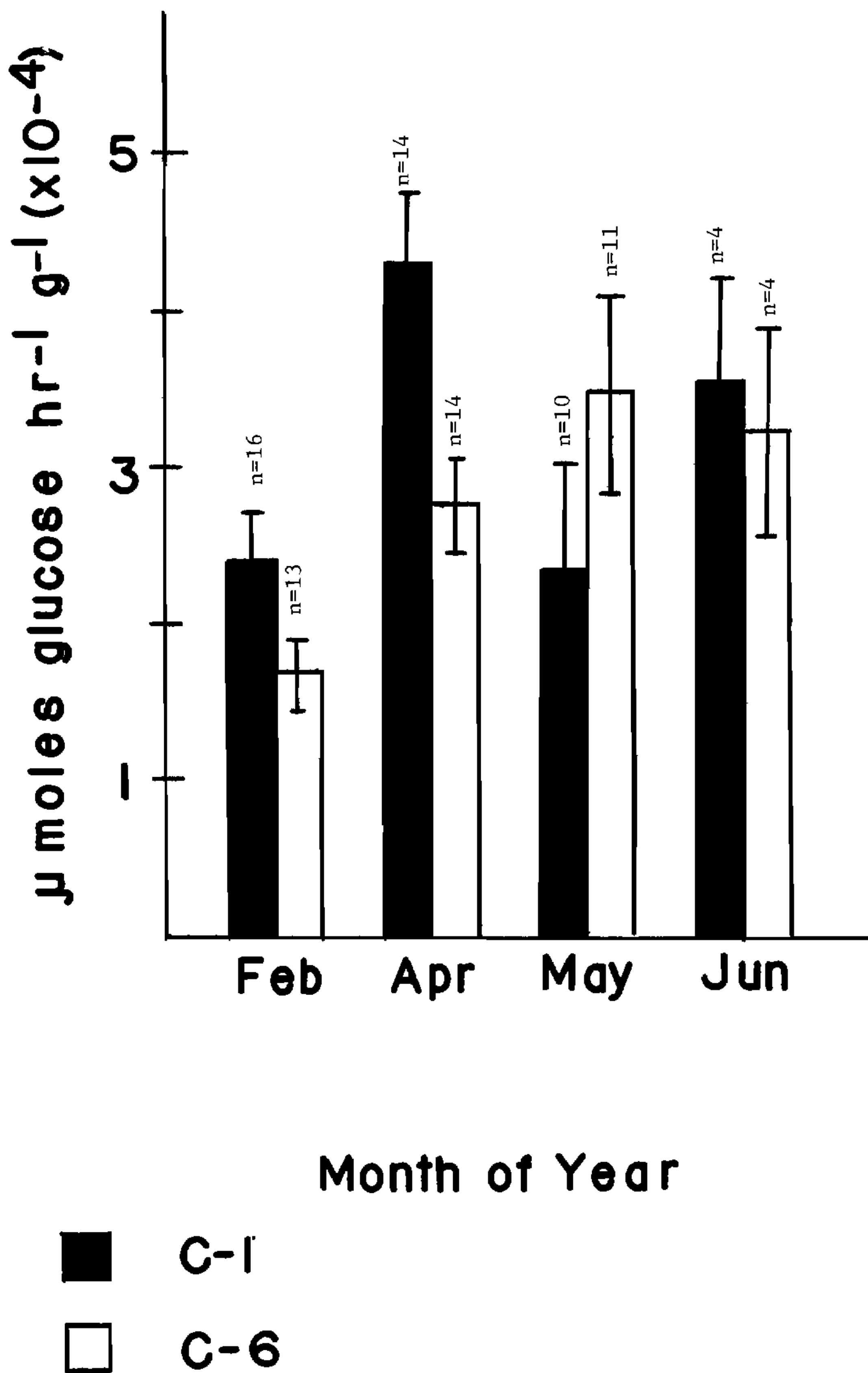
1 Values represent the mean $\pm$ standard error of the mean.

TABLE V. *In vivo* Utilization of C-1 and C-6 labeled Glucose during the Month of June by Frogs Acclimated to Warm Temperatures on 14:10 L:D Photoperiod

Group	Temperature	Photo-period (L:D)	# of Animals	Glucose Isotope	μ Moles Glucose[1] Utilized hr^{-1} g^{-1} X 10^{-4}
VII	20±2	14:10	4	C-1	3.52±0.70
VII	20±2	14:10	4	C-6	2.71±0.71

1 Values represent the mean ± standard error of the mean.

igure 3. Utilization of injected glucose-1-C^{14} and glucose-6-C^{14} molecules by the frogs. Values represent the mean $\pm$ standard error of the mean for all animals tested that month.


µ moles glucose hr-l g-l (x10-4)
5
3
1
n=16
n=13
n=14
n=14
n=10
n=11
n=4
n=4
Feb
Apr
May
Jun
Month of Year
C-1
C-6

measured throughout the course of this investigation. These values were used to determine the respiratory quotient (RQ) of the animals.

Between February 22 and March 2, 1979 oxygen consumption and carbon dioxide production were measured to test what effect temperature acclimation would have on overall metabolism in the frogs. Table VI summarizes the results of these experiments. No significant differences were found between those animals acclimated to warm temperatures and those acclimated to cold temperatures. Likewise, temperature did not produce significant differences in oxygen consumption or carbon dioxide production between animals receiving the C-1 and C-6 labeled glucose.

Experiments conducted in April were designed to demonstrate the effects of temperature and photoperiod on metabolism. The results are summarized in Table VII. There were no significant differences among any of the groupings.

Table VII shows the results from experiments conducted in May. There were not enough surviving animals acclimated to cold conditions to study the effect of photoperiod on metabolism of cold acclimated frogs. Again, in May there were no significant differences among any of the groupings.

The surviving animals from all previous studies were placed at 20 ± 2 C and on 14:10 L:D photoperiods on May 25. These animals were tested between June 28 and July 9, 1979. The results from this study are shown in IX.

Statistical comparisons were made between animals tested during each month of study. Animals acclimated at warm (20 ± 2 C) temperatures had a higher rate of CO_2 production in June than in any other month

TABLE VI. Respiratory Quotients for the Month of February for Frogs Acclimated to Temperature

Group	Temp. (C)	Photo-period (L:D)	# of Animals	Glucose Label	ml. O_2 Consumed hr^{-1} gm^{-1} [1]	ml. CO_2 Produced hr^{-1} gm^{-1} [1]	RQ [1]
I	20$\pm$2	14:10	8	C-1	0.219$\pm$0.028	0.175$\pm$0.025	0.79$\pm$0.06
I	20$\pm$2	14:10	10	C-6	0.203$\pm$0.037	0.132$\pm$0.020	0.69$\pm$0.06
II	7$\pm$2	14:10	7	C-1	0.179$\pm$0.021	0.149$\pm$0.026	0.82$\pm$0.07
II	7$\pm$2	14:10	6	C-6	0.163$\pm$0.01	0.129$\pm$0.010	0.78$\pm$0.04
All Warm Frogs			18		0.210$\pm$0.023	0.151$\pm$0.016	0.73$\pm$0.04
All Cold Frogs			13		0.172$\pm$0.12	0.140$\pm$0.014	0.80$\pm$0.04
All Frogs			31		0.194$\pm$0.015	0.175$\pm$0.029	0.76$\pm$0.03

1 Values represent the mean $\pm$ standard error of the mean.

TABLE VII. Respiratory Quotients for the Month of April for Frogs Acclimated to Temperature and Photoperiod

Group	Temperature (C)	Photo-period (L:D)	# of Animals	ml. O_2 Consumed hr^{-1} g^{-1} [1]	ml. CO_2 Produced hr^{-1} g^{-1} [1]	RQ[1]
V	20±2	8:16	9	0.226±0.032	0.183±0.024	0.82±0.04
III	20±2	14:10	7	0.165±0.015	0.133±0.016	0.81±0.07
VI	7±2	8:16	10	0.277±0.037	0.197±0.022	0.73±0.05
IV	7±2	14:10	4	0.238±0.022	0.175±0.016	0.74±0.02
All Warm Frogs			16	0.200±0.020	0.161±0.016	0.82±0.03
All Cold Frogs			14	0.266±0.027	0.190±0.016	0.73±0.03
All Frogs			30	0.230±0.017	0.175±0.012	0.78±0.03

1 Values represent the mean ± standard error of the mean.

TABLE VIII. Respiratory Quotients for the Month of May for Frogs Acclimated to Temperature and Photoperiod

Group	Temperature (C)	Photo-period (L:D)	# of Animals	ml. O_2 Consumed hr^{-1} g^{-1} [1]	ml. CO_2 Produced hr^{-1} g^{-1} [1]	RQ[1]
V	20±2	8:16	3	0.233±0.061	0.143±0.026	0.66±0.07
III	20±2	14:10	3	0.153±0.024	0.077±0.027	0.63±0.04
All Warm Frogs			6	0.193±0.034	0.110±0.023	0.65±0.04
All Cold Frogs			5	0.184±0.024	0.140±0.025	0.76±0.09
All Frogs			11	0.189±0.021	0.124±0.017	0.70±0.05

1 Values represent the mean ± standard error of the mean.

TABLE IX. Respiratory Quotients for the Month of June for Frogs Acclimated at 20±2 C and on 14:10 L:D Photoperiods

Group	Temperature (C)	Photo-period (L:D)	# of Animals	ml. O_2 Consumed hr^{-1} gm^{-1} [1]	ml. CO_2 Produced hr^{-1} g^{-1} [1]	RQ[1]
VII	20±2	14:10	14	0.257±0.024	0.209±0.023	0.81±0.04

1 Values represent the mean ± standard error of the mean.

(Figure 4). This rate was significantly higher than that found in February or May. The RQ value for frogs acclimated at warm temperatures (20 ± 2 C) was significantly higher in June, 0.81 ± 0.04 (mean$\pm$S.E.M.), than in February, 0.73 ± 0.04, or May 0.65 ± 0.04.

Animals acclimated to cold temperatures (7 ± 2 C) exhibited statistical differences only between February and April. Oxygen consumption in February, 0.172 ± 0.012 ml hr^{-1} g^{-1} (mean$\pm$S.E.M.), was lower than that of frogs in April, 0.266 ± 0.027 ml hr^{-1} g^{-1}. Carbon dioxide production also was lower in February, 0.140 ± 0.014 ml hr^{-1} g^{-1}, than in April, 0.190 ± 0.016 ml hr^{-1} g^{-1}. There were no significant differences, however, among the RQ values.

Statistical comparisons were then made between each month of study using all the animals regardless of the temperature of acclimation. Frogs consumed significantly more oxygen ($p \leq 0.05$) in June than in either February or May (Figure 5). Carbon dioxide production was higher in June than during any other month (Figure 5). This rate was significantly higher than that found in February or May. Frogs also produced significantly more CO_2 during April than in May (Figure 5). No significant differences were found to occur among the RQ values for these groups of animals (Figure 6).

Organ Weights

Figure 7 illustrates the changes in liver and fat body as per cent total body weight. The change in muscle size is shown in Figure 8. Changes in gonad size are shown in Table X.

In vivo Metabolism of Palmitic Acid

Animals were studied between March 12 and 13, and again between May 24 and 27 for the effect of temperature on lipid metabolism. Table XI

Figure 4. Oxygen consumed and carbon dioxide produced by warm (20±2 C) acclimated frogs. Values represent the mean ± standard error of the mean for each month.

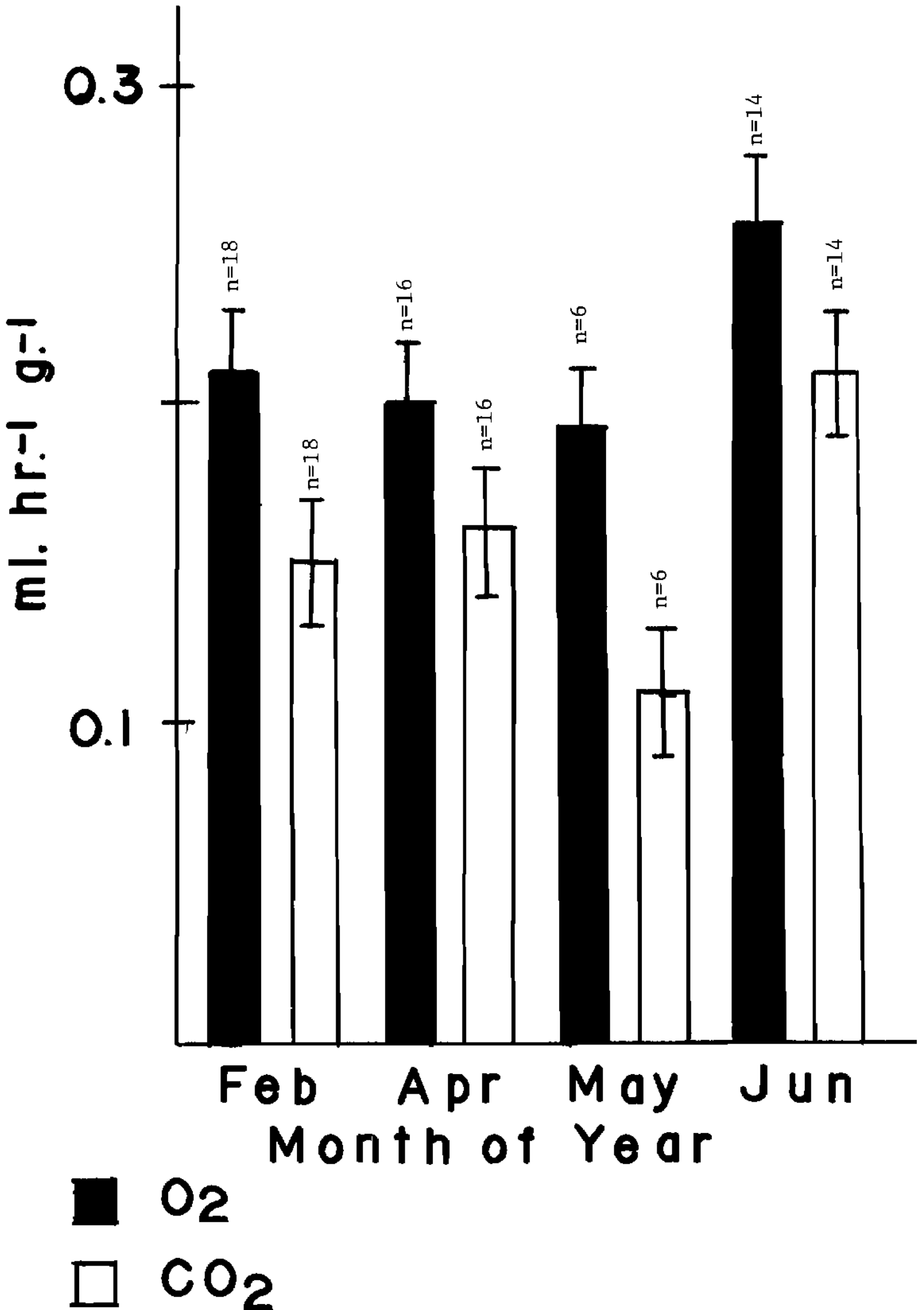

0.3
0.1
ml. hr.-1 g-1
n=18
n=18
n=16
n=16
n=6
n=6
n=14
n=14
Feb
Apr
May
Jun
Month of Year
O2
CO2

Figure 5. Oxygen consumed and carbon dioxide produced by all frogs tested each month. Values represent the mean ± standard error of the mean.

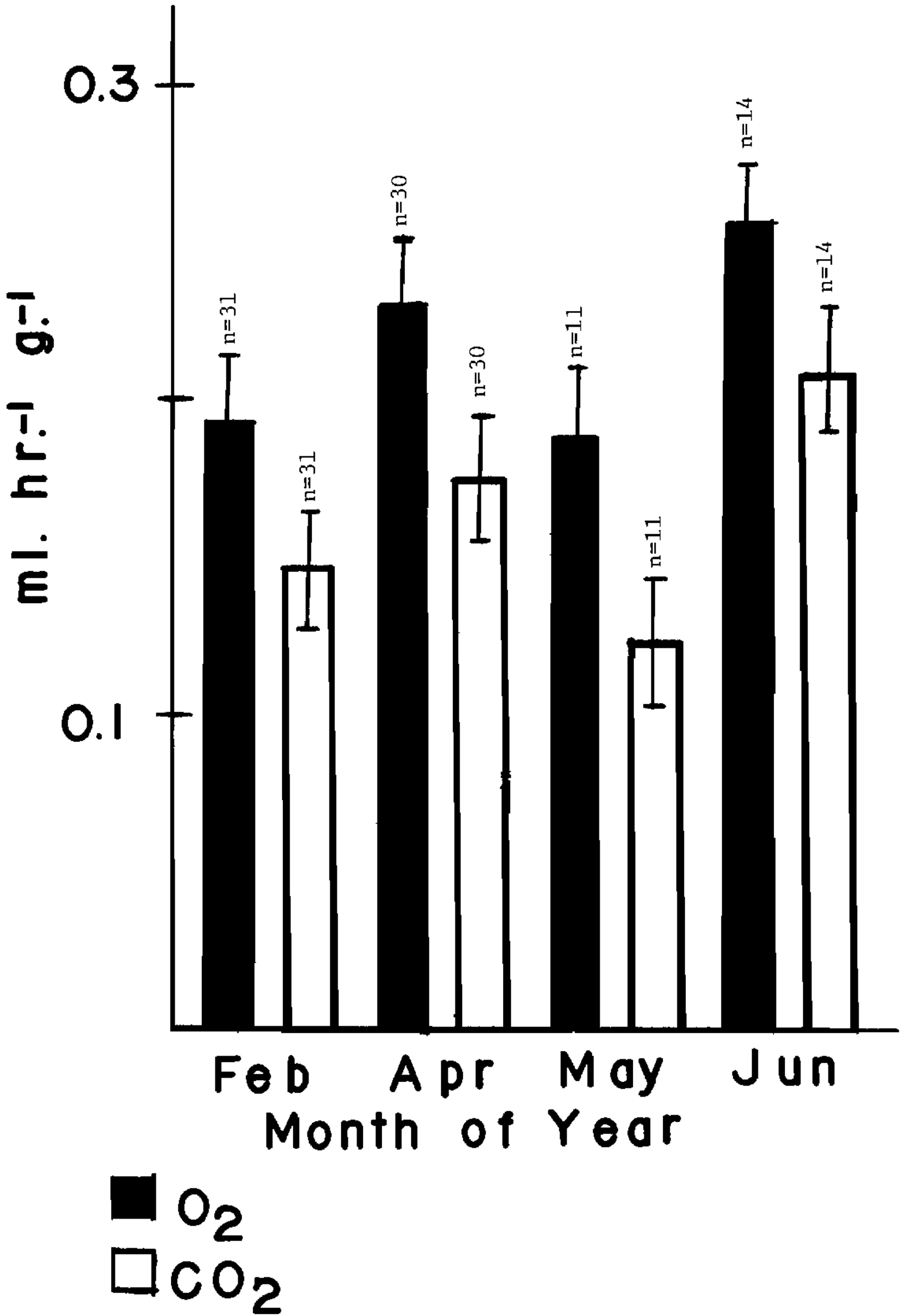

0.3
0.1
ml. hr.-1 g.-1
n=31
n=31
n=30
n=30
n=11
n=11
n=14
n=14
Feb
Apr
May
Jun
Month of Year
O2
CO2

Figure 6. Respiratory Quotients for all frogs tested each month. Values represent the mean $\pm$ standard error of the mean. R.Q. values for proteins, fats, and carbohydrates are 0.8, 0.71, and 1.0 respectively.

n represents number of animals tested each month.

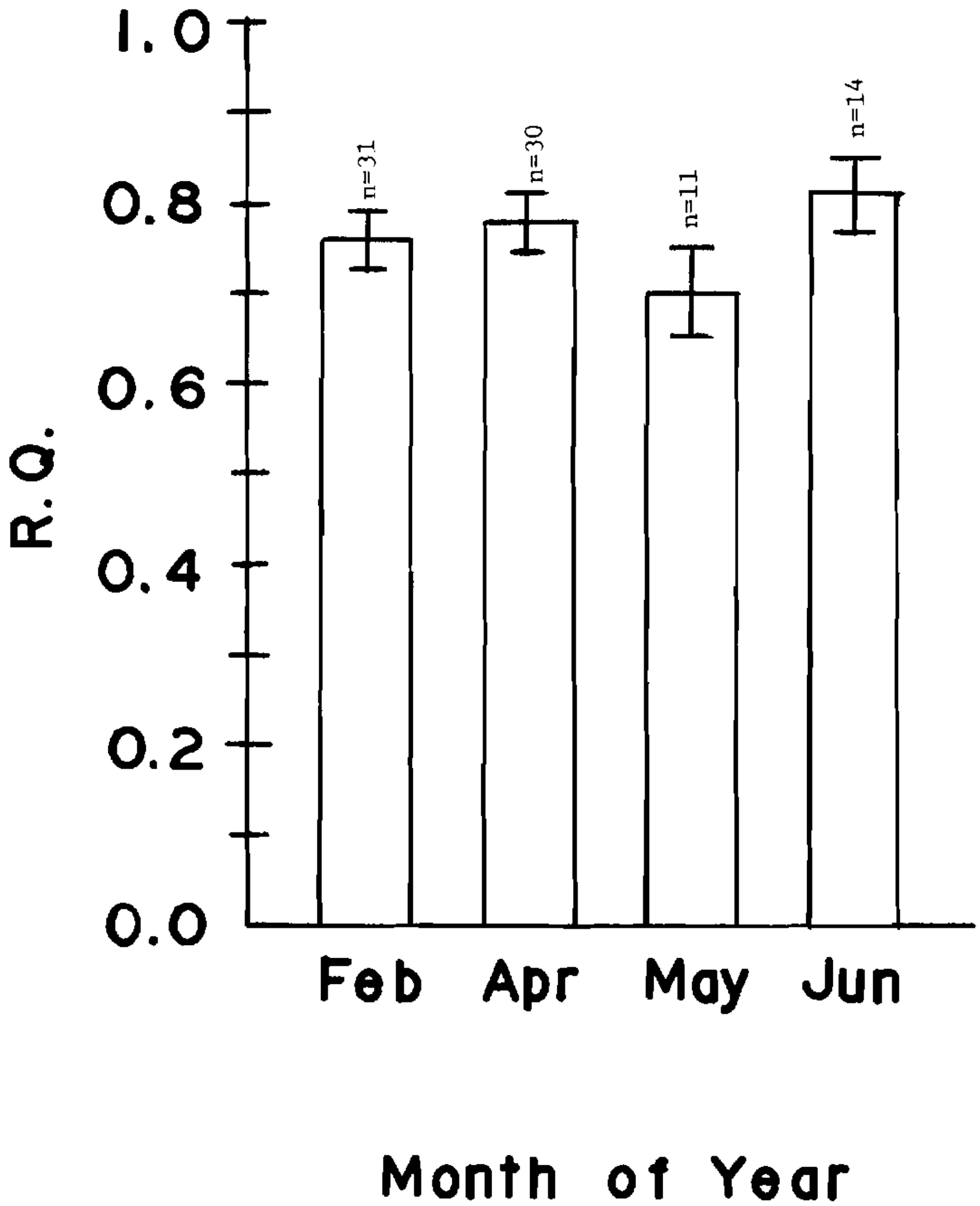
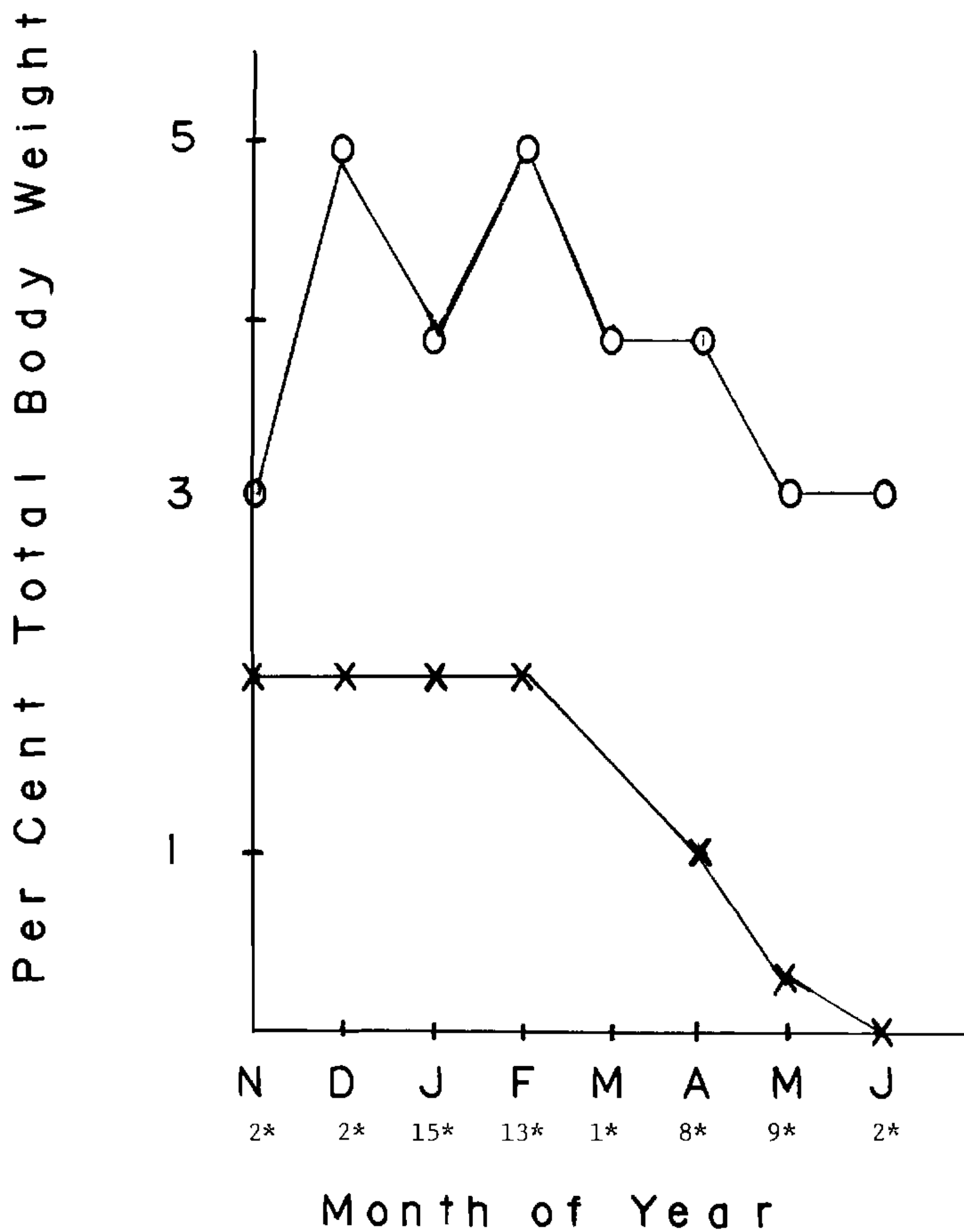

1.0
0.8
0.6
0.4
0.2
0.0
R.Q.
n=31
n=30
n=11
n=14
Feb
Apr
May
Jun
Month of Year

Figure 7. Liver and fat body weights expressed as per cent of the total body weight for all frogs autopsied each month. Each point is the average of all animals tested.

*represents number of animals tested each month



O - Liver

X - Fat Body

Figure 8. Gastrocnemius muscle weight expressed as per cent of the total body weight for all frogs autopsied each month. Each point is the average of all animals tested.

*represents number of animals autopsied each month

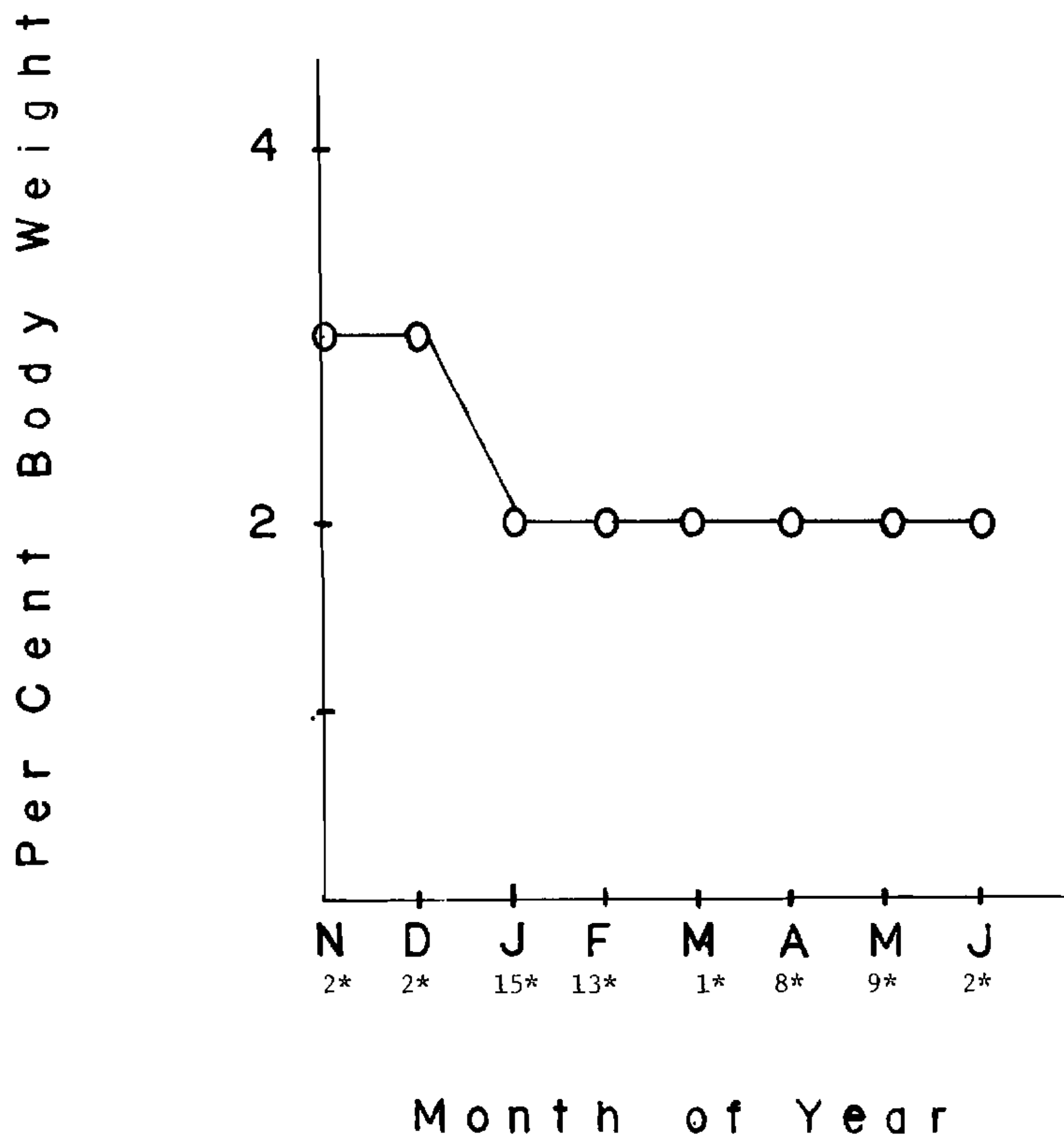

Per Cent Body Weight
4
2
N
D
J
F
M
A
M
J
2*
2*
15*
13*
1*
8*
9*
2*
Month of Year

TABLE X. Change in Gonad Size as Percent Total Body Weight

Month	Gonad Weight (% of Body Weight)			
	# of Animals	Males[1]	# of Animals	Females[1]
November	1	0.3		
December	2	0.3$\pm$.05		
January	7	0.5$\pm$.2	6	3$\pm$2
February	5	0.1$\pm$.02	8	6$\pm$2.6
March	1	0.1		
April	4	0.3$\pm$.06	4	2$\pm$.5
May	5	0.2$\pm$.04	4	8$\pm$4
June	2	0.2$\pm$.1		

1 Values represent the mean $\pm$ standard error of the mean.

TABLE XI. Metabolism of Palmitic Acid by Frogs Acclimated to Temperature

Month	Temp. (C)	# of Animals	μ Moles Palmitic Acid Utilized hr^{-1} g^{-1} X 10^{-4} [1]	ml. O_2 Consumed hr^{-1} gm^{-1} [1]	ml. CO_2 Produced hr^{-1} gm^{-1} [1]	RQ[1]
March	20±2	5	1.88±0.21	0.167±0.037	0.188±0.036	0.68±0.08
March	7±2	7	1.45±0.17	0.276±0.073	0.230±0.059	0.84±0.05
May	20±2	8	1.70±0.21	0.193±0.034	0.110±0.023	0.65±0.04
May	7±2	5	1.76±0.28	0.184±0.024	0.140±0.025	0.76±0.09
All March Frogs		12[2]	1.67±0.14	0.231±0.046	0.183±0.040	0.77±0.05
All May Frogs		12[3]	1.73±0.16	0.189±0.021	0.124±0.017	0.70±0.05

1 Values represent the mean ± standard error of the mean.

2 12 Animals were used in obtaining the R.Q. data, 16 animals were used in obtaining the Palmitic Acid data.

3 12 Animals were used in obtaining the R.Q. data, 13 animals were used in obtaining the Palmitic Acid data.

summarizes the results from these investigations. No statistical differences were found in the utilization of palmitic acid by animals at different temperatures or during the two months when testing occurred.

DISCUSSION

Many studies (Smith 1950, 1954; Fromm and Johnson, 1955; Mizell, 1965; Byrne and White, 1975) have shown that frogs caught in the wild undergo seasonal changes in the blood sugar level, liver glycogen, respiratory quotient, weights of fat body and liver, and body weights, as well as activities of the thyroid, adrenal and pituitary glands and of reproductive organs. One of the conclusions made from these studies was that winter frogs differed markedly from summer frogs in their relative reliance on carbohydrates and fats as primary energy sources. Data from spring and fall animals have shown some variability, presumably due to the fact that these animals are undergoing rapid metabolic shifts at this time.

Jungreis and Hooper (1970) suggested that the shift to carbohydrate metabolism in winter frogs is advantageous because the animals use a high level of pentose phosphate shunt activity in breaking down glucose. However, they presented no experimental evidence of increased pentose shunt activity in their animals.

Thyroxine has been found to increase glucose utilization through the pentose shunt in amphibian larvae (Dodd and Matty, 1964), in the liver of trout (Hochachka, 1962), and in the mammalian liver (Gordon and Goldberg, 1964). This suggests that hormonal changes could cause increased pentose shunt activity in the adult frog.

The primary purpose of the present study was to investigate the extent of pentose shunt activity in winter and spring frogs. The respiratory quotients of the animals were measured in order to monitor possible seasonal shifts in metabolism.

Respiratory Quotients. Respiratory Quotients (R.Q.) are obtained by calculating the ratio of the volume of carbon dioxide produced to the volume of oxygen consumed (CO_2/O_2). If the R.Q. is known, the relative amounts of carbohydrates, fats, and proteins being oxidized may be determined. The R.Q. has a value of 1.0 for carbohydrates, 0.8 for proteins, and 0.71 for fats (Gordon, 1972). When all three classes of compounds are being oxidized simultaneously, one cannot compute their proportional utilization from the R.Q. value alone. On an average mixed diet, man has an R.Q. value of 0.80 to 0.85 (Prosser, et al., 1950). The R.Q. value is at best only an estimate of the utilization of the various pathways of intermediary metabolism. Fromm and Johnson (1955) measured the changes in oxygen consumption, carbon dioxide production and respiratory quotients occurring over the course of one year. From this work, they concluded that *Rana pipiens* metabolize carbohydrates during the fall and winter, while changing to fat-based metabolism in the spring and summer.

The present research began in October 1978 when preliminary studies observed R.Q. values (see Appendix II) indicating that the frogs were metabolizing fats. From the preliminary studies, only during November 21-29, 1978, were R.Q. values (0.95 ± 0.02, mean $\pm$ S.E.M.) indicative of carbohydrate metabolism. Therefore, it would appear that the animals in this study never maintained the seasonal shift to carbohydrate metabolism described by Fromm and Johnson (1955). It was hoped that when the animals exhibited R.Q. values for carbohydrate metabolism, an increase in pentose shunt activity could be demonstrated by injecting the frogs with C^{14} labeled glucose.

Pentose Shunt. The pentose shunt is also known as the phosphogluconate pathway and as the hexose monophosphate shunt. Metabolism by the pentose

shunt is not the main pathway for obtaining energy from the oxidation of glucose in animal tissues. Instead, it is a multifunctional pathway specialized to carry out three main activities depending on the organism and its metabolic state (Lehninger, 1977). The primary purpose of the pentose shunt in most cells is to generate reducing power in the form of reduced nicotinamide adenine dinucleotide phosphate (NADPH). This reducing power is utilized in biosynthetic processes such as fatty acid synthesis (Prosser, 1973). The second function of the pathway (Lehninger, 1977) is to convert hexoses to pentoses, particularly D-ribose-5-phosphate, required in the synthesis of nucleic acids. A third function is the complete oxidation degradation of pentoses by converting them to hexoses, which can then enter the glycolytic pathway.

Many cells contain enzymes necessary for the pentose shunt in addition to those necessary for glycolysis and the citric acid cycle (Bohinski, 1974). All three of these pathways operate simultaneously, but to varying degrees.

An isotopic approach was used to determine the amount of glucose catabolism proceeding by the glycolytic versus the pentose phosphate pathway. For this study, animals were divided into two groups. One group was injected with glucose-1-C^{14} and the other with glucose-6-C^{14}. A comparison was then made of the initial rates at which C^{14} appeared in the CO_2 produced by the animals. The combined action of the glycolytic sequence and the tricarboxylic acid cycle would yield equal amounts of $^{14}CO_2$ from both C-1 and C-6 labeled glucose molecules. On the other hand, the pentose shunt would yield $^{14}CO_2$ only from glucose-1-C^{14}. Therefore, if an animal expressed high pentose shunt activity, a greater amount of $^{14}CO_2$ would be obtained from the animals injected with

glucose-1-C^{14} than animals injected with glucose-6-C^{14}. Figure 9 shows a diagrammatic sketch of glucose metabolism by the pentose shunt and the glycolytic pathway.

Effects of Temperature on Metabolism

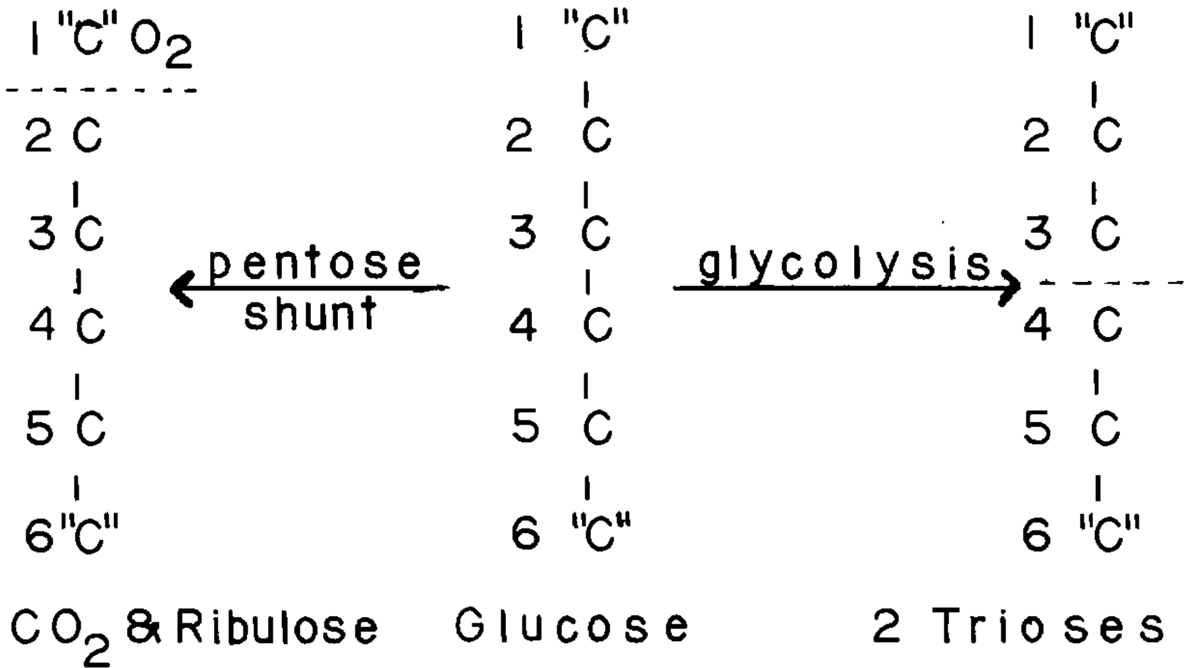
From December 22, 1978 to July 12, 1979, frogs were acclimated to warm (22 ± 2 C) and cold (7 ± 2 C) temperatures. During this period of time, respiratory quotients were measured and animals were tested for pentose shunt activity. The primary months of study were February, April, May and June.

Frogs studied in February had been acclimated to the two temperatures only and were fed weekly. Acclimation of the frogs did not produce any significant differences ($p \leq 0.05$) in the metabolism of the injected C^{14} glucose isotopes (Table II). Also, no differences were found in the amount of O_2 consumed, CO_2 produced or R.Q.'s between the warm and cold acclimated frogs (Table VI). The average R.Q. value of 0.73 ± 0.04 (mean$\pm$S.E.M.) for the warm acclimated frogs and 0.80 ± 0.04 for the cold frogs were suggestive of fat-based metabolism and not of overall metabolism of carbohydrates as was found for winter frogs by Fromm and Johnson (1955).

Frogs injected with C^{14} palmitic acid in March and May failed to show any changes in the utilization of the palmitic acid or in the O_2 consumption, as an effect of temperature acclimation (Table XI). Likewise, animals injected with C^{14} glucose did not show any increase in pentose activity with cold acclimation.

While the frogs in this study failed to express any significant changes in metabolic activity, previous workers have shown that temperature may affect metabolism in some ectothermic animals. Tindel (1956)

Figure 9. Diagrammatic representation of the metabolism of glucose by the pentose shunt and glycolytic pathways. The sketch shows where the molecules are first cleaved by each pathway. Marked carbons ("C") represent radioactive atoms. The two pyruvate molecules show how the cleaved glucose molecule would enter the Krebs Cycle for oxidative metabolism.


1 "C" O2
2 C
3 C
4 C
5 C
6 "C"
pentose
shunt
1 "C"
2 C
3 C
4 C
5 C
6 "C"
glycolysis
1 "C"
2 C
3 C
4 C
5 C
6 "C"
CO2 & Ribulose
Glucose
2 Trioses

1 "C" H3
2 C=O
3 C O O H
6 "C" H3
5 C=O
4 C O O H
2 Pyruvate Molecules

found that the relationship between temperature and the rate of gluconeogenesis in _R. temporaria_ was funadmentally linear, with the rate of gluconeogenesis rising rapidly between 13 and 21 C. He proposed that temperature might be an important factor in the regulation of carbohydrate metabolism in frogs. Also, studies on differently acclimated fish appear to show that pentose shunt activity increases during cold acclimation (Somero and Hochachka, 1971).

However, Jankowski (1960; cited by Hazel and Prosser, 1970) found that no increase in metabolism or O_2 consumption occurred in the muscle, liver and heart of _R. temporaria_ after cold acclimation. Hazel and Prosser (1970) concluded that enzymatic compensations are not expected at low temperatures for animals which become lethargic or torpid in the cold. Likewise, from their work with hormonal control of carbohydrate metabolism in _R. pipiens_, Byrne and White (1975) concluded that ambient temperatures are probably an inadequate explanation for the fine control shown by frogs in mobilizing their energy reserves. Rather, many studies of hormonal regulation of metabolism (Smith, 1953, 1954; Hermansen and Jørgensen, 1969; McNabb, 1969; Rinaudo, et al., 1969; Farrar and Frye, 1977) suggest that amphibians are able to exert some regulatory control over metabolism. Therefore, the failure of the frogs in the present study to exhibit temperature compensation in R.Q. or pentose shunt activity may be due to unmeasured adjustments in such regulatory control mechanisms.

Effects of Temperature and Photoperiod on Metabolism

Since no difference in metabolism was noticed in February between animals acclimated at warm and cold temperatures, the animals were divided into photoperiods of 14:10 (long day) and 8:16 (short day) L:D

within the two temperature groupings. However, no differences ($p \leq 0.05$) were found in the utilization of the injected C^{14} glucose molecules, O_2 consumption, CO_2 production, or R.Q. values with respect to either temperature or photoperiod in April (Tables III and VII) or May (Tables IV and VIII).

Although the literature on circadian and seasonal rhythms is voluminous, few investigators have examined rhythms of O_2 consumption in amphibians. Fromm and Johnson (1955) showed seasonal changes in the respiratory metabolism of curarized frogs. They found that frogs acclimated at 4 and 22-28 C and tested at 22-25 C, had a maximum oxygen consumption in spring, with a decrease during summer and a minimum in winter. The same cycle was found in frogs acclimated at both temperatures, with the values for the cold acclimated animals being lower in all seasons. During the present investigation, the frogs did not develop a statistically significant cycle in O_2 consumption or CO_2 production (Figure 5).

Since a seasonal cycle of O_2 consumption was not discovered in the animals of this study, a review of the literature may be helpful in explaining why such seasonal cycles did not occur. The effect of photoperiod, as well as seasonal variations in O_2 consumption in amphibians have been reported by previous investigators (Vernberg, 1952; Whitford and Hutchinson, 1965; Vinegar and Hutchinson, 1965). Guimond and Hutchinson (1968) showed that *Rana pipiens* had a significant rhythm of gas exchange under some temperatures and photoperiods. The mean hourly oxygen consumptions for frogs acclimated at 15 and 25 C were higher during the dark than light periods. Their animals were more active in the respirometers during the dark hours, and it was stated that the increase in O_2

consumption could possibly be due to the increased activity of the animals. In this study, the animal chambers of the respirometers were covered with dark plastic in order to keep the animals from being disturbed by movements outside the chambers. Since some findings indicate increased O_2 consumption in the dark, some error in O_2 consumption values may have been caused by the darkened chambers. However, this error should have been consistent among the groups. It is important to state that the purpose of placing the animals on short and long day photoperiods was to present them with an environmental key that might trigger a change in metabolism. This was especially important since temperature acclimation had failed to produce these metabolic changes earlier. While there may be daily cycles in O_2 consumption, this study did not find a change in metabolism to occur between different photoperiods.

Guimond and Hutchinson (1968) found that temperature caused a greater effect on the relative role of the pulmonary, cutaneous, and total gas exchange than the effects caused by photoperiod. These workers concluded that, in frogs, the skin is important for respiration at lower temperatures. Over 52% of the oxygen consumed and 75% of the carbon dioxide liberated took place through the skin at 5 C, compared to 34% of the O_2 consumed and 56% of the CO_2 exchanged at 25 C. At higher temperatures the lungs supplied the additional requirements. It is interesting to note that the oxygen consumption values obtained by Fromm and Johnson (1955) are generally lower than those reported by other workers (Brown, 1961). Brown (1961) suggests that the curarized frogs used by Fromm and Johnson were only respiring cutaneously, for the amount of d-turbocurarine used by these workers would have diminished the buccal-pharyngeal movements necessary for respiration via the lungs. Therefore, the changes

noted by Fromm and Johnson (1955) may have been changes in respiration via the skin and lungs and not changes in overall metabolism. This may explain why the present study was unable to repeat the seasonal cycles in metabolism expressed by Fromm and Johnson (1955). It is concluded that neither temperature nor photoperiod caused a change in metabolism in the frogs used for this investigation and therefore, may not provide the trigger for seasonal changes in metabolism in nature.

Seasonal Cycles in Energy Reserves and Metabolism

A number of workers have confirmed that a seasonal pattern exists in the use of energy reserves and metabolism in wild amphibians (Smith, 1950; Fromm and Johnson, 1955; Mizell, 1965; Hermansen and Jørgensen, 1969; Byrne and White, 1975; Farrar and Frye, 1979). Smith (1950), Mizell (1965), and Byrne and White (1975) monitored the changes occurring in the energy reserves of frogs throughout the course of one year. These investigators found that liver glycogen stores decrease during the winter. Smith (1950) found that as the liver weights decreased, liver glycogen content also decreased. This suggests that the animals are utilizing the liver glycogen stores in the winter. The frogs must be replacing these glycogen stores through glyconeogenesis of tissue protein. Otherwise, they would probably deplete their glycogen stores within five to seven days (Farrar and Frye, 1979).

The changes in liver and fat body weights during this study (Figure 7) are similar to the cyclic changes in these energy reserves noted by Smith (1950) and Mizell (1965). This indicates that the animals were using these energy stores, while being fasted in the laboratory, in the same manner as frogs captured in the wild. Figure 8 indicates that throughout this period the energy stores within the muscles were being

utilized at a fairly uniform rate. That is, the gastrocnemius muscle weights decreased in the same proportion as the overall body weight throughout the major portion of this study. What the data from these organs indicates is that the changes noted in the liver and fat body are unique to these organs while utilization of body protein, as indicated by the change in muscle weight. occurred at a more uniform rate.

Laboratory Acclimation. No agreement exists concerning whether seasonal changes continue in the laboratory-acclimated amphibian. However, the failure of the frogs in this study to show seasonal changes in O_2 consumption, R.Q., or pentose shunt activity might be due to the fact that the frogs were maintained in the laboratory rather than under natural conditions in the wild.

Since one of the purposes of this project was to reduce the variations caused by shipping and handling (Gibbs, et al., 1971) and the use of frogs from different species, locations, etc. (Farrar and Frye, 1979), the same group of frogs were maintained in the laboratory and used repeatedly throughout the course of this investigation. Hermansen and Jørgensen (1969) reported that laboratory conditioned toads (*Bufo bufo*) had a lower blood glucose level than found in freshly captured toads. They also found that seasonal variations in the laboratory animals were strongly reduced and sometimes absent. Farrar and Frye (1979) found that under laboratory conditions seasonal changes in blood glucose of *R. pipiens* were moderated. They also noted that their laboratory animals did not exhibit seasonal variations in liver glycogen content.

Gibbs (1973) used Tetracycline-HCl to treat frogs which had become ill due to bacterial infections. He noted that treated animals, which had been allowed to recover under optimal laboratory conditions, gave much better reproducibility in his experiments. However, seasonal

variations no longer occurred. This suggests that the use of Tetracycline-HCl to treat "red-leg" in many of the frogs used in this study may have contributed to the failure of these animals to show seasonal changes in metabolism.

It must also be noted that all frogs used in this experiment were captured at a very early age. These juvenile frogs may not have been subjected to natural conditions that could cause the animals to set their "biological clocks". Therefore, the use of young animals may also help to explain why seasonal variations noted in wild captured frogs were not found in this study.

Increased Pentose Shunt Activity in April

While no changes in metabolic activity were indicated by the respiratory quotients, injections of the frogs with C^{14} labeled glucose indicated that an increase in pentose shunt activity occurred during April. The frogs utilized significantly more ($p \leq 0.05$) of the injected C-1 labeled glucose (4.23 ± 0.34 µmoles $hr^{-1} g^{-1} x10^{-4}$ (mean$\pm$S.E.M.)) than the C-6 labeled glucose (2.71 ± 0.33 µmoles $hr^{-1} g^{-1} x10^{-4}$). This increase was not evident in either February, May, or June. The increased pentose shunt activity expressed in April was possibly caused by starvation and reproductive activity, or a combination of these effects.

Starvation. The increase in pentose shunt activity occurred in April after the animals had been fasted for six weeks. In May, after two and one-half months of starvation, there were no differences in the utilization of the two labeled glucose molecules by the frogs. The animals were then fed for one month and tested once more in June. Again, no differences were found in the utilization of the injected glucose molecules. Therefore, the increased pentose shunt activity during April

could possibly be attributed to the animals' attempts to compensate for the effects of relatively short-term starvation. Such compensation, if it occurred, must have been short-lived since it was not apparent after a longer period of starvation in May.

Fasting probably plays a role in the metabolism of frogs in nature, since there is not an over abundance of food during the winter months. It has been found that frogs do not feed (Holzapfel, 1937) or do not actively feed (Smith, 1950) during the winter. The cold-acclimated frogs in this study also refused to eat in January and February. Holzapfel (1937) stated that control frogs maintained at room temperature refused to eat. However, frogs maintained at room temperature (20 ± 2 C) in this study, continued to feed when placed in contact with live food. Another noted contradiction in behavior by the frogs in this study was observed when all of the cold-acclimated frogs did not bury themselves in the sand as reported by Jones (1968).

Previous investigations indicate that starvation causes a variety of changes within animals (Hill, 1911; Fromm and Johnson, 1955; Janssens, 1964; Stimpson, 1964; Seymour, 1973; Farrar and Frye, 1979). Starvation has been found to cause metabolism of proteins in goldfish (Stimpson, 1964) and the african lungfish (Janssens, 1964) and metabolism of triglycerides in eels (Larson and Lewander, 1973). Hill (1911) found that fasting caused a decrease in the utilization of the fat body in frogs after 31 days. He attributed this to the reduced heat of production (50%) which was found in these animals after the first 21 days of starvation. The frogs in this study failed to show conservation of the fat body (Figure 7). However, while not significant, the warm-acclimated frogs did show a reduction in O_2 consumption during the period

of starvation (Figure 4). This reduction in oxygen consumption was noted by Seymour (1973) in his work with the spadefoot toad. Oxygen consumption increased in June after feeding resumed (Figures 4, 5) in May.

On the other hand, workers have also found that feeding causes little or no change in frogs. Fromm and Johnson (1955) did not find any differences in metabolism between fed and unfed Rana pipiens. Farrar and Frye (1979) found that blood glucose, liver glycogen and muscle glycogen levels remained "remarkably" high in R. pipiens during short and long-term starvation. Farrar and Frye (1979) found that blood glucose levels become elevated after feeding but decline to stable levels within three to five days, and remain stable after fasting from five days to two and one-half months. Smith (1950) noted that between April and November blood glucose levels of frogs in the field were fairly high, but fell to a steady state after two weeks of captivity without food. The months between April and November are periods when the frogs are actively eating. While blood glucose levels may remain stable after prolonged starvation, Farrar and Frye (1979) found that many weeks of fasting may reduce muscle glycogen to 50% and liver glycogen to 20% of the initial post absorptive levels. Therefore, they stated that although frogs exhibit a remarkable tolerance to fasting, dietary status and feeding regimens may induce many variations in the energy reserves in these animals.

Because of the various reports, it is uncertain whether or not starvation alone causes a change in metabolism in frogs. In this study, starvation seemed to induce a change in metabolic activity by frogs maintained in the laboratory. It is believed that this response to

starvation also occurs in the wild in coordination with reproductive activity.

Reproductive Activity. The frogs in this study were never observed, through their behavioral actions or changes in gonad size, to exhibit any regular breeding habits. However, *Rana pipiens* usually undergo the onset of the breeding period in late March or early April (Mizell, 1965), immediately after emerging from hibernation. Frogs, however, do not begin to feed until the breeding season is over, usually by the end of May. Through this period Mizell (1965) noted that liver glycogen was at its lowest point in May, with the fat body continuing to decrease in size into June. Frogs in this experiment exhibited this same cycle in the fat body size (Figure 7). While liver glycogen was not measured, the liver sizes reached a minimum in May and remained stable through June (Figure 7). The decline in fat body size through May and June noted by Mizell (1965) might suggest a change in metabolism to a greater dependence on triglycerides.

If indeed the frogs do initiate breeding behavior in late March and early April, this may be a time when the animals also change their metabolic activity. Such a change might explain, at least in part, the increase in pentose shunt activity which occurred in this study in April but which was subsequently lost in May and June. The fasting of the animals in the laboratory coincides with their refusal to eat in the wild (Mizell, 1965). Therefore, the most likely explanation for the increased pentose shunt activity in April was a combination of the effects of starvation and reproductive activity. The inability to duplicate the seasonal changes in O_2 consumption and CO_2 production noted by Fromm and Johnson (1955) could have been caused by maintaining the frogs in the

laboratory throughout the period of study. However, no conclusions can be drawn without additional experimental evidence.

Summary and Conclusions

Throughout this study, the frogs failed to show the seasonal changes in O_2 consumption, CO_2 production, and respiratory quotients found by Fromm and Johnson (1955). Likewise, the frogs failed to change their metabolic activity when they were acclimated to cold (7 ± 2 C) or warm (20 ± 2 C) temperatures and placed on photoperiods of 8:16 or 14:10 L:D. The frogs showed an increase in pentose shunt activity during the month of April. This increase in pentose shunt activity was probably due to the combined effects of experimental fasting and the onset of the animals' natural reproductive activity. It is also believed that the frogs' failure to exhibit seasonal changes in metabolic activity, as noted by other workers (Smith, 1950; Fromm and Johnson, 1955; Mizell, 1965), was caused by laboratory confinement, and the young age at which these animals were acquired.

It must be noted that the common leopard frog, *Rana pipiens*, is a very complex animal. The frog must be able to change its behavior and physiological functions very rapidly and sustain these changes over prolonged periods. This animal has developed unique physiological and behavioral mechanisms in order to survive the harsh environmental changes it is faced with. Therefore, when working with the frog, one must be careful not to draw conclusions based upon the studies of other animals. For as more work is being done, what once seemed impossible, is now found to occur. It is truly a very fascinating world.

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APPENDIXES

APPENDIX I

Calculations for converting counts per minute to relative number of radioactive molecules.

To determine counting efficiency a small amount of radioactive material was added to each sample. The added radioactive material contained a greater number of counts than the original sample. The counting efficiency was then calculated by dividing the net increase in counting rate due to the internal standard by its known disintegration rate.

$$\text{Efficiency} = \frac{\text{(Count Rate Internal Standard + Sample) - Count Rate of Sample}}{\text{Disintegration Rate Internal Standard}}$$

The disintegration rate (disintegrations per minute, DPM) was then calculated as follows:

$$\text{Disintegration Rate Sample} = \frac{\text{Count Rate Sample-Background}}{\text{Efficiency}}$$

The number of labeled molecules present in the sample was then calculated as follows:

$$\text{moles Substrate} = \frac{\text{DPM Sample}}{(2.2 \times 10^6 \text{ DPM})^* \text{ Specific Activity of Substrate}}$$

*DPM in 1 μCi

APPENDIX II

Oxygen consumption, carbon dioxide production and respiratory quotients for frogs during the months of October**, November**, December**, and January**. (Animals were maintained on photoperiods of 14:10, L:D, at 20$\pm$2 C.)

Date 1978-79	# of Frogs	O_2 consumed* ml hr^{-1} g^{-1}	CO_2 produced* ml hr^{-1} g^{-1}	R.Q.*
Oct. 11-14	5	0.295$\pm$0.03	0.195$\pm$0.02	0.67$\pm$0.03
Nov. 21-29	13	0.193$\pm$0.20	0.181$\pm$0.03	0.95$\pm$0.02
Dec. 5- 8	16	0.222$\pm$0.04	0.150$\pm$0.02	0.69$\pm$0.01
Dec. 11-19	34	0.250$\pm$0.03	0.219$\pm$0.17	0.81$\pm$0.03
Dec. 22-23	11	0.225$\pm$0.02	0.161$\pm$0.02	0.72$\pm$0.04
Jan. 23-29	11	0.206$\pm$0.03	0.149$\pm$0.03	0.70$\pm$0.05

* mean$\pm$standard error of the mean

** results obtained during these months were from preliminary investigations.