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Effects of Prolonged Starvation of Male *Ambystoma tigrinum* in Post-Reproductive Condition

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ABSTRACT. — We investigated the effects of extended starvation upon male *A. tigrinum* by maintaining them in aquaria without food for a period of up to 70 days. Serum lipase activity increased significantly during each of the first four weeks of starvation. At 35 days lipase activity reached a maximum level that was maintained the remainder of the 70 days. Hepatic glycogen steadily decreased from an average value of 3.32 g glycogen/100 g liver at the time of capture to less than 0.50 g glycogen/100 g liver after 70 days. Visible fat body content varied erratically for the samples of this study and showed no clear pattern except for the very significant reduction in mean fat body mass from the time of capture to the 70 day measurements. Body mass decreased to 45.5% of the initial weight during the study but, as was the case for fat bodies, did not show a sequential decrease among samples. Gonadal tissue was catabolized from 1.64% of body weight to 0.25% of body weight within 21 days of starvation and shows no further reduction as a percentage of body composition. Although absolute liver weight decreased during the study, it remained an extremely consistent 2.66 ± 0.22% of body weight throughout the entire study.

Starvation represents a significant component of the life history of many amphibians including the tiger salamander (*Ambystoma tigrinum*). During winter hibernation in temperate regions, starvation continues for prolonged periods yet the depletion of endogenous energy stores is minimized by the greatly reduced metabolic requirements when body temperature is below 6°C (Fromm and Johnson, 1955). A potentially more critical period without food for male *A. tigrinum* occurs during the peak of reproductive activity when metabolic requirements are high and prey availability is low (Sever and Dineen, 1978), stressing the animal’s energy reserves to provide optimal activity of the gonads and appropriate behavioral responses (Kumpf, 1934; and O’Donnell, 1937). Since these two periods of obligatory and volitional starvation occur either sequentially or nearly so, the ability of this species to utilize endogenous energy sources efficiently is essential.

Data concerning the energy metabolism of *A. tigrinum* are restricted to effects of anoxia (Rose et al., 1971), activity energy proportionment (Hutchison et al., 1977) and the characteristics of fat bodies and free fatty acids and their function in ovarian metabolism (Rose and Lewis, 1968; and Lewis and Rose, 1969). Study of energy metabolism of reproductively active male *A. tigrinum* are lacking. The purpose of this study is to identify the extent to which endogenous energy sources are available in male *A. tigrinum* during the post-reproductive period of the annual cycle and to determine the ability of these salamanders to utilize these sources during an additional period of starvation beyond that occurring naturally.

MATERIALS AND METHODS

Forty-eight male *A. tigrinum* were captured by seining from a ¼ ha, 60 cm deep pond in South Bend, Indiana on 25 March 1981. Sever and Dineen (1978) had previously determined that emigration from the pond was initiated by this time in previous years. Additionally many egg masses were present in late stages of development, therefore we assumed that these salamanders were near the end of their reproductive activity. The salamanders were placed in 10 gallon aquaria which contained a
E. D. MOULD AND D. M. SEVER

gravel-sand mixture topped with an upper layer of soft topsoil to allow for burrowing. Water was added periodically to keep the substrate moist, but no food was provided and a screen was placed over the aquaria to insure complete starvation during the entire study period. The aquaria were located in a basement at a temperature ranging from 16°C to 21°C during the study which was representative of the temperature variation in the vicinity of the pond.

At the end of capture and at 7 day intervals for the ensuing 35 days, four salamanders were randomly sampled from the aquaria. A final group of four salamanders were maintained in the aquaria without food for 70 days to observe the effects of prolonged starvation. The salamanders selected for sampling were quickly decapitated with a minimum of struggling and a 0.2-0.4 ml blood sample was collected in non-heparinized sterile containers directly from the severed heart and allowed to separate. Wet masses of the entire body, visible fat deposits, testes and associated ducts, and the tail (cut just posterior to the vent) were determined on an analytical balance. Stomach contents were removed, weighed and then visually separated into nutritive constituents and soil categories. Sera and liver samples were stored in air-tight containers at -20°C until analyses were performed and carcasses were preserved in 10% formalin.

Lipase activity of serum was determined by a modification of the fatty acid liberation method of Tietz and Fiereck (1966). One tenth ml serum was mixed in a 5 ml test tube with 0.25 ml water, 0.10 ml trizma buffer (Sigma Chemical Co.) and 0.30 ml Sigma Lipase Substrate. The reagents were then shaken thoroughly for 5 seconds and placed in a 37°C water bath for 6 hours. At the completion of the incubation period the mixture was poured into a small Erlenmeyer flask using 95% ethyl alcohol as a quantitative rinse. One drop of thymolphibalein indicator solution was then added to the mixture and then titrated to a light blue endpoint with 0.05 N NaOH solution. Lipase activity was expressed in Sigma-Tietz units which is exactly equal to the ml of 0.05 N NaOH needed to titrate the mixture. Liver glycogen was determined using the standard anthrone procedures of Seifter et al. (1950).

RESULTS AND DISCUSSION

Lipase activity increased after the first week of starvation then reached a plateau with no significant increase until after 35 days of starvation when the highest level of lipase activity was observed (Table 1). After 70 days without eating there was no significant change in lipase activity suggesting that the values reached at 35 days represent maximal values for lipase activity for A. tigrinum in this condition. Therefore, lipase activity apparently remains high in these salamanders even after lipid deposits are exhausted as long as fasting is maintained as observed in the 70 day subjects. These data are consistent with the demonstrated increased activity of hormone-sensitive lipase during fasting mediated by pituitary hormones (Hollenberg, 1965; Butcher, 1968; and Huttunen et al., 1970). There is a notable decrease in the variability of lipase activity as starvation ensues, which may indicate that variability in animal condition prior to the onset of the experiment was a determinant in the rate of enzymatic response. This is especially true if carbohydrates are present in the blood of some of the salamanders which would result in increased insulin secretion thereby inhibiting triglyceride catabolism and facilitating fat deposition (Renold, 1965; and Unger and Eisen Trout, 1969). After 21 days of starvation the variability in lipase activity was reduced to a standard deviation of only ±0.14 units. We have no explanation for the apparent synchrony in the increase of lipase activity from the 21 day to the 35 day level. It is notable
that the reduction in variability of lipase activity corresponds to the period when stomach contents (not including soil) were not significantly different from zero (Table 1). Stomach content analysis indicated a large variance between individuals on the day of capture, presumably as a function of vigor and length of reproductive activity and consequent nutritional history. Total nutritive emptying of the gastrointestinal tract occurred between 14 and 21 days after capture. All of the salamanders consumed copious amounts of soil during the first 21 days of starvation (Table 1).

Endogenous energy stores in amphibians reside in small amounts of readily available carbohydrates in the form of glycogen and as lipids stored in fat deposits (Brown, 1964). Hepatic glycogen is the most indicative reservoir of endogenous carbohydrates for long term utilization by A. tigrinum since 86% of the total organ glycogen exists in the liver (Rose et al., 1971). Hepatic glycogen averaged 3.32 g glycogen/100 g liver for the salamanders sacrificed at the time of capture (Table 1). Assuming 4.1 kcal/g glycogen (Brown, 1973) and a liver weight of 1.5 grams, stored glycogen in these salamanders accounts for a maximum of only 0.013 kcal if the glycogen was converted to energy. Smith (1950) found that hepatic glycogen varied from an annual low of 1 g glycogen/100 g liver in April to a high of 15 g glycogen/100 g liver in October for Rana temporaria. Thus the stored carbohydrates of these salamanders in post-reproductive condition are relatively low.

Lipid deposits in urodeles are restricted to two tail deposits, the cardiac region, and those associated with the gonads (Pond, 1978). Although the presence of all potential fat storage sites were investigated, only gonadal fat bodies were grossly conspicuous in the salamanders of this study. Gonadal fat body mass averaged a high of 1.8 mg fat/g BW at the initiation of the study.

<table>
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<tr>
<th>Table 1. Mean body mass, serum lipase activity, nutritive and soil stomach contents, hepatic glycogen, visible fat body mass, and liver mass of male Ambystoma tigrinum sampled during forced starvation.</th>
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<td><strong>Length of starvation (days)</strong></td>
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and a low of 0.10 mg fat/g BW for the salamanders starved 70 days (Table 1). Between these two extremes there is no clear pattern indicating a steady decline as would be expected for starved active salamanders. Since the stomach content data indicate that starvation was indeed occurring (Table 1), it must be concluded that the variability in the data are a result of variable endogenous lipid storage prior to the initiation of the experiment. Since the successful mating by male *A. tigrinum* is skewed in favor of a few individuals (Sever and Dineen, 1978), a variability in physiological condition and body composition would be expected in a random sampling as conducted in this study. While measurement of fat body content may serve as a useful indicator of animal condition on a seasonal basis using many subjects, it may not indicate the immediate physiological status of an individual in a given time frame as conclusively as enzymatic indicators since they respond more acutely to the lack of food.

There has been much conjecture concerning the apportionment of gonadal fat deposits for nutritive maintenance or gonadal development. The well documented inverse relationship between size of gonadal fat deposits and reproductive activity led some early investigators to speculate that these deposits are primarily reserved for reproductive metabolism (see Adams and Roe, 1929). Fitzpatrick (1976) postulated that reproductive activity may occur only when absolute levels of lipids are sufficient, regardless of their precise location. Gonadal deposits are likely utilized for both maintenance and reproduction in amphibians since they exhibit significant decreases during both dormancy and reproductive activity in *Desmognatus ochrophaeus* (Fitzpatrick, 1973). *Amphiura means* (Rose, 1967), *Scaphiopus couchii* and *S. hammondii* (Seymour, 1973). While gonadal fat reserves persisted longer than all other fat body deposits of the male salamanders of this present study, there was a drastic reduction in even these deposits after 70 days of starvation (Table 1). Apparently these reserves of fat are spared even after the catabolism of other tissues (notably including the gonads themselves) has been initiated. However, during prolonged starvation these high energy reserves are also catabolized by male *A. tigrinum* to maintain essential life functions. Thus, these data are consistent with the apparent dual use of gonadal fats by other amphibian species.

After 70 days of starvation, body mass decreased to 45.9% of the initial mass before starvation (Table 1). However, as was the case for fat body, body mass did not sequentially decrease among sampling units. Sampling of heavier individuals at later intervals distorted the weight loss relationship. Gonadal tissue was catabolized from approximately 1.64% of body weight to a level of 0.25% of body weight within 21 days of starvation. This proportion of gonadal tissue was maintained through the remainder of the 70 days of the experiment. Although absolute liver weight decreased from 1.33 grams to 0.49 grams during the study, it remained an extremely constant 2.66 ± 0.22% of body weight.

As was expected for salamanders at the completion of their reproductive activity, these animals were in poor condition even at the onset of the experiment. Both available fat reserves and hepatic glycogen were low at the initiation of the study. Despite this fact, these salamanders showed a remarkable ability to not only withstand prolonged starvation, but also to remain active during this period as burrowing in the aquaria topsoil was extensive throughout the length of the experiment. It has been suggested that *A. tigrinum* exhibit lek type reproductive behavior (Sever and Dineen, 1978). Since mating in *A. tigrinum* occurs immediately after hibernation, males would necessarily be required to endure prolonged volitional starvation in order to
be reproductively successful. This study
confirms the ability of these salamanders to survive extended starvation
even after energy reserves are already low.

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