Seasonal Changes in Energy Reserves in the Common Frog, *Rana tigrina*

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Summary Liver glycogen and lipid, ovarian glycogen, and lipid index were used as criteria to elucidate energy changes associated with reproduction and biphasic pattern of dormancy (summer and winter) in the female frog, *Rana tigrina*. Liver glycogen showed 2 peaks; the ovarian glycogen showed reciprocal relationship with that of liver. The liver lipid and glycogen showed parallel trend.

Key words: energy reserves, frog.

Frogs are widely used in many different experiments; therefore, some basic knowledge of the physiology of the frog and its annual variations would seem necessary. Seasonal variations in the frog have been studied quite extensively. Seasonal variations in the liver and fat body of the common frog *Rana temporaria* have been studied by Smith (1950), in different endocrine glands by Hanke and Weber (1964), van Kemenaade and van Dongen (1965), and Oordt et al. (1968), in sex organs by Juszcyk (1959). The size of the erythrocyte appears to be related to general metabolic activity (Sinha, 1983; Sinha and Ahmad, 1984; Sinha and Singh, 1987), and estrogen treatment significantly enhanced lipid contents of the liver and ovary in the frog *Rana esculenta* (Sinha, 1982).

The frogs of the species *R. tigrina* in Patna (25°37′N, 85°12′E) do not seem to hibernate because they do not have the thermoregulatory centre but they undergo a period of dormancy during winter. For this reason, the term “wintering” is used here to mean the form of behaviour typical to the winter period and decreased rate of metabolism caused by low temperature of the environment (8–12°C). The term “wintering” has also been used for *Rana esculenta* (Sinha, 1983). The frog *R. tigrina* also undergoes a period of summer dormancy in May and June, when the temperature is high (34–40°C), and for this reason, the term “aestivation” has been used.

Healthy female frogs (*Rana tigrina*) weighing 250–280 g were obtained from
local ponds in and around Patna (25°37'N, 85°12'E), brought to the laboratory and kept in the large aquaria for about 3 days until sacrificed. The frogs were maintained in the laboratory as described earlier by Sinha (1983). The frog was weighed accurately, then dissected and the liver, ovary, and fat body were taken out, placed on a filter paper to soak up the moisture, and weighed accurately. The studies were made during May and June (aestivation), July and Aug. (breeding), Sept. and Oct. (prowintering), Nov.–Jan. (wintering) and Feb.–April (postwintering).

The glycogen content of the tissues (liver and ovary) was determined according to the method of Kemp and Andrienne (1954) with some modification of Sinha and Kanungo (1967). The total lipids in the tissues were determined according to the method of Bligh and Dyer (1959). The lipid index (L.I.) was determined by dividing the total fat body by body weight and then multiplying by 100 (Sinha, 1982).

The standard deviation of the mean of a set of eight determinations and levels of significance were calculated according to the method of Siegel (1956). Values of 5% or lower were taken as significant differences.

The liver glycogen revealed two significant peaks: 1) during the period of aestivation (May and June) and 2) during the period of wintering (Fig. 1). The ovarian glycogen had also 2 peaks: 1) during the beginning of the breeding period and 2) during the postwintering period (Fig. 1). It was interesting to note that there was a reciprocal relationship between the liver and ovarian glycogen except during the beginning of the prowintering period when the glycogen content was minimum in the tissues (Fig. 1).

The liver fat followed a parallel pattern to that of liver glycogen (Fig. 1). The liver lipid was minimum in the prowintering period and maximum during the aestivation and wintering periods (Fig. 1). The L.I. was nil during the aestivation and breeding periods (May–Aug.) and the synthesis initiated in September and reached a peak in December and thereafter decreased and reached minimal level in April (Fig. 2).

The glycogen and lipid reserves of the tissues decreased (Fig. 1) during breeding in summer, implying that the primary metabolic storage products in the female R. tigrina are the lipids and glycogen. Since reproduction requires large amounts of energy in most animals, the frog R. tigrina utilizes the tissue fat for energy and it has been reported that for continued fatty acid oxidation, a steady supply of carbohydrate in the form of oxaloacetate is essential (Sinha and Sinha, 1984). Therefore, liver glycogen built up during aestivation might well assist fat utilization by providing the required supply of oxaloacetate. During aestivation, carbohydrate reserves are maintained over exceedingly prolonged periods of starvation in lungfish (Prosser, 1973). The accumulation of liver glycogen in the aestivating frog R. tigrina may also be due to a very low basal metabolic rate. The arousal from aestivation is very rapid, probably to meet the high energy drain during reproduction glycogen immediately after aestivation in contrast to the gradual arousal in the wintering frog.
During summer the metabolic machinery is geared towards liver glycogen storage leading to metabolic dependence for carbohydrate and fat for reproduction. The metabolic dependence of carbohydrate for reproduction is substantiated by 1) depletion of ovarian glycogen during the breeding period owing to the minimal level in the post breeding period, and 2) the reciprocal relationship of liver and ovarian glycogen (Fig. 1). It is therefore suggested that there is some exchange of glycogen from the liver to the ovary. On the contrary, during the second half, that is, during winter, there is an energetic shift away from utilization towards storage. This is evidenced by: 1) marked increase in the fat body (lipid index), 2) increase in liver fat, 3) moderate increase in liver glycogen. The phenomenal increase of the above constituents during the postbreeding and prewintering period may be of great adaptive significance. The frog *R. tigrina* undergoes winter dormancy for a period of about 3 months (Nov.-Jan.) and therefore, the accumulated metabolic reserves are consumed gradually during the winter dormancy. It is suggested that during winter the metabolic machinery is geared towards fat storage for sustained low
metabolism.

To return back to a comparison of energy reserves (fat and glycogen) during aestivation and wintering of *R. tigrina*, it seems clear that two different kinds of adaptations are involved. The adaptive basis of these different patterns of energy reserves shows that a selection has favoured for aestivation during which glycogen accumulates for immediate use for energy production during breeding. On the contrary, the extensive fat deposition (high L. I.) during the prewintering period (Fig. 2) would be needed for ensuring winter dormancy similar to hibernating mammals, which is longer than summer dormancy. It is suggested that there is no advantage for fat accumulation during aestivation when the environmental temperature is high and the frog is under the minimal basal metabolism lasting only for about 2 months, in contrast to the low temperature and longer period (3 months) of the wintering frog. This must have been accomplished by the evolution mechanism of unknown nature which results in accumulation of liver glycogen during aestivation and accumulation of body fat during prewintering. Thus, the accumulation of energy for wintering and rebuilding gonads is fundamentally important for propagating *R. tigrina* in our tropical country.

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