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Oxidation of glucose-U-^{14}C and synthesis of glycogen in different tissues of the garden snail, *Cryptozona ligulata* with reference to aestivation and starvation

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Abstract. The per cent decrease of glycogen content in all the tissues investigated is more in 20-days starved snails than the 4-months aestivated snails when compared to active snails. Recovery of administered glucose-U-^{14}C in the respiratory CO_2 is 42·27% in active snails, whereas it is 8·81% and 26·09% in aestivated and starved snails respectively. Maximal levels of incorporation of labelled glucose were found at 18 hr in all the tissues and the rate of incorporation was greatly elevated in the tissues of aestivated and starved snails. The causes for the difference in the rates of incorporation and the utilization of glycogen in active, aestivated and starved snails are discussed.

Keywords. Oxidation; glucose-U-^{14}C; glycogen synthesis; *Cryptozona ligulata;* aestivation; starvation.

1. Introduction

A number of gastropod snails are known to aestivate during adverse environmental conditions. The slow utilization of glycogen, decreased oxidative metabolism and Kreb's cycle enzyme activities have been observed during aestivation of *Pila globosa* (Sreenivasa Reddy 1974; Raghupathirami Reddy 1965). Meenakshi (1964) concluded that the aestivating *Pila virens* does not consume oxygen and that aestivation metabolism involves anaerobic glycolysis. However, later studies involving administration of ^{14}C-glucose and trapping the evolved $^{14}CO_2$ indicated that the snail, *P. globosa* could be partially aerobic (Raghupathirami Reddy and Ramamurthi 1973). Similar pattern was suggested by Coles (1968) in the aestivation of *P. ovata* where the aestivated snail needed about 17% of oxygen requirement of active snail. Von Brand *et al* (1957) reported that starvation and dehydration caused a decline in oxygen utilisation in the aquatic species *Australorbis glabratus.* In view of lack of information about the synthesis of glycogen in aestivated and starved snails, the present work is undertaken to study the quantitative distribution and *in vivo* synthesis of glycogen in different tissues and the oxidation of glucose-U-^{14}C in active, aestivated and starved garden snail, *Cryptozona ligulata.*

2. Materials and methods

Details of collection and maintenance of active and aestivated snails have been described earlier (Krupanidhi and Naidu 1976). The snails aestivated for 4 months were selected in the present investigation. Some of the snails were allowed to starve but kept active by sprinkling water periodically. The snails starved for 20 days were used in the present study.

One μ ci of glucose-U-^{14}C (specific activity: 210 m ci/m M, isotope division, Bhabha Atomic Research Centre, Trombay, Bombay) in a total volume of 30μ l was administered into each snail with the help of 'Hamilton' microsyringe through a puncture in the soft part of the body.

The CO_2 liberated by two such snails was trapped in 100 ml of saturated potassium hydroxide, precipitated as barium carbonate and processed for measurement of radioactivity as described by Hu (1958) and Bergreen *et al* (1961). Radioactivity was measured with the gas flow proportional counter (ECIL, Hyderabad).

At 6 hr time interval snails were sacrificed (5 at each interval) and different tissues viz., hepatopancreas, foot muscle and the rest of the body were quickly dissected out, weighed and homogenized in 10% trichloro acetic acid. After centrifugation to 1 volume of supernatant 5 volumes of ethanol was added and kept for overnight at 4°C. The precipitate was washed and reprecipitated in ethanol and dissolved in known quantity of distilled water and an aliquot was used for the estimation of glycogen (Carroll *et al* 1956). Another aliquot was transferred to a stainless steel planchet and dried under infrared lamp and radioactivity was measured in proportional counter using duplicate samples and corrected for self absorption. Counts were taken 5 times and the counts per minute were calculated.

3. Results and discussion

The hepatopancreas had the highest content of glycogen compared to the other tissues (table 1) and this may be attributed to the major storage site and an active role of this tissue in general metabolism (Sreenivasa Reddy and Swami 1976). The glycogen content decreased significantly in all the tissues both during aestivation and starvation. The per cent decrease of glycogen content in all the tissues is more in 20 days starved snail than the 4 months aestivated snail (table 1) indicating extremely slow utilisation of glycogen during aestivation.

Table 1. Levels of glycogen in different tissues of active, aestivated and starved snail, *C. ligulata*. Values expressed as mg/g wet weight tissue, are mean ± S.D. of 5 individual observations.

State of the snail	Hepatopancreas		Foot muscle		Rest of the body	
Active	3·189 ± 0·55		1·768 ± 0·16		1·554 ± 0·14	
Aestivated	2·634 ± 0·21		1·026 ± 0·082		0·829 ± 0·069	
Starved	1·032 ± 0·13		0·853 ± 0·097		0·796 ± 0·035	
Comparison of means (students t-test) and changes in %						
	P	%	P	%	P	%
1–2	<0·02	—17·4	<0·001	—42·0	<0·001	—46·7
1–3	<0·001	—67·0	<0·001	—51·8	<0·001	—48·8

The label of injected glucose-U-^{14}C appeared in respiratory CO_2 within a short time (table 2). The expiration of labelled CO_2 showed a stepwise increase with passage of time. In the active snails of *C. ligulata*, 24 hr after the administration of labelled glucose, 42·27% of injected radioactivity was found in CO_2 indicating the rapid oxidation of glucose than the palmitic acid-1-^{14}C (26·08%) (Raghavaiah *et al* 1977) suggesting carbohydrate oriented metabolism in *C. ligulata* which is in agreement with other pulmonates (Emerson 1967). There is a drastic depression of oxidation of injected labelled glucose in 4 months aestivated snails (8·81%). Raghupathirami Reddy and Ramamurthi (1973) also showed that $^{14}CO_2$ output was lower in aestivated *Pila globosa*. The increased glycolytic rate (Sreenivasa Reddy 1974) and the depression in the activities of respiratory enzymes (Raghupathirami Reddy 1967) during aestivation possibly favoured the depression in the oxidation of glucose which probably aids in the economy of organic reserves during aestivation. While in the 20 days starved snail, 26·09% of injected radioactivity was found in the respiratory CO_2. The decrease in the oxidation of labelled glucose in the starved snail when compared to the active snail may be due to the decline in oxygen utilization (Von Brand *et al* 1957).

In the active snail, 6 hours after the administration, glucose-U-^{14}C incorporation into glycogen of the gram wet weight of the hepatopancreas was 3 times more than that of the foot muscle (figure 1). In aestivated and starved snails the incorporation of labelled glucose into glycogen of the hepatopancreas was only slightly more than that of the foot muscle. These results indicate greater glycogen synthetic potential of

Table 2. $^{14}CO_2$ output in active, aestivated and starved snail, *C. ligulata* injected with glucose-U-^{14}C. Values represent recovery of radioactivity (cumulative counts) as per cent of total radioactivity administered.

State of the snail	6 hr	12 hr	18 hr	24 hr
Active	19·29	24·33	31·81	42·27
Aestivated	4·04	5·86	7·24	8·81
Starved	13·74	18·86	22·28	26·09

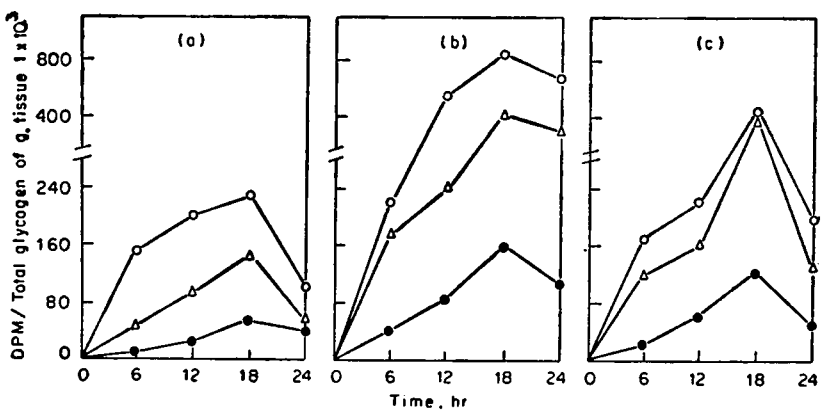

Figure 1. Rate of incorporation of glucose-U-^{14}C (expressed as disintegrations/min/total glycogen of g tissue) into glycogen of various tissues of active (a), aestivated (b) and starved (c) *C. ligulata*. Hepatopancreas O——O; Foot Muscle Δ——Δ; Rest of the body ●——●.

the foot muscle both during aestivation as well as starvation. At 12 hr all the tissues showed a further rise in radioactivity. In active, aestivated and starved snails the maximal levels of incorporation were found at 18 hr in all the tissues and the incorporation from high to low was hepatopancreas > foot muscle > rest of the body. But the rate of incorporation was greatly elevated in all the tissues of aestivated and starved snails. At 24 hr there was a decline in radioactivity of all the tissues and the decline was sharp in active and starved snails and gradual in aestivated snails. In active snail at 6 hr and 12 hr, the level of specific activity (dpm/mg glycogen) was in the following order from high to low: hepatopancreas > foot muscle > rest of the body (figure 2). In the aestivated and starved snails the level of the specific activity from high to low was foot muscle > hepatopancreas > rest of the body. Maximal level of specific activity was found at 18 hr for all the tissues and the foot muscle glycogen recorded the highest specific activity. At 24 hr all the tissues showed lower level of specific activity. There was a sharp fall in specific activity in the tissues of active and starved snails whereas a gradual fall was observed in the tissues of aestivated snails. A rapid increase and sharp fall in radioactivity in the tissues of starved snails as compared to the active snail indicates greater turnover of glycogen. The rapid turnover and low level of glycogen in the foot muscle of the starved snail may indicate that the synthesis and breakdown of glycogen form the major energy cycle during the locomotor activity. The very high specific activity in the tissues of the starved snail may be due to the tremendous depletion of glycogen (table 1). In the tissues of the aestivated snail there was a steep rise and gradual fall in specific activity indicating greater synthesis and lower turnover of glycogen. Increased glycolytic rate, decreased oxidative metabolism and Kreb's cycle enzyme activities during aestivation (Sreenivasa Reddy 1974; Raghupathirami Reddy 1965) might have suppressed the aerobic oxidation of glucose which might result in the low turnover of glycogen. Therefore it has been concluded that aestivation metabolic economy in *C. ligulata* is geared towards conservation of nutrients. Enzymes involved in the synthesis of glycogen may be functioning normally, whereas the activities of enzymes

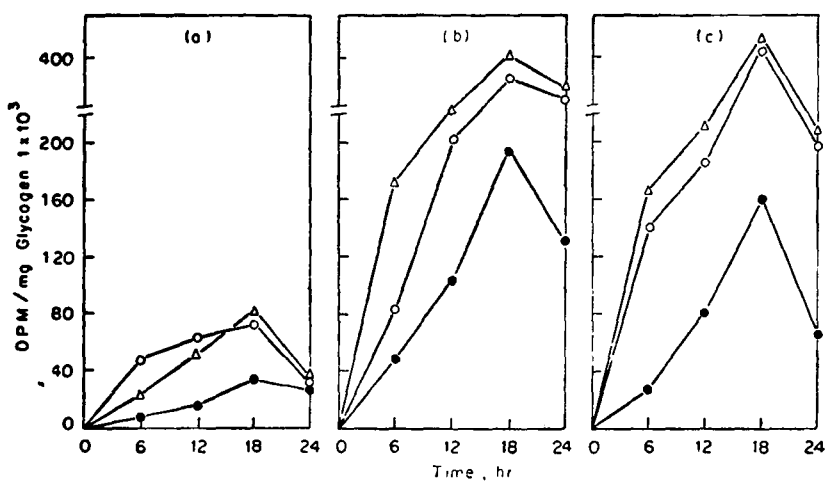

Figure 2. Rate of incorporation of glucose-U-^{14}C (expressed as disintegrations/min/mg glycogen) into glycogen of various tissues of active (a), aestivated (b) and starved (c) *C. ligulata.*

concerned with the break down of carbohydrate might have been drastically depressed as reflected by the high specific activity and lower turnover of glycogen in the tissue of aestivated snails.

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