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Glycogen level and glycogen phosphorylase activity in the eggs of silkworm *Bombyx mori* L.

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Abstract. The glycogen content of the eggs of silkworm *Bombyx mori* L. was analysed at intervals during the course of embryonic development and diapause. Changes in the glycogen levels were also examined in acid treated eggs in which diapause has been broken. These studies showed significant differences in glycogen utilisation pattern in the diapause and non-diapause eggs. The activity of the enzyme glycogen phosphorylase was also found to exhibit different patterns in the two types of eggs. Our studies indicate that this enzyme plays an important role in the metabolic changes which occur during diapause in silkworm eggs.

Keywords. *Bombyx mori*; glycogen; glycogen phosphorylase.

1. Introduction

Glycogen is stored in the eggs of the silkworm *Bombyx mori* L. and is made use of as and when energy is required during the course of diapause as well as embryonic development (Chino 1957). In many other insects also utilisation of glycogen has been found to exhibit a definite pattern which is indicative of metabolic regulation corresponding to the needs of growth and activity (Karlson and Sekeris 1964).

The embryonic development in *B. mori* is interesting because in certain races the embryonic development proceeds uninterrupted while in some, it is characterized by diapause and the diapause eggs can be artificially treated to continue embryonic development without interruption. Therefore, silkworm eggs provide an ideal material for examining the adaptability of glycogen metabolism and its role in diapause. There have been some studies earlier, demonstrating the changes in the glycogen content in silkworm eggs during diapause (Chino 1957, 1958). During the present studies, changes in the glycogen levels during the embryonic development in locally available non-diapause eggs have been examined in detail. Changes in glycogen level in diapause eggs during the course of diapause and also following acid treatment to break diapause have been studied. These studies would explain whether differences, if any, in the pattern of glycogen utilisation in diapause and non-diapause eggs are due to the phenomenon of diapause or due to any inherent differences in these two types of eggs.

The initial and key control step in the utilisation of glycogen is a phosphorolytic reaction catalysed by the enzyme glycogen phosphorylase. Glycogen phosphorylase is an allosteric enzyme capable of occurring in two forms namely an active a-phosphorylase and an inactive b-phosphorylase which are interconvertible (Steele 1982). The vertebrate phosphorylases have been extensively studied (Graves and Wang 1972; Busby and Radda 1976). Similar studies have been carried out in a number of insects and these studies have been reviewed by Steele (1982). However,

not much is known about this enzyme in silkworms. The activity of glycogen phosphorylase has been studied at intervals in all the 3 types of eggs to examine whether the enzyme activity can always account for changes in glycogen levels and to see how the activity of the enzyme differs in diapause and non-diapause eggs.

2. Material and methods

2.1 *Materials*

Bivoltine (NB_4D_2) and multivoltine (Pure Mysore) silkworm races were maintained under standard conditions. Diapause eggs of the bivoltines and non-diapause eggs of multivoltines were used for the experiments. Loose eggs were prepared on polythene sheets and kept at 25°C with a relative humidity of 75%.

2.2 *Breaking of diapause*

For breaking diapause, 20 h old diapause eggs were treated with HCl solution of specific gravity 1·075 at 46·1°C for 3–4 min, washed thoroughly with water and dried. The eggs were then kept at 25° ± 2°C.

2.3 *Determination of glycogen in eggs*

Glycogen was determined by the method of Hassid and Abraham (1957) with slight modification.

2.4 *Enzyme preparation*

A 10% (w/v) homogenate of the eggs were prepared in ice cold distilled water with a glass homogenizer fitted with teflon pestle. The homogenate was filtered through a cotton pad and centrifuged at 5500 g for 15 min at 0°C. The supernatant was filtered through Whatman No. 1 filter paper and the resultant filtrate was used as the enzyme source.

2.5 *Determination of enzyme activity*

The activity of phosphorylase was assayed according to the procedure of Cori *et al* (1973). The inorganic phosphate was estimated by Fiske and Subba Row (1925) method. The enzyme activity is expressed as μ mol P_i/min/mg protein. Protein concentration was determined according to the method of Lowry *et al* (1951) using Bovine serum albumin as standards.

All the above experiments were conducted in 7–8 samples and the mean values expressed along with the standard deviation.

3. Results

3.1 *Studies on glycogen levels*

At the time of egg laying, the level of glycogen was found to be more in diapause eggs than in non-diapause eggs (figures 1 and 2).

In non-diapause eggs, the glycogen level decreased very gradually during the first 3 days following oviposition and on the 3rd day, the glycogen content of the eggs was not much different from that of the newly laid eggs. Following this period, there was a more significant decrease and glycogen decreased to 60% of the initial content around the 9th day.

In diapause eggs after acid treatment, with the onset of development glycogen level followed a pattern very similar to that observed in non-diapause eggs (figure 2). In diapause eggs, a pattern altogether different from the above two types was seen (figure 3). The glycogen content started decreasing markedly and suddenly 1 day after oviposition and it was reduced to 25% of the initial level within 4 days after oviposition. The glycogen level decreased to about 17% of the initial level by the 10th day and it remained at this level for a long time (figure 4).

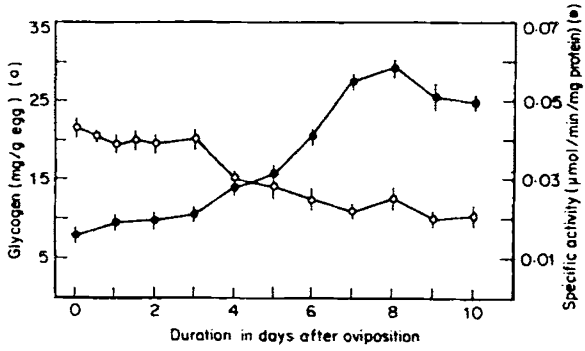

Figure 1. Changes in the glycogen level and glycogen phosphorylase activity during embryonic development in non-diapause eggs.

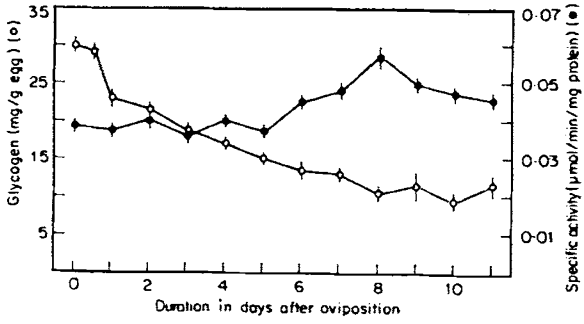

Figure 2. Changes in the glycogen level and the glycogen phosphorylase activity during embryonic development in bivoltine eggs after acid treatment.

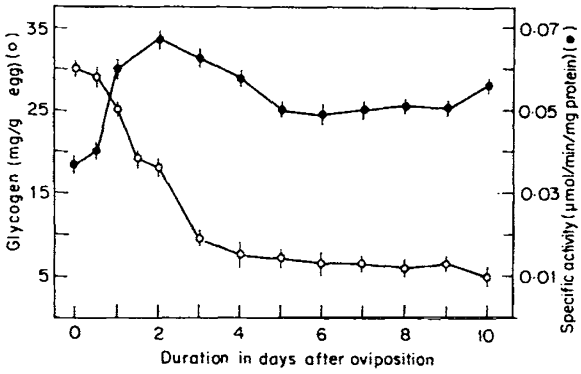

Figure 3. Changes in the glycogen level and the glycogen phosphorylase activity during diapause in bivoltine eggs.

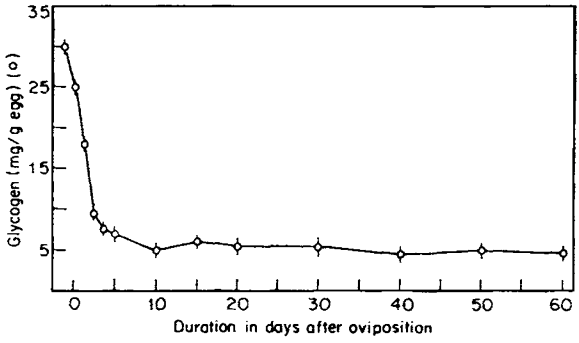

Figure 4. Changes in the glycogen content of the bivoltine eggs during diapause.

3.2 *Studies on phosphorylase activity*

In non-diapause eggs, the phosphorylase activity was low to start with after egg laying (figure 1). The level started increasing about 3 days after oviposition and was highest at the time of hatching.

In diapause eggs after acid treatment, the total phosphorylase activity was comparable to that observed in non-diapause eggs. There was an increase in the level of activity about 5 days after acid treatment and a maximum level was obtained around the 8th day after oviposition (figure 2).

In diapause eggs, the level of activity soon after oviposition was slightly higher than that observed in non-diapause eggs. But the activity suddenly increased 12 h after oviposition to reach a peak on the 2nd day following which the activity decreased to 74% of the peak level which was more than the initial level and around this increased level it was maintained over a long period (figure 3).

4. Discussion

It is evident from our studies that the glycogen content of the diapause eggs at the time of oviposition is much higher than that of non-diapause eggs. This may be an

adaptive phenomenon. Because, in non-diapause eggs the embryonic development proceeds and completes in about 10 days while in diapause eggs, considerably long period precedes the development and a demand for energy can be expected to occur during diapause even though to a smaller degree compared to that during embryonic development.

The glycogen level gradually decreased in non-diapause eggs and similar decrease in glycogen level was also observed in diapause eggs after acid treatment, showing that glycogen may be an important source of energy during growth and differentiation in silkworm eggs. During the early phase of embryonic development, the non-diapause eggs provide a marked contrast to diapause eggs. While in non-diapause eggs, the glycogen level decreases very gradually, in diapause eggs rapid fall in glycogen level is observed. Thus, in diapause eggs though growth and development are temporarily suspended, glycogen is being broken down for some purpose. This marked decrease in glycogen level also coincides with the onset of diapause. These observations agree with those of Chino (1957) and Yamashita *et al* (1975).

The activity of the enzyme phosphorylase shows a reciprocal relationship with the level of glycogen in all the 3 types of eggs. In diapause eggs phosphorylase activity reaches a very high level 2 days after oviposition explaining the rapid rate of glycogen break down associated with the initiation of diapause.

Newsholme and Start (1973) have shown that glycogen phosphorylase plays an important role in the metabolism of glycogen in many species of animals. Our studies also indicate that glycogen phosphorylase plays an important role in the utilization of glycogen at the initiation of diapause.

The pattern exhibited by phosphorylase activity, wherein it increases accompanied by decrease in glycogen level seems to be a general pattern in silkworms since the same was also reported in the eggs of *Philosamia ricini* (Pant and Nautiyal 1974). Interestingly, when non-diapause eggs of silkworm were subjected to anoxia, the glycogen phosphorylase activity was found to be abruptly elevated (Yamashita *et al* 1981), showing that there is always a rapid break down of glycogen associated with the onset of diapause and that phosphorylase acts as a key enzyme involved in this glycogen utilisation. Hence, it is suggested that glycogen phosphorylase plays a key role in regenerating the glycosyl units either as a source of energy or as building blocks for biosynthetic reactions in silkworm eggs during the embryonic development and during diapause.

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References

Busby S J W and Radda G K 1976 Regulation of the glycogen phosphorylase system—From physical measurements to biological speculations; *Curr. Top. Cell Regul.* **10** 89–160

Chino H 1957 Carbohydrate metabolism in diapause eggs of the silkworm *Bombyx mori*. I. Diapause and the change of glycogen content; *Embryologia* **3** 295–316

Chino H 1958 Carbohydrate metabolism in the eggs of the silkworm *Bombyx mori*. II. Conversion of glycogen into sorbitol and glycerol; *J. Insect Physiol.* **2** 1–12

Cori G T, Illingworth B and Keller P J 1973 Muscle phosphorylase; *Methods Enzymol.* **1** 200–205

Fiske C H and Subba Row Y 1925 The colorimetric determination of phosphorus; *J. Biol. Chem.* **66** 375–400

Graves P J and Wang J H 1972 Glucan phosphorylases—chemical and physical basis of catalysis and regulation; *Enzymes* **7** 435–482

Hassid and Abraham 1957 Chemical procedures for analysis of polysaccharides; *Methods Enzymol.* **3** 34

Karlson P and Sekeris C E 1964 Comparative Biochemistry (New York: ACAD Press) Vol. 6 pp. 180–220

Lowry O H, Rosebrough N J, Farr A L and Randall R J 1951 Protein measurement by the Folin phenol reagent; *J. Biol. Chem.* **193** 265–275

Newsholme E A and Start C 1973 *Regulation in metabolism* (London: John Wiley and Sons) pp 80–98

Pant R and Nautiyal G L 1974 Changes in protein, glycogen, free sugar content and active phosphorylase activity during embryogenesis of *Philasamia ricini*; *Proc. Indian Acad. Sci.* **B80** 121–126

Steele J E 1982 Glycogen phosphorylase in insects; *Insect Biochem.* **12** 131–147

Yamashita O, Suzuki K and Hasegawa K 1975 Glycogen phosphorylase activity in relation to diapause initiation in *Bombyx* eggs; *Insect Biochem.* **5** 707–718

Yamashita O, Yaginuma T and Hasegawa K 1981 Hormones and metabolic control of egg diapause of the silkworm *Bombyx mori* (Lepidoptera: Bombycidae); *Entomol. Gener.* **7** 195–211