Change of Glycogen Content in the 'diapause' Egg and in the 'non-diapause' Egg activated by a Hydrochloric Acid Treatment in Bombyx mori L.

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Introduction

It was well known that almost all glycogen initially present in the 'diapause' egg of the *Bombyx* silkworm was rapidly broken down with onset of diapause and it was resynthesized concurrently with the termination of diapause (NAKATA, T. '52, CHINO, H. '57). The end-product of this breakdown of glycogen in the 'diapause' egg was not lactic acid but two polyols, sorbitol and glycerol (CHINO, H. '58).

In order that the 'diapause' egg of the silkworm was made to develop without onset of diapause, an effective method was employed practically, where the 'diapause' egg was treated with warm hydrochloric acid for several mintes at about 20 hours after the oviposition.

In present experiment, change of glycogen content in the 'diapause' egg of *Bombyx* silkworm activated by treatment with hydrochloric acid was mainly observed, and a quite interesting result was obtained.

Materials and Methods

'Diapause' eggs from the hybrid between Chinese and Japanese races were used as materials.

To prepare the glycogen in the eggs, an adapted method based on that of FUJII ('61) was used, where the eggs were ground with 2ml. of 30% KOH in a glass tube and added 2 or 3 drops of saturated KCl and 2 volumes of 95% alcohol after boiling for 20 minutes in a boiling water bath, then they were cooled in a room temperature, after 2 hours or more the mixture was centrifuged at 3000 RPM. for 30 minutes. The sediment was dissolved in warm distilled water and added 2 volumes of 95% alcohol and heated in boiling water bath until the boiling occurred, then centrifuged again at 3000 RPM. for 30 minutes after cooling to a room temperature. The sediment was heated on a

boiling water bath until alcohol was completely evaporated. The preparation for determination of glycogen was made through the treatment where the sediment obtained by above process was dissolved in hot distilled water and after cooling to a room temperature distilled water was added to this solution to make 15ml.

In order to the determination of glycogen the method of MORRIS, D. L. ('48) was employed, i, e. 2 volumes of 0.2% anthrone solution where 200mg of anthrone was dissolved in 100ml of 95% sulphuric acid was added to the test sample, and the colour developed in the mixture was measured colorimetrically by a spectrophotometer with wave length of 620mμ. In order to obtain the best results of determination, the reaction mixture was heated in boiling water bath for 5 minutes and then cooled in a running water bath. The standard solution for the determination was the solution where contained 100μg glycogen.

In present experiment such numbers of eggs were used for determination of glycogen as their glycogen content was between 10 and 100μg, for the Lambert–Beer's law of this method was applicable to the glycogen content ranging between 10 and 100μg. In most cases the numbers of eggs used in each determination was 20 eggs, but in some cases 40 eggs were used.

To activate the 'diapause' eggs of *Bombyx*, a treatment with warm hydrochloric acid was used, where the 'diapause' eggs were immersed into the hydrochloric acid solution (specific gravity 1.075) for 5 minutes in 46°C. at about 20 hours after oviposition, then they were incubated after washing with water.

Results and Discussion

1. *Glycogen contents during diapause in eggs of Bombyx mori L.*

In one experiment, 'diapause' eggs were kept intact in seasonally changed room temperature for long period, and their glycogen contents were determined during long period (from middle of July to next April). After the considerable long exposure to the low temperature of winter season, glycogen in egg began to increase.

In this stage some eggs were incubated in 25°C. and their developments were observed.

In another experiment, eggs were kept 5°C. from 1 day after oviposition and their glycogen contents were determined during long period. After the glycogen in egg began to increase, some eggs were incubated in 25°C. and their developments were observed.

The results were shown in tables 1, 2 and figure 1.

In tables 1, 2 and figure 1, it was clearly shown that the glycogen of the 'diapause' egg in seasonal temperature which was high temperature in July

Table 1. Glycogen contents of the 'diapause' eggs of *Bombyx mori* L. kept in seasonal temperature.

Age of eggs (day)	Glycogen contents μg/egg	mg/1g eggs	Note
1	26.3	36.0	7/VII oviposition.
2	19.2	32.0	
3	8.0	12.9	
5	3.0	4.9	
10	3.2	5.3	
14	2.3	3.7	
17	1.17	1.82	
20	1.25	2.08	
30	0.54	0.88	
50	0.53	0.91	
75	0.63	0.98	
100	1.47	2.32	
150	1.05	1.74	Hatching did not occur.
175	5.74	8.94	〃
187	9.73	17.8	〃
205	10.2	16.9	〃
223	14.7	26.3	Hatching occurred.
242	13.7	25.9	〃
270	30.0	50.0	〃

Table 2. Glycogen contents of the 'diapause' eggs of *Bombyx mori* L. Kept in 5°C.

Age of eggs (day)	Glycogen contents μg/egg	mg/1 g eggs	Note
1	26.3	36.0	7/VII oviposition.
2	19.2	32.0	Eggs transferred in 5°C.
6	11.3	19.0	
8	7.6	12.2	
12	7.1	11.8	
17	6.5	11.1	
20	6.7	11.6	
30	2.7	4.7	
35	2.3	3.8	
40	1.9	3.3	
45	1.4	2.4	
50	0.85	1.5	
65	5.6	9.7	Hatching did not occur.
75	7.2	13.1	〃
85	6.7	12.2	〃
100	8.3	15.1	〃
110	11.3	20.4	Hatching occurred.
125	15.4	25.3	〃

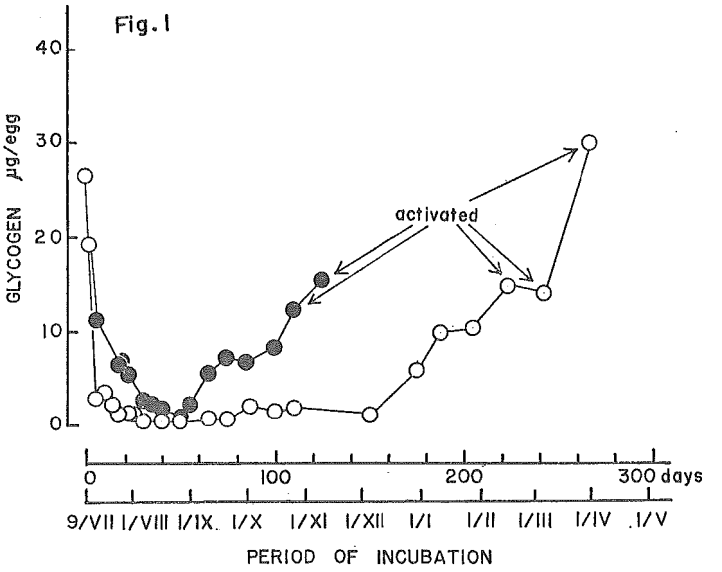

Fig. 1. Glycogen content of 'diapause' egg of *Bombyx mori*. The hollow circle and the solid circle represent the glycogen content of the eggs kept intact in room temperature varying with the seasonal temperature change and the glycogen content of the eggs kept in 5°C. respectively.

decreased rapidly after the oviposition, and the glycogen was kept in very low level for long period, then it increased again after the considerablly long exposure to the low temperature of winter. In these tables and figure, it was also shown that the glycogen of the 'diapause' egg in 5°C. decreased more slowly than that of the eggs in higher temperature, and it increased again after the shorter period without the decrease to the minimum level of the previous case.

From these results it was clearly shown that the glycogen contents of the 'diapause' eggs of *Bombyx mori* L. and its change with diapausing process of the eggs were fundamentally consisitent with those obtained by NAKATA, T. ('52) and by CHINO, H. ('57).

It was shown in the results from the incubation of the 'diapause' egg in 25°C. that the hatching of the egg occurred when the glycogen increased to the level above 20 mg per 1 g eggs or 11 μg per one egg.

2. *Change of glycogen content in the 'non-diapause' egg activated by a hydrochloric acid treatment in Bombyx mori L.*

Glycogen contents in the 'non-diapause' eggs by a hydrochloric acid treatment in *Bombyx mori* were determined in following cases. (a) The eggs were incubated in 25°C. after the treatment. (b) The eggs were incubated in 5°C. after the treatment. (c) The eggs were incubated in 25°C. for 24 hours then

incubated in 5°C. after the treatment. As the control cases the glycogen contents in the 'diapause' eggs incubated in 25°C. and in 5° C. without the hydrochloric acid treatment were determined.

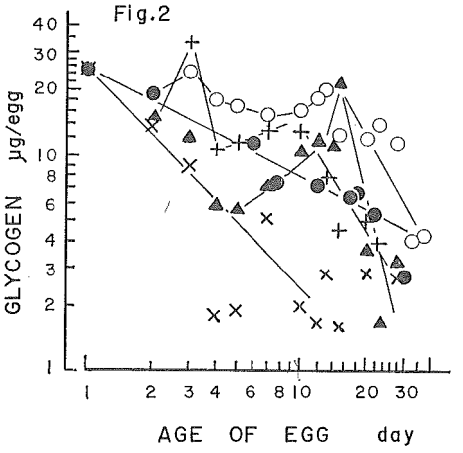

Fig. 2. Glycogen contents of the 'diapause' egg and 'non-diapause' egg activated by a hydrochloric acid treatment in *Bombyx mori*. Signs represent as follows——× : glycogen content of the 'diapause' egg incubated in 25°C., ● : glycogen content of the 'diapause' egg incubated in 5°C., ○: glycogen content of the 'non-diapause' egg incubated in 5°C. after the acid treatment, ▲ : glycogen content of the 'non-diapause' egg incubated in 25°C. after the acid treatment, + : glycogen content of the 'non-diapause' egg incubated in 25°C. for 24 hrs. then incubated in 5°C. after the acid treatment.

Table. 3. Glycogen contents of the eggs of *Bombyx mori* L. incubated in 25°C.

Age of eggs	Intact eggs (A)	HCl-treated eggs (B)	Difference between B and A	Logarithmic value of difference
day	μg/egg	μg/egg	μg/egg	
1	25.0	25.0	0.0	
2	13.5	15.1	1.6	0.205
3	8.7	12.2	3.5	0.545
4	1.8	5.8	4.0	0.603
5	1.9	5.7	3.8	0.580
7	4.1	9.3	5.2	0.716
10	2.0	10.5	8.5	0.930
12	1.6	12.0	10.4	1.016
13	2.9	10.9	8.0	0.903
15	1.5	21.6	20.1	1.303
20	2.8	3.7	0.9	$\bar{1}$.955

The results were shown in tables 3, 4 and figure 2. In the figure 2, it seems to be obvious that glycogen in the 'diapause' egg decreases reciprocally to the age of the egg, and the glycogen content is nearly represented by the following mathematical expression, $y=ax^{-b}$, where x represents the age of the egg in day, y represents the glycogen content of the egg in μg/egg, a and b represent the constant values respectively. The glycogen content obtained from the determination was nearly consistent with that calculated from the expressions, $y =$

Table 4. Glycogen contents of the eggs of *Bombyx mori* L. incubated in 5°C.

Age of eggs	Intact eggs (A)	HCl-treated eggs (B)	Difference between B and A	Logarithmic value of difference
day	μg/egg	μg/egg	μg/egg	
1	25.0	25.0	0.0	–
2	17.7	18.5	0.8	1.913
3	14.4	24.5	10.1	1.005
4	12.5	17.8	5.3	0.725
5	11.2	17.1	5.9	0.772
7	9.5	15.2	5.7	0.756
10	7.9	16.4	8.5	0.955
12	7.5	18.4	10.9	1.037
13	6.9	19.5	12.6	1.100
15	6.5	12.5	6.0	0.778
20	5.6	12.2	6.6	0.820

$25x^{-1}$ in the eggs incubated in 25°C. and $y=25x^{-0.5}$ in the eggs incubated in 5° C.

It was also shown in figure 2 that the glycogen content in the 'non-diapause' egg increased with some latent period in all cases. However, a remarkable difference in the glycogen change at early period of the incubation was found among three cases. While the peak of the increase of glycogen in the 3-day-old egg in 25°C. was not so obvious, the peak of the increase of glycogen in the 3-day-old egg in 5°C. was quite obvious.

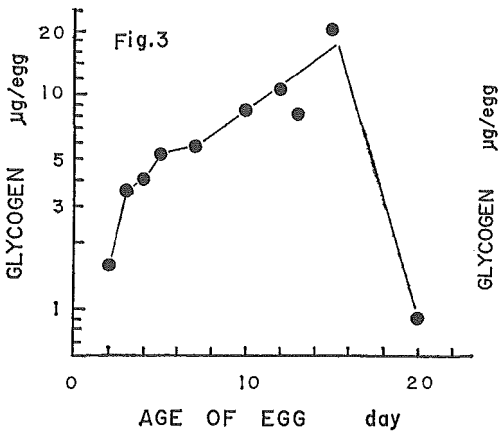

Fig. 3. Differences of glycogen contents between the 'non-diapause' eggs and the 'diapause' eggs in 25°C. incubation.

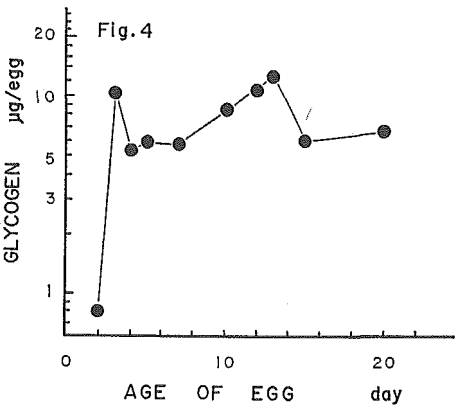

Fig. 4. Differences of glycogen contents between the 'non-diapause' eggs and the 'diapause' eggs in 5°C. incubation.

The differences of glycogen contents between the 'non-diapause' eggs activated by hydrochloric acid treatment and the 'diapause' eggs in 25°C. were shown in figure 3. These differences were also shown in table 3. The differences of glycogen contents between the 'non-diapause' eggs and the 'diapause' eggs in 5°C. were shown in figure 4, and in table 4. In figures 3, 4 and tables 3, 4, it was found an interesting fact that an almost equal increase of glycogen occurred between 7-day-old and 13 or 17-day-old 'non-diapause' eggs in both 25°C. and 5°C. incubations. In this period the logarithmic values of glycogen differences between 'non-diapause' eggs and 'diapause' eggs increased proportionally to the ages of the eggs.

In these figures it was shown that in later stage the glycogen of the 'non-diapause' egg decreased. It is probable to consider that this decrease of glycogen in later stage of the 'non-diapause' egg is due to the breakdown of glycogen for the development of the egg, for the glycogen decreased rapidly in 25°C. where the egg developed rapidly on the one hand, and the glycogen decreased more slowly in 5°C. where the egg developed very slowly on the other hand.

The remarkable increase of glycogen of the early stage in the 'non-diapause' egg activated by a hydrochloric acid treatment was also shown clearly in the figure 4.

In the results obtained from the present experiments, it seems to be suggested a possibility that the four different reactions were responsible for the change of glycogen in the 'non-diapause' egg activated by a hydrochloric acid treatment, where the first reaction was responsible for the breakdown of glycogen with onset of the diapause in the 'diapause' egg, the second reaction was responsible for the decrease of glycogen in the developmental process of the egg, the third reaction was responsible for the increase of glycogen occurred in the 'non-diapause' egg activated by a hydrochloric acid treatment at early age of the egg, and the last reaction was responsible for the increase of glycogen occurred in the later age of the egg. According to CHINO, H. ('60), there were polyol dehydrogenases catalysing four different reactions in the silkworm egg, and all these polyol dehydrogenases were found not only in the diapausing egg but also in the developing egg where the accumulation of sorbitol and glycerol is never seen. In this connection, it may be possible to consider that a quite interesting result was obtained from present experiments.

Summary

(1) Glycogen contents of the 'diapause' eggs of *Bombyx mori* L. were determined for long period in the next two cases, where 'diapause' eggs were kept intact in seasonal room temperature from July to next April on the one hand,

and the other they were kept in 5°C.

(2) Glycogen of the 'diapause' egg in higher temperature decreased rapidly at early period from the oviposition, and it increased gradually after the eggs were exposed to the low temperatures of winter season for a considerable long period. In the 'diapause' eggs kept in the low temperature, 5°C. from the next day after the oviposition, glycogen decreased more slowly and it increased again after the shorter period without the decrease to the minimum level of the previous case.

(3) When the silkworm eggs of which glycogen increased over the definite level were incubated in 25°C, hatching occurred normally. Presumablly, it may be supposed from the present results that such glycogen level is about 11μg/egg.

(4) In regard to the glycogen change in 'diapause' egg of *Bombyx mori* L. the present results were fundamentally consistent with those obtained by NAKATA, T. ('52) and by CHINO, H. ('57).

(5) In relation to the activation of 'diapause' egg by a hydrochloric acid treatment, the change of glycogen content of the egg of *Bombyx mori* in early period of incubation was investigated.

(6) In the 'diapause' egg, it was observed that glycogen decreased reciprocally to the age of the egg, and the content of glycogen was nearly represented by the following mathematical expression, $y=ax^{-b}$, where x represents the age of the egg in day, y represents the glycogen content in μg/egg, a and b are constant values. In 25°C. the expression was $y = 25x^{-1}$ and in 5°C. it was $y = 25x^{-0.5}$.

(7) Glycogen of the eggs which were treated with hydrochloric acid about 20 hours after oviposition increased with considerable rate after some latent period, and the rate of the increase was almost equal regardless of the temeperature of their incubation.

(8) It was suggested from the results that there was a possibility that four different reactions were responsible for the change of glycogen in the 'non-diapause' egg activated by a hydrochloric acid treatment.

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*=in Japanese