

STUDIES ON THE RHYTHMICITY OF HATCHING IN SILKWORM EGGS, *BOMBYX MORI L.*

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INTRODUCTION

It is well-known fact that silkworm eggs hatch with a diel periodicity every daybreak. Such a diel rhythmicity implies reasonably that the light stimulation (the sun light) should be a controlling factor for hatching, and it has been known practically by experience since considerable old times in Japan. A pretty many investigations*, therefore, have been carried out on incubation methods or hatching phases of silkworm eggs. Among these, many papers** are treated on effects of light on hatching when the eggs are subjected to light at various embryonic stages. From the results it becomes clear that the light stimulation is an important factor to cause hatching.

Silkworm eggs, however, can hatch rhythmically without light stimulation at the stage of larval body completion (TANAKA 1957). Researching on the effectiveness of light and dark upon the hatching phase of silkworm eggs at later growth stages, TAKEUCHI (1956, 1957 a b c) has revealed that the rhythmicity with a regular cycle exists in the hatching feature regardless of their races. A similar phenomenon is observed in the moulting phase of the larvae (F. TANAKA 1956) and also in the imaginal emergence of silkworms (Tsuboi 1910, Kimura 1952). But these studies have hardly paid special concern to the rhythmicity. Then the question whether the daily rhythmicity is brought by an endogenous stimulus, by an exogenous one or by the above dual ones has been left unexplained. Then, tried to clear up these problems, the author carried out several experiments, in which the occurrence of the rhythmicity of hatching was examined under the constant condition and under shift of environmental factors, using the eggs of various strains of silkworms. Consequently it has been ascertained that silkworm eggs have a hereditary character which persists generally about 18-hr rhythmicity of hatching. Further he analyzed the actions of light and dark, of warm and cool or of combined condition of each factor upon the manifestation of the rhythmicity.

These experimental results are given in the present paper.

Before going further the author wishes to acknowledge the indebtedness to Dr. N. YAGI for his cordial guidance throughout the study and reading the original manuscript. Thanks are also due to Dr. N. KOYAMA and Dr. E. NAGASHIMA for their kind advices and supports for the study. Further the author desires to express his gratitude to Ass. Prof. H. MACHIDA who gave him encouragement in many ways, and to Mr. I. CHINO who kindly proposed a lot of silkworm eggs for the author's use,

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CHAPTER I RACIAL DIFFERENCES IN HATCHING PHASES

Up to the present, hatching phases of silkworm eggs have been studied mainly on those of so-called "normal races", the eggs of which take purplish black colouration. Tried to clear up the racial differences of hatching phases, the author carried out the following experiments using various varieties which differ genetically in egg colour, moltinism or voltinism, from the normal races. Besides a comparison was made of the hatching phase in *Bombyx mori* with that in *B. mandarina*.

MATERIALS AND METHOD

Each group of about 1000 hibernated eggs of silkworms and of about 180 eggs of 2nd generation of *B. mandarina* was pasted on a thick paper and incubated under the constant conditions of temperature (25°C), R. humidity (80%) and light (dark, 10 lux, 100 lux), respectively.

The incandescent lamp of Matsuda was used as the light sources. Number of larvae hatched out was counted, swept off from the paper with a feather-brush, at every interval of 3 hours from the beginning to the end of hatching. Especially in the constant dark condition, a deep care was taken, at the counting, not to give any light stimulation upon the eggs. The hatchabilities in all experiments were expressed with percentage.

1. Rhythmicity of Hatching in a Hybrid

The eggs of a hybrid, *Taihei* × *Choan* (Japanese bivoltine race × Chinese bivoltine race) were used for the materials. The incubation of eggs began in May, 13th.

The results obtained are shown in Fig. 1.

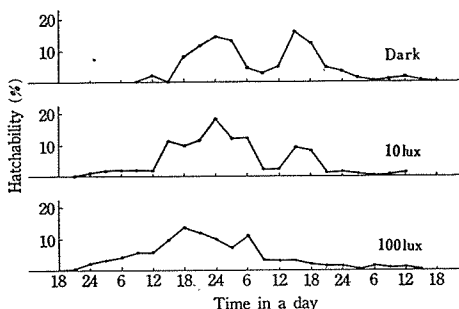


Fig. 1 Rhythmic phase of hatching in *Taihei* × *Choan*

The initial hatching appeared after 9 days and 6 hours in 100 lux régime, after 9 days and 9 hours in 10 lux régime, and after 9 days and 18 hours in the dark condition. The fact indicates that the light promotes the growth of eggs. The light, notwithstanding, seems also to disturb the hatching phase, because the termination of hatching in each experiment is almost at the same time.

In the dark condition, the rhythmicity of hatching is apparently recognized; two modal peaks of hatchability appear

rhythmically with 18-hr interval. On the other hand, in the light conditions the daily rhythmicity can hardly be seen though small modal peaks of hatchability exist indistinctively.

2. Rhythmicity of Hatching in the Three Main Races

As above mentioned, the eggs of the hybrid of silkworms hatch with about 20-hr rhythmicity under the continuous darkness. Then the author performed the following experiments to detect the differences of hatching phases among Japanese, Chinese and European races. The incubation of eggs of each race was commenced at 12 a. m. May, 28.

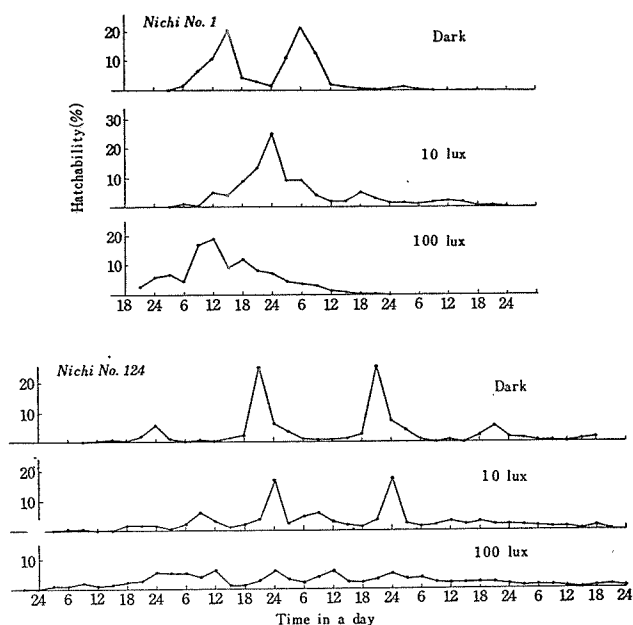


Fig. 2 Rhythmic phases of hatching in Japanese races

Japanese race : *Nichi-No. 1* (univoltine) and *Nichi-No. 124* (bivoltine) were used for the materials. Fig. 2 shows the experimental results.

In the case of *Nichi-No. 1* we can observe two marked peaks of hatchability in the dark condition, a modal interval between the peaks being about 15 hours. It suggests the existence of rhythmicity in hatching. In 10 lux condition, however, two typical peaks as seen in the dark one disappear and the hatching rhythm is hardly distinguishable, the first and the second modal intervals being 12 hours and 24 hours, respectively. In 100 lux condition the interval between modal peaks becomes very narrower and the hatching rhythm cannot be recognized.

In the case of *Nichi-No. 124* an attention should be paid to longer duration of

hatching than the former variety. Under the dark condition there exist certainly four rhythmical peaks with 24-hr interval. This rhythmical phase continues, being a little deformed, under 10 lux incubation, but under 100 lux one the hatching period is quite postponed as the modal peaks flattened. The rhythmicity of hatching seems to be indefinite in higher illumination.

Above facts show that the manifestation of hatching of silkworm eggs differs according to the varieties in the same race.

Chinese race : The eggs of *Shi-No. 108* (bivoltine) was used for the materials.

As indicated in Fig. 3, the regular rhythm of hatching appears conspicuously in the dark incubation. In 10 lux incubation this tendency is still maintained, but not clearer than the former, the modal interval of hatchability reducing to 18 hours. In

100 lux incubation, however, the eggs hatch irregularly and the rhythmic character in hatching seems to disappear.

European race: The eggs of *O-No. 19* (univoltine) were used for the experimental materials (Fig. 3). In the dark régime the hatching phase of the variety is more uneven than that of aforementioned races, the rhythmical modes of hatching being not so significant. Further any appreciable regularity in hatching cannot be recognized.

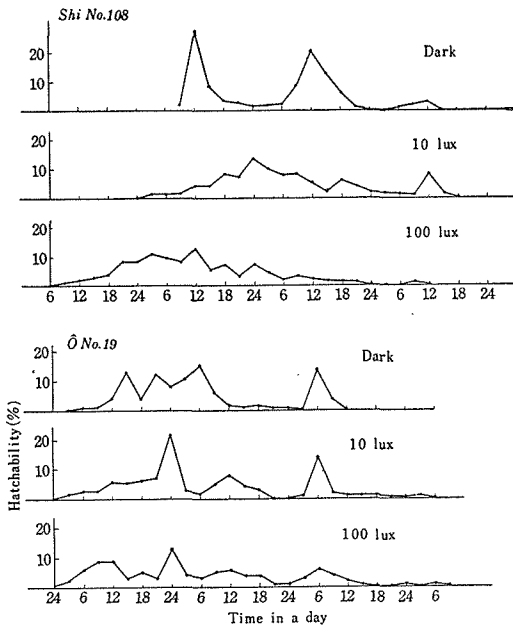


Fig. 3 Rhythmic phases of hatching in Chinese and European races

3. Egg Colour Mutants and the Rhythmicity of Hatching

From above experiments the author got the knowledge that most of varieties the egg of which take black colouration, had the rhythmic character in hatching.

In this time the author researched comparatively the hatching phases in the egg colour mutants such as w_1 , re and b_1 . The results are shown in Fig. 4.

White egg mutant (w_1): Under the dark incubation two modal peaks of hatchability with 15-hr interval appear, but the rhythmicity is not clear. Under 10 lux illumination the peaks of hatchability become much lower, though about 15-hr interval is faintly kept. The rhythmic feature of hatching is completely inhibited by 100 lux light in which the hatching duration is more or less accelerated.

Red egg mutant (re): Any rhythmical hatching is indistinguishable not only in the case of dark régime but also in the case of light régime, though the higher the

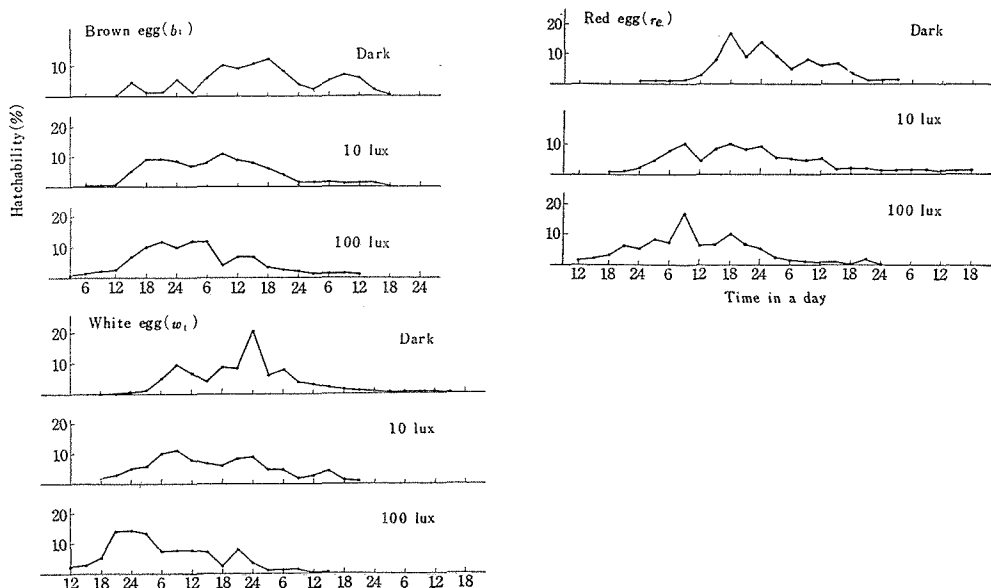


Fig. 4 Hatching phases in egg colour mutants

light intensity the quicker the hatching time.

Brown egg mutant (b_1): The hatching phase is generally irregular. Under the dark condition some modal peaks of hatchability are observed, but they do not continue the definite cycle. Under 10 lux and 100 lux conditions we cannot find any rhythmic feature of hatching.

Thus the author has obtained the conclusion that the regular rhythmicity of hatching hardly occurs in the egg colour mutants. This fact will be caused by some essential differences between the abnormal coloured eggs and the normal black eggs.

4. Moltinism and the Rhythmicity of Hatching

The normal races of silkworms are generally four moulter. Besides some mutants are known which pass hereditarily three exuviations or five ones. The three moulter is shorter and five moulter is longer than the four moulter in whole larval period.

In this experiment the author studied the hatching phase in less or more moulter using *Kyokko* and *Gominhaku*, the eggs of which are black as same as the normal eggs.

The incubation was undertaken from May, 28th. The results are illustrated in Fig. 5.

Three moulter (*Kyokko*): Under the dark and 10 lux incubations there appear the modal peaks of hatchability with 9 or 12-hr intervals, but the regular rhythmicity

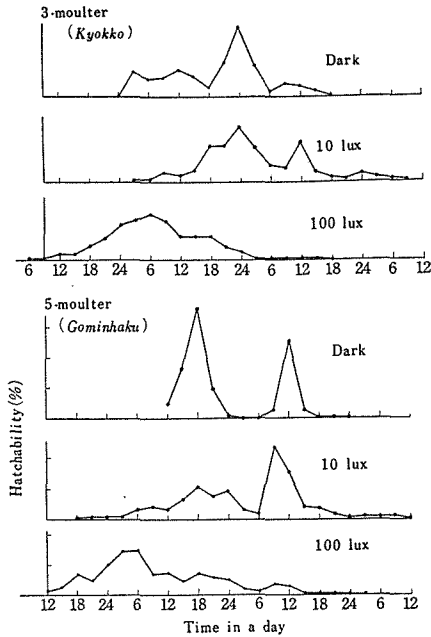


Fig. 5 Hatching phases in 3 and 5-moulters character as in the normal four moult, shows 18-hr rhythmicity in the dark condition while the three moult scarcely exposes such a rhythmicity.

5. Voltinism and the Rhythmicity of Hatching

In this section the hatching phases of the sub-multivoltine and the multivoltine strains were researched comparing with those of uni- and bi-voltine strains.

The incubation carried out from May, 28. The results obtained are given in Fig. 6.

Sub-multivoltine strain (Daizo): This strain is one of Chinese races and shows naturally two or more voltinism. There appear two modes of hatchability under the

of hatching unlikely exist. Under 100 lux condition the large promotion of hatching is seen, but the modal peaks disappear.

Five moult (Gominhaku): In the dark condition the start of hatching is delayed as compared with the case of light conditions. The hatching phase, however, is well characterized with 18-hr rhythmicity. In 10 lux condition the first mode of hatchability diminishes, yet the rhythmicity with 15-hr cycle is faintly maintained. One hundred lux illumination accelerates the hatching, in which the sharp rhythmic feature cannot be observed.

From abovementioned results it is suggested that the hatching feature in the five moult, with almost the same

character as in the normal four moult, shows 18-hr rhythmicity in the dark condition while the three moult scarcely exposes such a rhythmicity.

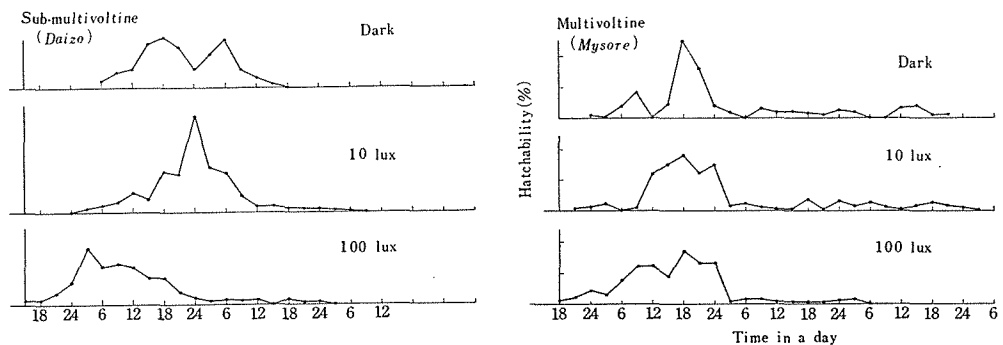


Fig. 6 Hatching phases in multivoltine strains

dark incubation, but the interval between them is 12-hr. Therefore it is quite doubtful whether such a cycle should be called the real rhythmicity of hatching in silkworms. A discussion on this problem will be provided in the later chapter. Under the light conditions any rhythmic sign of hatching could not be recognized.

Multivoltine strain (*Mysore*): *Mysore* used for the experimental materials is a strain of Indo-Chinese races and has w_3 gene. The hatching phase is very much irregular and the rhythmicity unlikely exists neither under the dark condition nor under the light condition.

As explained above, it is obvious that the same rhythmical hatching as seen in the normal races does not occur in the present strain.

6. Rhythmicity of Hatching in *Bombyx mandarina*

In the normal black eggs of silkworms the rhythmic hatching took place generally with 18~21-hr cycle. Then, in this experiment a comparative study on hatching phase was done between *Bombyx mori* and *B. mandarina*; the latter has been believed as an ancient type of domestic silkworms. The eggs of 2nd generation

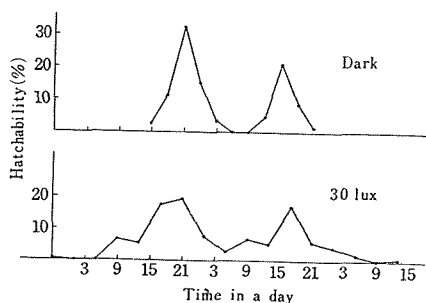


Fig. 7 Rhythmic phase of hatching in *Bombyx mandarina*

from the same batch (about 180 eggs were used for each experiment) were incubated under the continuous darkness and under the illumination (30 lux). The incubation was carried out from July, 7 and from July, 12 and the eggs began to hatch after 8 days, respectively.

The results obtained are shown in Fig. 7. The regular rhythmicity of hatching with 18-hr cycle was presented under the

dark condition and further the rhythmic sign had been persisted even under the illumination, though the manifestation a little disturbed by light.

7. Consideration and Conclusion

It is, therefore, evident that the eggs of *B. mandarina* have also an endogenous character of hatching with 18-hr rhythmicity, which is much stronger than that of *B. mori*.

In above several experiments we have observed that the eggs of normal races of silkworms expose the rhythmicity with 18~21-hr interval when they hatch under the absence of light. Similar results were reported by *Takeuchi* (1957 a b c) in *Bombyx mori*. Occurring under the entire constant condition, the rhythmicity involves to be depended upon the endogenous factor in population. Under the constant illuminations, however, the rhythmical cycle is often shortened or becomes irregular and sometimes disappears. There is, nevertheless, a few races which keep the faint

regularity of rhythmicity in 10 lux illumination as *Taihei* × *Choan* and *Shi-No. 108* or even in 100 lux as *Nichi-No. 124*, so that we cannot help accepting the existence of racial differences in the rhythmicity of hatching among eggs of the normal races.

The above results show that the continuous illumination to the eggs accelerates the threshold of hatching, besides prolongs the duration of hatching. In Japan so many investigations have already been carried out on the influences of light upon the growth of silkworm eggs from the applicative point of view in the incubation. WATANBE (1934) and TAKEUCHI (1957 b) pointed out that the growth of eggs was rather inhibited after the eye spot stage while hastened remarkably at just hatching time, by the presence of light. Taking their statements into consideration irregularity or disappearance of the rhythmicity of hatching seen in the constant light condition may be caused by the disturbance of egg growth, being promoted or controlled by the light action. This problem will be analyzed in Chapter III.

Table 1 Comparison of hatching features among the eggs of various varieties

Varieties used	Genetic characters				Rhythmicity in hatching			Mode of interval
	Race	Egg colour	Moltin ism	Voltin ism	Dark	10 lux	100 lux	
<i>Taihei</i> × <i>Choan</i>	Jap × Chin.	black	4	2	+++	+	±	15-18hr
<i>Nichi-No. 1</i>	Japanese	"	4	1	+++	+	-	15-18
<i>Nichi-No. 124</i>	"	"	4	2	+++	+++	+	24
<i>Shi-No. 108</i>	Chinese	"	4	2	+++	+	-	18-24
<i>Ô-No. 19</i>	European	"	4	1	±	±	±	15-21
* <i>w</i> ₁	Japanese	white	4	1	±	-	-	15
* <i>re</i>	"	red	4	1	-	-	-	-
* <i>b</i> ₁	"	brown	4	1	±	-	-	9-15
<i>Kyokko</i>	"	black	3	1	±	-	-	9-12
<i>Gominhaku</i>	"	"	5	1	+++	±	-	18
<i>Daizo</i>	Chinese	light brown	4	sub-multi.	+	-	-	12
<i>Mysore (w₃)</i>	Indo-Chin.	"	4	multi.	±	-	-	9-18
<i>Bombyxmandarina</i>		brown	4	2	+++	++(30 lux)		18

Remarks. +++ clearly present, + faintly present, ± almost absent. - absent
 * In the hydrochlorized eggs of these strains, the rhythmic sign was sometimes recognizable.

The interval between modal peaks of rhythmic hatching differs, more or less, according to the varieties, but it can be recognized to be nearly the diel periodicity, 18-hr rhythm in mean value. As above described, the rhythmicity of hatching is generally seen in the normal races while hardly in the egg colour mutants such as *w*₁*, *re**, *b*₁*, and *Mysore (w₃)**. Such a difference involves that the egg colour,

* The hydrochlorized eggs of these mutants showed sometimes a faint rhythmic phase of hatching. The fact suggests that the rhythmic character of hatching is kept commonly in silkworms eggs, differing so much among varieties and between the hibernated eggs and the hydrochlorized eggs (2nd generation).

namely the quantity and quality of the serosal pigments of tryptophan origin, has a close relation to the possession of the rhythmic character. The problem, however, must be resolved in the future investigation.

European race, *Ô-No. 19* shows an indistinct rhythmicity though black in the egg colouration. The cause has yet been unknown, but it is plausible enough that the growth of eggs of *Ô-No. 19* which are naturally slow and diverse, relates to the cause. Similar indiscernible rhythmicities in *Daizo* (sub-multivoltine) and *Kyokko* (three moult), secreting very actively the growth hormone (MOROHOSHI 1950), are assumed to be affected by much faster growth than the normal races. As this hormonal action, according to *Morohoshi's* interpretation, controls the egg growth from the early embryonic stage, it will strongly exert an influence on the hatching phase of eggs. Conclusively the author wants to conceive that the existence of rhythmic sign in hatching is certainly related to the colouration of the serosal pigments and also to the activity of the growth hormone.

By the way the rhythmic phenomena in hatching of the eggs or in emergence of moths (KIMURA 1952, PICTET 1918, SCOTT 1936, MORIARTY 1959) appear as a mass pattern, so that it seems to be different far from the daily periodicity in the same individual, for example in the pigment migration of Arthropod eyes (CONGDON 1907, DEMOLI 1911, WELSH 1930-51, BENNETT 1932, BENNETT & MERRICK 1932, NAGANO 1943-1950, WEBB & BROWN 1953, KOYAMA 1955, YAGI, KOYAMA & FURUHATA 1956), in the activity of some insects (GUNN 1940, MELLANBY 1940, SUGIYAMA & MATSUMOTO 1955) etc. Notwithstanding, the rhythmicity in hatching is probably formed in the end as a totality of individual rhythmicity in the same egg.

CHAPTER II ENVIRONMENTAL FACTORS AND THE RHYTHMICITY OF HATCHING

In this chapter the author studies the effectiveness of environmental factors such as temperature, humidity and light upon the hatching phase of silkworm eggs.

1. Thermal Conditions and the Rhythmicity of Hatching

In all the previous experiments incubations of the eggs were carried out under the thermal condition of 25°C, which makes the eggs of the next generation go to diapause (taking black colouration, the diapause eggs of silkworm are generally called "black eggs"). Then higher and lower temperatures than 25°C were taken as the thermal factor.

The materials used were the hibernated eggs of *Shi-No. 108*, *Shi-No. 122*, *Daizo*, *Taihei* × *Choan*, *Choan* × *Taihei* and *Honen* × *Kenpaku*, which were incubated under the conditions of 18°C, 12°C, and 30°C. The 18°C régime is known as the temperature inducing non-diapause eggs in the next generation. In this

experiment the eggs have been kept under the temperature of 18°C since the initial day of incubation. When the silkworm eggs are incubated continuously under such an inadequate temperature for growth as 12°C or 30°C, they fall in death or in abnormal growth (MIZUNO 1926). Then the eggs were subjected to above each temperature only from just before hatching after being incubated under the constant condition of 25°C. Numbers of eggs used were about 1000 per an experiment. They were pasted on a thick paper. New born silkworms were counted every interval of 3 hours from the beginning to the end of hatching, the hatching phase being expressed with percentage. The author performed these experiments either in dark or in light conditions, but in the latter the rhythmicity of hatching hardly occurred as in the previous experiments, so the results got only under the incubation of darkness will be described in the present chapter.

(i) Rhythmicity of hatching under the condition of 18°C

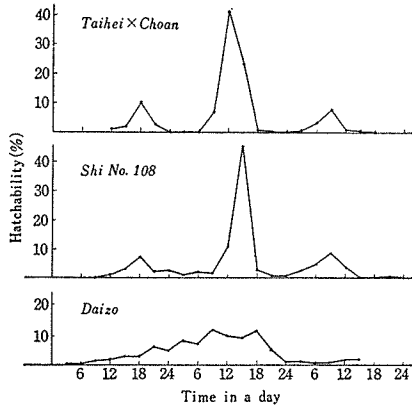


Fig. 8 Hatching phases under cool incubation (18°C) with darkness

The results obtained are shown in Fig. 8. *Taihei x Choan* begins to hatch from 16 days after incubation, about 18-hr rhythm of hatching can be seen. The second modal peak of hatchability is highest and consists of about 70% individuals of whole eggs.

In *Shi-No. 108*, which begins to hatch almost from the same time as in the above hybrid, the rhythmic hatching with 18-hr cycle appears. In *Daizo*, however, no rhythmicity of hatching cannot be recognized though the initial day of hatching is earlier than the former two.

(ii) Rhythmicity of hatching when subjected to 12°C

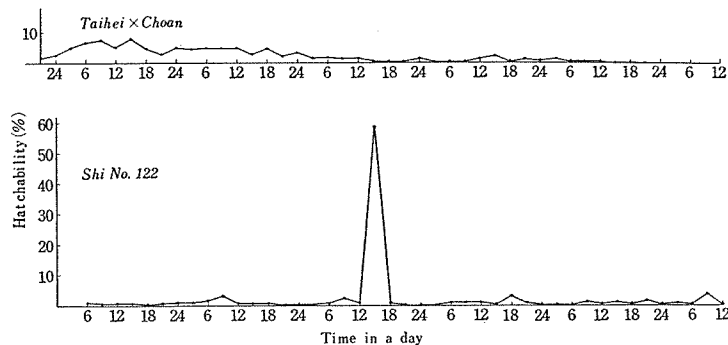


Fig. 9 Hatching phases in the eggs put in coolness (12°C) with darkness at their full-grown stage

The present data (Fig. 9) show the hatching phase when the eggs were subjected to 12°C from just before hatching after the constant incubation of 25°C. The duration of hatching is markedly delayed, taking more than 5 days in both *Taihei* × *Choan* and *Shi-No. 122*, and the rhythmic phases of hatching cannot be distinguished at all though in the latter about 60% of whole eggs hatch at a time.

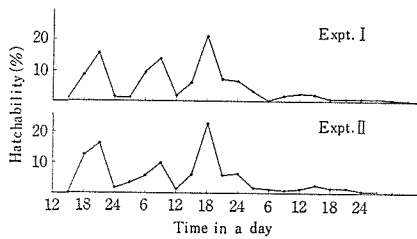


Fig. 10 Hatching phase in the eggs of *Honen* × *Kenpaku* put in warmth (30°C) with darkness at their full-grown stage

(iii) Rhythmicity of hatching when subjected to 30°C

As same as in the above experiment the eggs were subjected to 30°C from just before hatching. In this case we can observe three evident peaks of hatchability with 9~12-hr rhythmicity. The hatching, the phase of which is shortened in all ways, ends within about 2 days.

(iv) Consideration

From the above results the existence of rhythmicity is also recognized under the constant lower temperature (18°C) than 25°C in constant darkness.

It is, therefore, suggested that the rhythmic character in hatching is hardly affected by higher or by lower thermal conditions of normal range, within which the cyclic interval takes always 18~21 hours. However, *Daizo* being a peculiar example, as indicated in Chapter I, does not manifestate the rhythmicity. The hatching phases are summarized in the following table.

Table 2 Thermal conditions and the rhythmicity of hatching (under constant darkness)

Temperatures		Races	Rhythmicity in hatching	Interval between modes	Duration of hatching
through embryonic stage	just before hatching				
25°C	→ 30°C	<i>Honen</i> × <i>Kenpaku</i>	+++	9 - 12 hr	54 - 74 hr
18	→ 18	<i>Choan</i> × <i>Taihei</i>	+++	18 - 21	54
"	→ "	<i>Shi-No. 108</i>	+++	18 - 21	66
"	→ "	<i>Daizo</i>	-		60
25	→ 12	<i>Taihei</i> × <i>Choan</i>	-		130
"	→ "	<i>Shi-No. 122</i>	+	27 - 30	130

Remarks : Expressions in "Rhythmicity in hatching" are all the same as in Table 1.

When an extraordinary temperature such as 30°C is given to the eggs just before hatching, the modal interval of hatching is rather shortened to 9~12 hours, keeping the constant rhythmicity. On the other hand, when subjected to much lower temperature such as 12°C, the rhythmicity of hatching is hardly observed. It is conclusively appreciated that the rhythmicity of hatching with 18~21-hr cycle occurs

regularly near in the range of optimum temperature of egg growth. The hatching rhythm is plausible to be arisen correspondingly with a physiological change along egg growth, but the problem will be discussed in Chapter III.

2. Relative Humidity and the Rhythmicity of Hatching

In the present experiment the effect of moisture upon the hatching phases in the eggs of *Shi-No. 108* and *Choan* × *Taihei* was researched with regard to three conditions of relative humidities such as 100%, about 75% and about 30%, which were made with saturated vapour, saturated water solutions of NaCl and of CaSO₄, respectively. Each group of about 1000 eggs being pasted on a thick paper was confined and incubated under the regulated condition of humidity with the temperature of 25°C. The numbers of new born larvae were counted every interval of 3 hours and expressed with percentage.

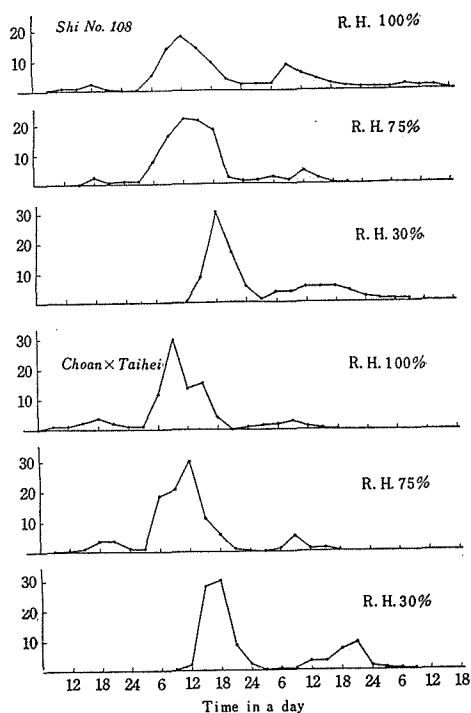


Fig. 11 Rhythmic phases of hatching under several conditions of humidity (dark, 25°C)

The results obtained are denoted in Fig. 11. Under the condition of R. H. 100% the duration of hatching is comparatively longer in *Shi-No. 108* (78 hours) than in *Taihei* × *Choan* (57 hours) and 21~24-hr rhythmicity of hatching is recognized in the both strains.

Under the condition of R. H. 75%, the hatching phase is almost similar to the above experiment, the hatching duration being shortened to 54 hours.

On the other hand the commencement of hatching is markedly delayed (about 20 hours) under the condition of R. H. 30%. The two modal peakes of hatchability appear in the both strains but without normal interval of 18~21-hr.

Abovementioned results tell us that the rhythmic character of hatching appear in each condition of relative humidity under the constant darkness. R. H. 100% and R. H. 30%, however, cause a small shortening and widening of the rhythmic interval of hatching

mode, respectively. As already reported on eggs of *Bombyx mori*, *Antheraea pernyi* and *Philosamia cynthia* (KOGURE • YAMAMOTO 1928, KURASAWA • KANAZAWA • IKEUCHI 1937, YAMAZAKI 1939, FUKUDA KITSUDA 1949, HODAI 1949), the optimum humidity for egg

growths exists within R. H. 70~95%. Then an extraordinary humidity, lower or higher, will have related to the change of the normal cycle in hatching making the egg growth abnormal.

3. Ultraviolet Ray and the Rhythmicity of Hatching

In the previous chapter the incandescent lamp was used as a light source. Then the author carried out a comparative investigation in hatching phase making use of ultraviolet ray. The materials used were the eggs of *Taihei* × *Choan* and *Zenko* (black egg strains).

The eggs were subjected constantly to the illumination from the mercury lamp of winding tube type, which has two large absorption band in the wave length viz. 430m μ and 360m μ .

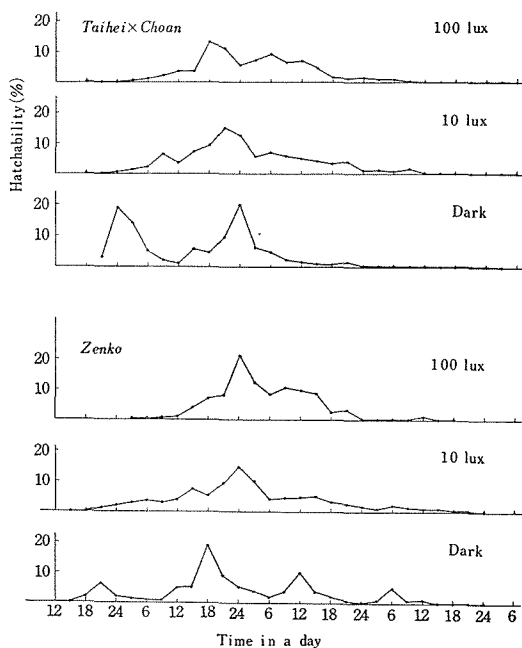


Fig. 12 Hatching phases under ultraviolet ray

The results obtained are shown in Fig.12. Under the constant dark condition the eggs expose 18~24-hr rhythmicity of hatching. But the rhythmic sign disappears completely under the conditions of illumination, besides the beginning of hatch is postponed.

The rhythmicity of hatching, which could be maintained under 10 lux of incandescent lamp light (Chapter I), becomes almost indistinguishable in the case of the fluorescent light. This is assumed to be attributed to stronger action of the latter than the former.

It is well known that the light promotes the embryonic growth of silkworm eggs, but the effect more or less varied according to the embryonic stage (TANAKA 1951, KAWAI • EBANA 1951, KOIZUMI 1954). HOTTA and KAWABATA (1955) reported that when the eggs were continuously exposed to an incandescent or a fluorescent lamp light, the positive effect was recognizable until the blastokinesis and the blue spot stages, while effect turned to negative from the blue spot stage. In this case the fluorescent light was more effective than the incandescent light, too. The present results show that the fluorescent light induces a small delay of hatching. Considering the light stimulation inhibited strongly the embryonic growth from the blue spot stage, the author's results are in concordance with theirs.

4. Conclusion

The effect of environmental factors such as temperature, relative humidity and light upon the rhythmic sign of hatching in silkworm eggs were dealt with in this chapter comparing with results obtained Chapter I.

From the results it can be concluded that the rhythmic character of the eggs, when incubated under the constant dark condition, appears with comparatively strong stability and keeps generally 18~21-hr rhythmicity inspite of different conditions of temperature and humidity. However, an extraordinary condition of temperature or humidity brings a little disturbance in the interval between modal peaks of hatchability.

Meanwhile the light, if casted continuously on the eggs, causes an inhibitory influence upon the incidence of rhythmic hatching, which seems to be more controlled by the fluorescent lamp light than the incandescent lamp light.

CHAPTER III EXPERIMENTAL ANALYSIS ON THE ENDOGENOUS CHARACTER IN THE RHYTHMICITY OF HATCHING

As described in Chapter I and II, the eggs of silkworms manifest regularly the rhythmic hatching under the constant darkness. The author has pointed out that such a regular rhythm occurs with 18~21-hr cycle in normal strains, showing more or less racial differences and considered that the rhythmicity may be affected by the endogenous character of the eggs, from the cyclic signs of hatching under various conditions of incubation.

With attempt of revealing the endogenous mechanism in regard to the rhythmic hatching, the author investigated expressive processes or changes of the rhythmic character of hatching by subjecting the eggs to various régimes, viz. alternation of light and dark, chilling and photoperiodic treatment. Further the physiological activity of the eggs which has probably an essential relationship to cause the rhythmic manifestation was measured using dehydrogenase activity as an indicator. The results obtained are described in the respective section.

1. Effect of Alternation of Light and Dark upon the Rhythmicity of Hatching

When the eggs were subjected continuously to illumination through their whole stages, the endogenous rhythmicity of hatching was inhibited (Chapter I), so its appearance was assumed to be predominantly controlled, but not absolutely, by continuous illumination.

In the present experiment the effect of alternation of light and dark upon the manifestation of the periodic hatching was researched making use of the eggs at each growth stage.

MATERIALS AND METHOD

About 2000 hibernated eggs of *Taihei* × *Choan* were used for each régime, which is shown in Table 3.

RESULTS

Observing the results in Experiment I (Fig. 13), II (Fig. 14 a) and III (Fig. 14b),

Table 3 Experimental régimes (30 lux, 25°C, RH 80%)

		Embryonic stages				
		Longest embryo	Blasto-kinesis	Eye spotted	Just before hatching	Hatch
Experiment I	Al	L				→
	Ad	D				→
	B1	D	→ L			→
	Bd	L	→ D			→
	C1	D		→ L		→
	Cd	L		→ D		→
	D1	D			→ L	→
	Dd	L			→ D	→
Experiment II	al	L				→
	ad	D				→
	bl	L12: D12	→ L			→
	bd	L12: D12	→ D			→
	cl	L12: D12		→ L		→
	cd	L12: D12		→ D		→
	dl	L12: D12			→ L	→
	dd	L12: D12			→ D	→
Experiment III	Long day	L15: D9			→ D	→
		L18: D6			→ D	→
		L21: D3			→ D	→
	Short day	L9: D15			→ D	→
		L6: D18			→ D	→
		L3: D21			→ D	→
		L8: D8			→ D	→
						→ D

Remarks : L—light, D—dark, Numbers show "Hours" of each condition.

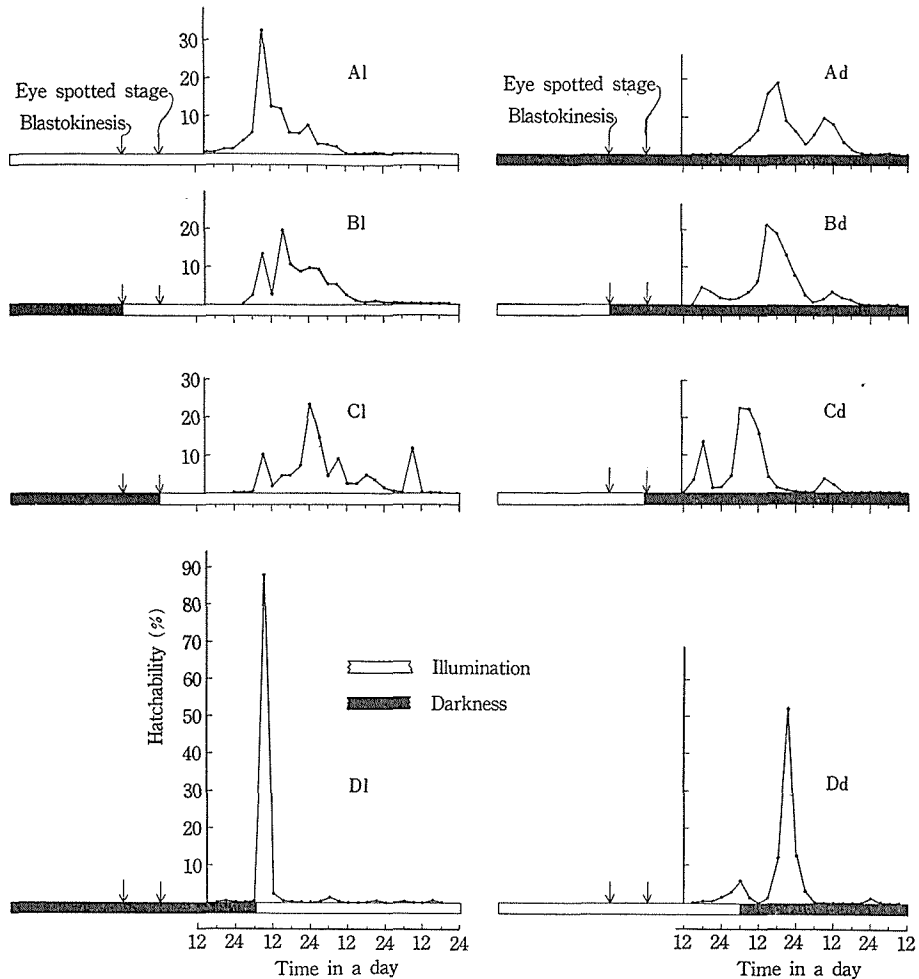


Fig. 13 Effects of light and dark upon hatching phases when each condition is given to various embryonic stages of the eggs (*Taihei* × *Choan*)

we can recognize just same phenomena as already demonstrated not only under the continuous illumination (Figs. A1 and a1), but also under the continuous darkness (Figs. Ad and ad); the rhythmical feature of hatching is suppressed in the former while exposed in the latter. Now comparison will be made among each régime.

Light effect at the blastokinesis stage (Figs. B1, b1, Bd, bd)

In B1 régime (D→L) the rhythmicity can hardly be detectable, but in b1 régime (L12:D12→L) a rhythmic like sign but the modal interval shortened to 12 hours has been disclosed. This maintenance is presumed to be brought by the after effect of the artificial conditioning through previous embryonic stages. In Bd (L→D) and bd (L12:D12→D) régimes the rhythmicity with 18~21-hr cycle is clearly seen, presenting three modal peaks. The phenomenon seems to bear on the repossession of the rhy-

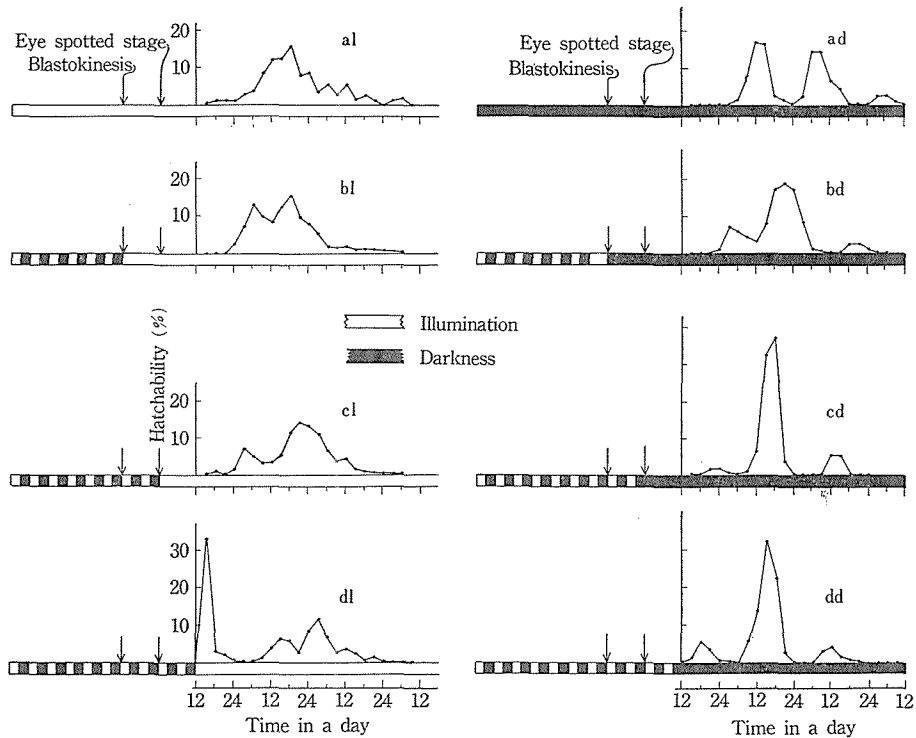


Fig. 14 a Effects of alternation of light and dark upon hatching phases when the condition is given to various embryonic stages of the eggs (*Taihei* × *Choan*)

thmic character in the continuous darkness.

Light effect at the eye spot stage (Figs. Cl, Cd, cl, cd)

In Cl régime (D→L) the modal peaks of hatchability appear with shortened cycle such as 9~15 hours, which was usually observed when the rhythmic manifestation was slightly inhibited. In cl régime (L12:D12 →L), however, 18-hr rhythmicity takes place evidently, which is plausibly caused by the after-effect of the artificial conditioning as to light because the rhythmic sign cannot be recognized in Cl régime.

On the other hand, in Cd (L→D) and cd (L12:D12→D) régimes the regular rhythmicity of hatching is exposed.

Light effect at the stage just before hatching (Figs. Dl, Dd, dl, dd)

In Dl régime (D→L) a simultaneous hatching occurs as subjected to light, the later modal peaks being scarcely detectable. In dl régime (L12:D12→L) the first peak of hatchability appears in the highest degree, after 24 hours the second peak, and after 12 hours the third peak being succeedingly seen. From this fact the proceeding alternation of light (12hr) and dark(12hr), almost same to the diel periodicity, acts the after-effective factor making the eggs hatch rhythmically even under illumination, if the alternation is continued for considerable long time. In Dd régime

(L→D) a large modal peak is presented, delayed about 15 hours after putting in dark. In dd régime (L12:D12→D), however, the eggs hatch quite regularly with 21-hr cycle. There exists also the after-effective sign of the alternation of light and dark in this case.

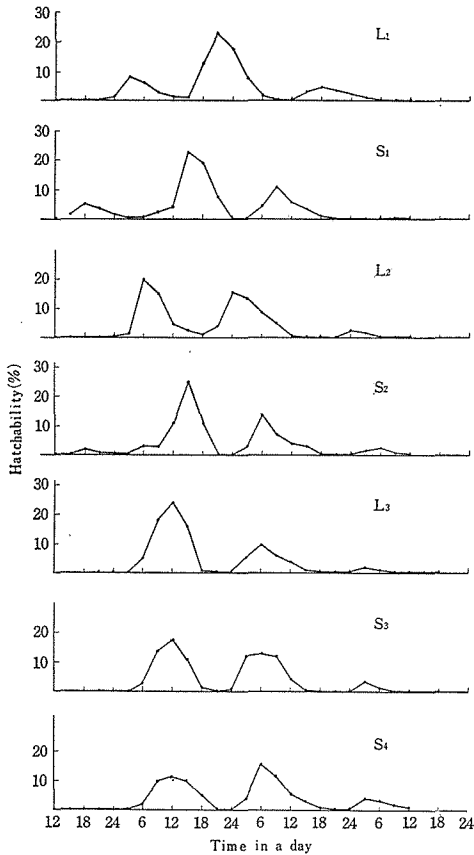


Fig. 14 b Rhythmic phases of hatching under darkness when photoperiodic conditions are given to the eggs before their full-grown stage (*Taihei* × *Choan*)

Illumination	Darkness	Illumination	Darkness
L ₁ 15hr	: 9hr	S ₁ 9hr	: 15hr
L ₂ 18	: 6	S ₂ 6	: 18
L ₃ 21	: 3	S ₃ 3	: 21
		S ₄ 8	: 8

(12 hr) is given at each stage of the eggs, there occurs always the after-effective phenomenon in the hatching phase. Then it can be stated that the proceeding conditioning almost same to the diel periodicity has well been maintained in the eggs, the hatching of which depends certainly upon the exogenous stimuli. The daily rhythmicity of hatching seen in the natural condition must be arisen more regularly

Photoperiodic treatment through embryonic stages (Fig. 14 b)

In above two régimes (Dd, dd), the eggs have suggested a tendency to expose the rhythmicity of hatching when kept under darkness from just before hatching, whatever the light condition through embryonic stages may be. This suggestion has been proved as crucial fact by the present experiment. Observing Fig. 14b, we can hardly detect the differences in the rhythmic phases of hatching among photoperiodic régimes.

CONSIDERATION

As above mentioned, the rhythmic feature of hatching is much controlled by continuous illumination, viz. the longer the illumination, the more the feature becomes faint, but the eggs can recover, even though put previously in light, the rhythmic character of hatching when put back in dark. The fact suggests that the eggs have an endogenous character to hatch rhythmically, though checked by long illumination, so the character becomes free if the illumination is taken off.

When the artificial environment such as alternation of light (12 hr) and dark

by such a fact.

If the eggs subjected to light or dark at just before hatching, most of all individuals go to hatch at the same time, making a large modal peak. Especially in D1 régime (D→L), about 90% of eggs hatch immediately after light casting. This fact indicates that the lightening at the hatching time should be a principal factor making the eggs hatch as already been pointed out by many researchers. In addition, that the sensitivity to light stimuli in the eggs becomes much stronger after the eye spot stage (TAKEUCHI 1958, KOIZUMI 1958) may be concerned to the burst of hatching. Meanwhile, in Dd régime (L→D) the burst of hatching occurs a little later than in D1 régime. To this phenomenon an explanation has been given; that the absence of light acts as an inhibitory condition to hatching. If it be, however, considerable disturbance would be arisen in the hatching phase as if the eggs were put under the continuous illumination. The author, therefore, wants to propose another opinion that certain endogenous factor inhibited by long illumination becomes free from the suppression of light when confined in the darkness, and the eggs response to the endogenous factor making them hatch, but some duration is requested for recovering the endogenous stimulation.

2. Effect of Low Temperatures upon the Rhythmicity of Hatching

In the previous section we could know the effect of light upon the rhythmicity of hatching, which was inhibited by long stimulation while being soon repossessed under darkness. Then, in this experiment the author researched the effect of several low temperatures, to which the eggs at various stages were subjected, upon the rhythmic phase of hatching.

MATERIALS AND METHODS

About 2000~3000 of the hibernated and the hydrochlorized eggs of *Taihei* × *Choan* and *Nichi-No. 115* were used for the materials, which were incubated constantly under darkness or under illumination. Low temperatures were made with Hitachi Refrigerator, in which the eggs were put at various embryonic stages. Full methods about the experiments are described in the respective topic.

RESULTS

(i) Effect of chilling at the beginning of incubation upon the rhythmicity of hatching

a. In the case of the hibernated eggs

The eggs grown to the longest embryo were chilled with $5\pm 1^{\circ}\text{C}$, which is known as the critical low temperatures for the growth of silkworm eggs. After this treatment they were divided into three groups, each of which was taken out of the chilling room every 8 hours, being kept under the thermal condition of 25°C with light or with dark.

Under the dark incubation (Fig. 15) the rhythmicity of hatching with 18-hr

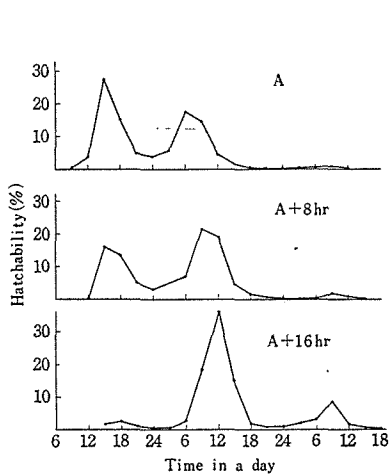


Fig. 15 Chilliness (5°C) at the beginning of incubation and the rhythmic phases of hatching under darkness (*Taihei* × *Choan*)

A: removed to 25°C after 5-hr chilling,
 A+8hr: do, after more 8-hr chilling than A,
 A+16hr: do, more 16-hr chilling.

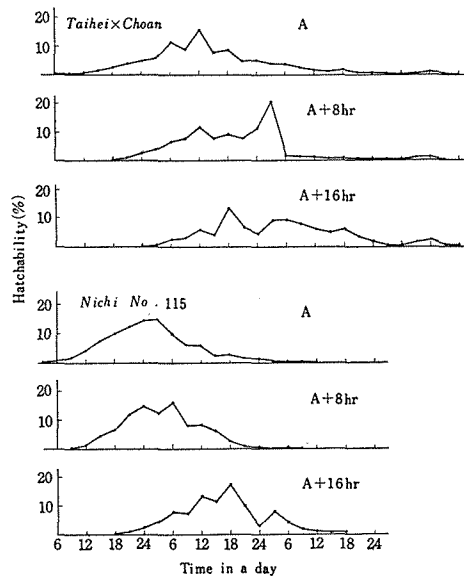


Fig. 16 Chilliness (5°C) at the beginning of incubation and the hatching phases under illumination

Expression of each régime is as same as in Fig. 15.

cycle was exposed in every régime. In A régime (removed to 25°C after 5-hr chilling) two modal peaks appeared as same as in A+8-hr régime (removed to 25°C after more 8-hr chilling than A), but the highest peak seen firstly in the former, moved to the second in the latter, in which the third peak is observed. Further, in A+16-hr régime (removed to 25°C after more 16-hr chilling than A) the highest peak became larger than in A and A+8-hr régimes, a small peak appearing at its both sides. From these facts it is assumed that each highest peak is gradually delayed to appear and the modal peak tends to appear at the definite time even if the eggs experienced 8~16-hr chilling. The same tendency is shown in the next experiment.

The newly hatched larvae from the eggs composing each peak in A+8-hr régime and A+16-hr one (see Fig. 15) were reared and the sex ratio was discriminated at the fourth instar.

Table 4 Sex ratio of the eggs composing each modal peak

Régimes	A+8hr (Fig. 15)				A+16hr (Fig. 15)			
	1				2			
Order of peak	1	2	3		fore part	middle part	hind part	(Total)
♀	246	358	39	68	214	139	159	(512)
♂	221	405	41	53	137	208	248	(593)

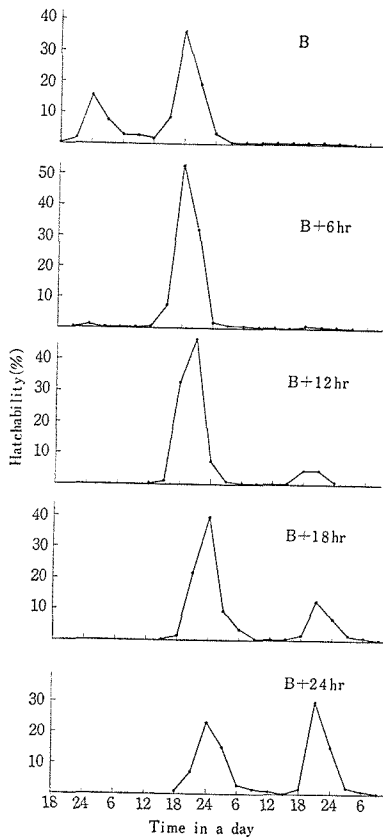


Fig. 17 Chilliness (5°C) at the beginning of incubation and the rhythmic phases under darkness (hydrochlorized eggs of *Taihei* \times *Choan*)
 B : removed to 25°C after 18-hr chilling,
 B+6 hr : do, after more 6-hr chilling,
 than B, B+12 hr : do, more 12-hr chilling
 B+18 hr : do, more 18-hr chilling, B+24
 hr : do, more 24-hr chilling.

hr chilling) they were reduced to only one peak, which stood at the same position as that of the second peak in B régime. As chilling duration became longer, the second peak was gradually formed, at last the formal similarity to B régime in the hatching phase occurred in B+24-hr régime, which experienced more 24-hr chilliness than B régime.

These facts show that the rhythmic hatching with 18~21-hr cycle is regularly exposed in every régime and the cyclic peaks tend to appear at the definite positions.

(ii) Effect of chilling at the middle or the last periods of incubation upon the rhythmicity of hatching

a. Chilling treatment at each embryonic stage

When each group of the hibernated eggs reached respectively to the fourth day, the seventh day (just after blastokinesis) and the ninth day (one day before hatching)

The results (Table 4) showed that there is no appreciable relation between sex ratio and the eggs composing each peak.

Under the light incubation (Fig. 16), the start of hatching was gradually delayed accompanying with the time when the eggs were removed to 25°C ; the duration of normal incubation in each régime is about 8 days 18 hours in *Taihei* \times *Choan*, being about 8 days 11 hours in *Nichi-No. 115*. Thus, in this case we can see the phenomenon that the growth of the eggs is completely inhibited by the low temperature such as 5°C and that the rhythmicity is always checked by continuous illumination.

b. In the case of the hydrochlorized eggs

The phenomena seen in the hibernated eggs were researched also in the hydrochlorized eggs shortly after oviposition. The material eggs were chilled for 18 hours under 5°C after hydrochlorization, being divided into 5 groups. Each group was taken out of coldness every 6 hours and incubated under the thermal condition of 25°C with darkness. The results are denoted in Fig. 17.

In B régime (removed to 25°C after 18-hr chilling) two modal peaks were observed, but in B+6 hr régime (removed to 25°C after 24-

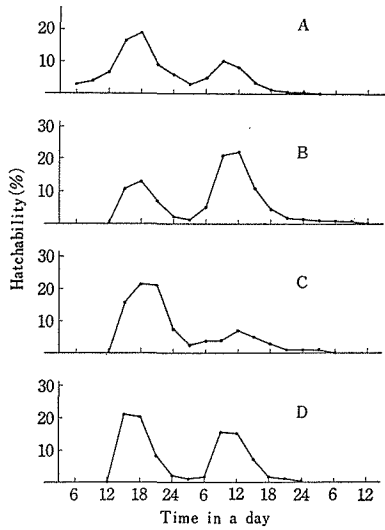


Fig. 18 9-hr chilliness (5°C) at various embryonic stages of the eggs and the rhythmic phases of hatching under darkness (*Taihei* × *Choan*)
 A : Control, B : Chilled at 4th day of incubation, C : do, at 7th day, D : do, at 9th day.

3°~5°C respectively for 7 hours, 14 hours and 21 hours, besides under 0~2°C and -4 ~ -6°C for 14 hours. The hatching phases are shown in Fig. 19.

The rhythmicity of hatching with 18~24-hr cycle was significantly presented almost all régimes. In addition the author performed an extraordinary chilliness such as -7 ~ -9°C for the eggs, but in such a case no rhythmicity was detectable, besides percentage of hatchability fell to 25%.

Speaking on the times when the modal peaks appeared, in 3~5°C 7-hr régime (subjected to 3~5°C for 7 hours) both peaks were manifested almost the same times as in Control régime. The result is same enough

under the thermal condition of 25°C, it was subjected to the chilliness such as 3° ~5°C for 9 hours. The results obtained are illustrated in Fig. 18.

In each case two modal peaks of hatching appeared with about 18-hr interval and the time when each peak is presented is almost simultaneous.

Thus the rhythmicity of hatching is scarcely disturbed but regularly manifested, even though 9-hr chilliness is given to the eggs at various embryonic stages.

b. Chilling duration and lower temperatures less than 5°C

Each group of the hydrochlorized eggs aged 8 days from incubation was put under

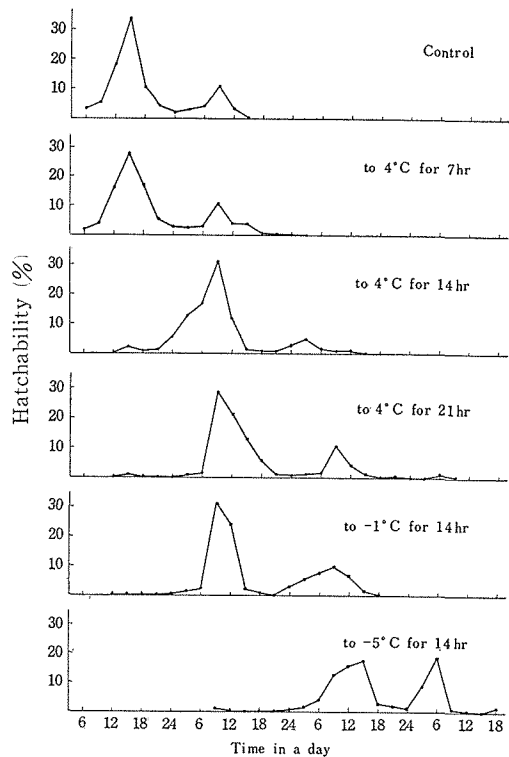


Fig. 19 Duration and degree of chilliness and the hatching phase under darkness when the eggs chilled at 8th day of incubation (*Taihei* × *Choan*)

as in the previous experiment (Section a).

In 3~5°C 14-hr régime (subjected to 3~5°C for 14 hours) the largest peak which stood at the same position of the second peak in the former régime appeared, being delayed one cycle.

In 3~5°C 21-hr régime (subjected to 3~5°C for 21 hours) and also in 0~2°C 14-hr régime (subjected to 0~2°C for 14 hours) the same tendency was indicated.

In -4~-6°C 14-hr régime (subjected to -4~-6°C for 14 hours), however, the modal peaks were exhibited, being delayed more one cycle, viz. more two cycles than Control and 3~5°C 7-hr régimes.

From the above facts it can be recognized that less than 7-hr chilling (3~5°C) hardly effects on the incident of rhythmic hatching, while longer chillings such as 14 and 21 hours cause one-cycle delay of the modal peaks. Furthermore, such a low temperature as less than -4°C seems to cause more one-cycle delay of the hatching rhythm, but in less than -7°C-chilling no rhythmical character is observed because the vitality of the eggs may be completely checked by such a temperature.

CONSIDERATION

The critical low temperature in the growth of silkworm eggs is known to exist nearly at 5°C (MIZUNO 1926, SUGIYAMA 1960). If so, a corresponding delay must reasonably be brought in the rhythmical phase of hatching when the eggs are put in the chilliness less than 5°C for considerable long time. Under such a speculation, the author has been carried out the above experiments. Several régimes, in which chilliness was given to the eggs at various stages, show that each cyclic peak of hatchability tends to appear at the definite time without relation to chilling duration. This fact suggests that the physiological rhythm* in the eggs has probably maintained without stopping even though under very low temperatures which may check the morphological growth, viz. the endogenous rhythmic factor in the eggs would be continued unless attacked by the extraordinary low temperatures to stop completely the egg vitality.

The rhythmic feature of hatching is presumed to be more clearly manifested by such an essential stability, which is very much controlled by illumination. Then, under illumination the rhythmic sign of hatching disappears and the hatching phase coincided with the growth of the eggs is to be exposed as shown in Fig. 15.

Now, in Fig. 20 the author intends to show the rhythmic manifestation of modal peaks, occurring at the definite time, which can be used as an indicator of the rhythmicity of hatching. The chilling treatment at the beginning of incubation makes the egg growth prolong just for chilling duration, but the positions where the modal peaks appear are kept at the definite times, as diagrammatically denoted

* MORI (1948) has proposed an opinion that the original factor to cause the periodic activity of animals may exist in the physiological condition of each individual.

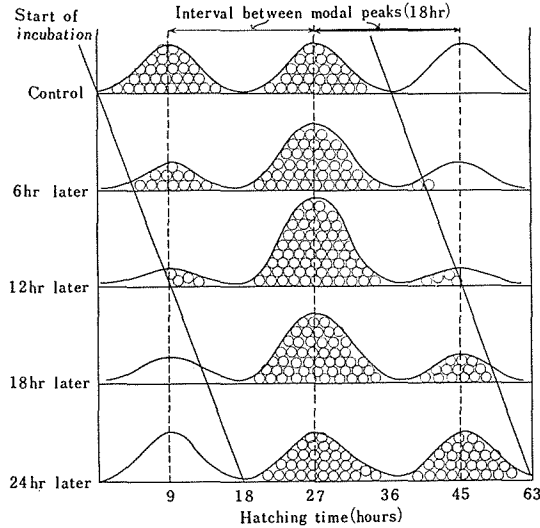


Fig. 20 Diagrammatic explanation on the occurrence of cyclic modal peaks of hatching under darkness when each incubation has a different start.

in the figure. Taking the chilling treatment at the last period of incubation into consideration, the rhythmic phases of hatching in Fig. 18 (C) and (D) take quite resemblance to Control that is nonchilling. Less than 9-hrchilliness, if given to the period when the larval bodies come to completion, does not give any effect upon the rhythmic appearance of hatching. Thus, that the rhythmic phase differs according to the chilling time may be based on the differences of cold resistance or of thermal sensitivity among the embryonic stages.

Regarding to the relation between sex ratio of the eggs and the time of hatching, MONZINI (1927) and MOROHOSHI (1949) reported that the female larvae hatched faster than the male one, MUROGA (1941), NAGATOMI (1943), MATSUO (1951) and TAKEUCHI (1957) did that the tendency was not always constant according to the races, besides SHIMIZU and HORIUCH (1960) stated that in the original race the female hatched faster than the male. Nevertheless the author's experiment has proved there is no sexual difference between the individuals composing each modal peak. Therefore each peak of hatching with rhythmic interval is obvious not to be composed of the sexual difference of the eggs.

3. Time of Oviposition and the Rhythmicity of Hatching

In the previous experiments (Section 2) it was suggested that there existed the definite occurrence of hatching cycles in the same population even though the eggs had experienced different start of incubation, or longer or shorter chilliness, and the

physiological rhythmicity in the eggs had well been maintained even under about critical low temperature. In the present experiment the manifestation of rhythmical hatching with special reference to numbers of the modal peaks was investigated on each group of the eggs layed by one moth at different times, viz. differing at growth stage.

MATERIALS AND METHODS

The materials used were *Taihei* × *Choan* (F₂), the eggs of which were divided into two groups, namely one group (A) consists of early oviposited eggs (oviposited from 2 p. m. to 5 p. m., September 30) and another (B) consists of later ones (oviposited from 5 p. m. to next 5 a. m.). The hydrochlorization of the eggs in each group was performed at 4 p. m. October 1. The treated eggs were incubated under 25°C with darkness and observed the hatching process.

RESULTS

As denoted in Fig. 21, the rhythmicity of hatching was recognizable either in A régime or in B one; the former's second modal peak being completely coincided with the latter's first peak. Each phase of hatching was formed with 2 modal peaks, so 3 modal peaks (C) will reasonably be arisen if the eggs of A and B are incubated being mixed up.

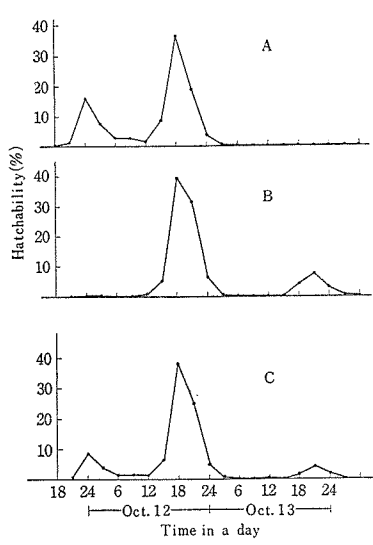


Fig. 21 Rhythmic phases of hatching in the eggs having different oviposited time
 A : oviposited, 2~5 p. m. Sept. 30, B : do, 5p. m. Sept. 30~5 a. m. Oct. 1, C : Total appearance of hatching of A and B

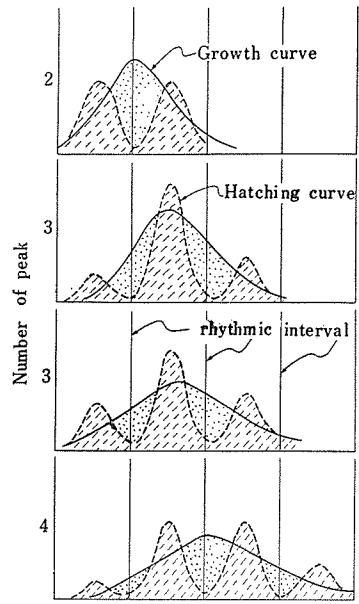


Fig. 22 Diagrammatic explanation on the relation between growth variance of the eggs and number of cyclic modal peaks of hatching under darkness

CONSIDERATION

In the same population the difference in the egg laying times is assumed to relate to the occurrence of modal peaks in hatching, viz. the numbers of the modal peaks being decided by variance of the egg ages. Thus the explanation that the more the egg ages are varied, the more the modal peaks appear, will be given diagrammatically in Fig. 22. If the constant dark incubation of the eggs is begun at the same time, in common sense hatching phase would be expected to appear as unimodal curve in accordance with the distribution of the egg ages. However, the contradictory fact to the common sense, namely the curve being divided into several peaks with the rhythmic interval, is considered to be nothing but verification that the egg hatching is essentially affected by the rhythmic factor underlying in the eggs.

The numbers of the modal peaks differ sometimes according to the races. In the present studies, the most consisted of 4 peaks, which was observed in *Nichi-No. 124* (Fig. 2).

4. Latent Activity of Hatching and the Rhythmicity of Hatching

In Chapter I we observed the phenomenon that the hatching of the eggs was generally more delayed in continuous darkness than in continuous illumination, further in this chapter (Section 1) that a considerable delay in hatching occurred when the eggs were put in darkness at just hatching period after kept under illumination during the whole embryonic stages.

These phenomena suggest that there is a latency for hatchability under dark condition. Then the author studied on the latency relating to the rhythmic appearance of hatching in this section.

MATERIALS AND METHODS

Each group of 1000 eggs in the same population of *Taihei* × *Choan*, which were formally hydrochlorized, was incubated under constant darkness. When the eggs reached to the larval body completion, each group was exposed to 50 lux lamp light for 4 hours with every interval of 4 hour. The hatching phases were expressed with percentage of hatchability. Curve A (Fig. 23) means the percentage of hatched larvae in one group at every time and Curve B shows the increasing degree of hatchability, viz. the present percentage minus the previous one.

RESULTS

The aquired results are shown in Fig. 23 and Table 5. The eggs subjected to the light (Curve A•B) requested about 28 hours for hatching termination and the hatching phase showed nearly a monomodal curve. However, in the eggs kept under constant darkness (Curve C), a usual cyclic phase of hatching took place. In the

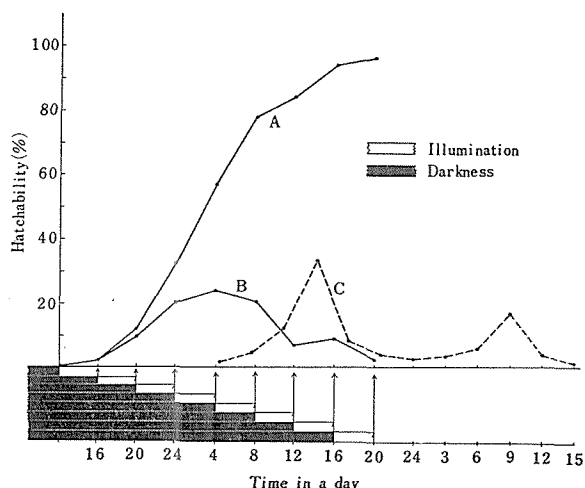


Fig. 23 Latent activity of hatching of silkworm eggs

- A : Increase of hatchability in one group of the eggs at every time when subjected to 4-hr illumination
 B : Increasing degree of hatchability as same as the above A
 C : Hatching phase under continuous darkness

dark régime the hatching began 14 hours later and ended 19 hours later, than the light régime.

CONSIDERATION

In this experiment the latent period of hatchability was measured with relation to the rhythmicity of hatching. From the results obtained it is conspicuous that the eggs just before hatching have latent activities of hatching, and can immediately hatch when subjected to light while not soon hatch when kept under darkness. Occurrence of hatching under constant darkness must be induced by the endogenous factor, that is physiological condition which stimulates, instead of light stimuli,

Table 5 Hatchability of eggs when subjected to 50 lux lamp light

No.	Time of illumination (o'clock)	No. hatched	No. of tested eggs	Hatchability (%)	Increase of hatchability (%)
1	May 30. 12~16	26	957	2.6	—
2	16~20	122	896	12.2	9.6
3	20~24	297	912	32.6	20.4
4	May 31. 0~4	519	910	57.0	24.4
5	4~8	783	1120	77.9	20.9
6	8~12	829	986	84.1	6.2
7	12~16	806	857	94.0	9.9
8	16~20	952	988	96.2	2.2

Remarks: Beginning of incubation—May 21. Temperature 25°C R. H. 80%

the eggs hatch during the latent period of hatchability. In this process some duration (14~19 hours) is probably requested to recover the regular physiological condition in the eggs. A delay of hatching appeared when the eggs were put in dark (Section 1, Chapter III) is also considered to be brought by the same mechanism. According to TAKANASHI (1946) and KOIZUMI (1961), the photosensitivity of the eggs just before hatching differs considerably among strains of silkworms, so the latent activity of hatching would be shown with wide variance by using light stimulation, which is doubtless to be the principal stimulus making the eggs hatch, as already described.

Conclusively it can fairly be stated that the hatching of the eggs occurring under darkness is induced by the endogenous stimuli which arise rhythmically in each egg. Of course the stimuli must be the rhythmic change of physiological activity in the eggs. If not so, the hatching feature should show a formal similarity to Curve B without rhythmicity.

Such a problem will be dealt with in the following section (5).

5. Succinic Dehydrogenase Activity through Incubative Duration

As previously indicated, occurrence of hatching under darkness was likely induced by the endogenous stimuli, which should be the rhythmic change of physiological activity in the eggs. In the silkworm eggs KAWASE (1954) and NITTONO et al. (1952), NISHIZAWA (1955) and KAWASE (1955) studied on the activities of cytochrome-c oxidase and of dehydrogenase, respectively, and pointed out that the activities had close relation the growth phase of the eggs, whereas no attention was paid to the diel change of the activity.

Then, in the present experiment the author measured the enzyme activity by using succinic dehydrogenase as an indicator.

MATERIALS AND METHODS

In the first experiment the hibernated eggs of *Taihei* × *Choan* were used for the test. The measurement of the activity of succinic dehydrogenase was carried out every 4 hours from 7th day to 9th day of incubation.

In the second experiment the hydrochlorized eggs of *Nichi-No. 124* × *Shi-No. 124* were used. The activity was measured every 6 hours through whole incubative duration. The incubation of the eggs was done under 25°C and R. H. 80 % with continuous darkness.

Thunberg tube method was applied for measuring the activity by making use of 2, 3, 5-triphenyltetrazolium chloride (TTC), 0.6g (0.5g*) of the eggs was homogenated and centrifuged together with 7cc (5cc*) of Sørensen phosphate buffer (pH 7.4). The supernatant liquid was poured into the main chamber of Thunberg tube. Then each 2cc of 10 M. TTC and 10 M. sodium succinic acid was taken into the

* showing the case of the second experiment

side chamber, being mixed up under vacuum, and kept under 35°C for 6 hours (5 hours*). Resulted formazan (TPF) was absorbed with ethyl acetate and its quantity was determined by AKA Colourimeter (480 μ filter was used). Fig. 23 and 24 show average values which were got by assaying 3 tubes per an experiment in the same time.

RESULTS

In the hibernated eggs (Fig. 24) the activity of succinic dehydrogenase increases gradually as the egg growth, becoming very stronger at 8 p. m., 27th and 4 p. m., 28th. An interval between these two peaks is about 20 hours, coinciding nearly

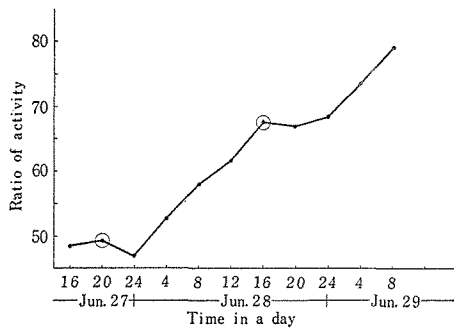


Fig. 24 Activity of succinic dehydrogenase at 7th to 9th days of incubation (hibernated eggs of *Taihei* × *Choan*)
○ : Peak of activity

with regular interval seen in the rhythmic phase of hatching.

In the hydrochlorized eggs (Fig. 25) a zigzag curve appears, but considerable higher peaks can be revealed in it, viz. at 4th, 6~7th, 8th and 9th days in incubation, among which the sharpest is assumed to become the blastokinesis stage. In the curve 18~24-hr rhythm can be detectable though its sign is very faint. This will perhaps be caused by longer interval (6 hours) of the measurement than in the first experiment (4 hours).

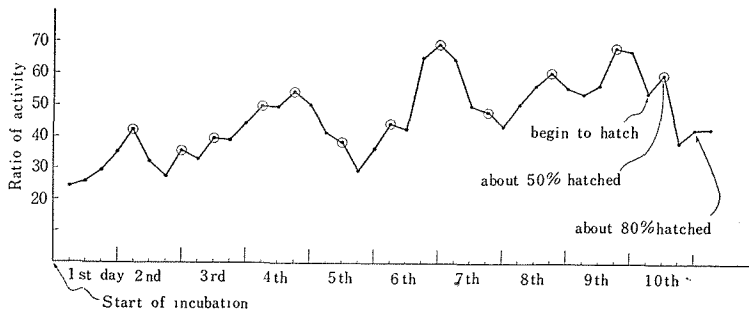


Fig. 25 Activity of succinic dehydrogenase during incubation (hydrochlorized eggs of *Nichi-No. 124* × *Shi-No. 124*)
○ : Peak of activity

CONSIDERATION

We have observed in the present experiment that the activity of succinic dehydrogenase increases gradually from the beginning time of incubation, falls down

temporally at the blastokinesis stage, later increases largely until just before hatching when it shows the strongest and suddenly decreases from just hatching time. This tendency is almost agreed with that in some enzyme activities of silkworm eggs (NITTONO et al. 1952, NISHIZAWA OGIWARA 1955, NISHIZAWA•KOBAYASHI 1955, KAWASE 1954•1955). These, however, deal with the activity at a definite time in every day, and any change of the activity within a day is not evident. According to this experiment, a rhythmic sign likely exists in the activity of the dehydrogenase. Being about 20 hr, the cycle interval is supposed to correlate to the endogenous rhythmicity of hatching under darkness.

The activity of succinic dehydrogenase should be an important factor, but not the only factor, which indicates the physiological activity of the eggs. It is, therefore, plausible that the rhythmicity of hatching under darkness is induced by the endogenous stimuli such as the rhythmic change of the activity of the dehydrogenase.

Considering from the daily rhythmicity in emergence (KIMURA 1952) and moulting phase (TANAKA 1956), the physiological rhythmicity seems to continue through whole life of silkworm.

6. Conclusion

The existence of the endogenous rhythmicity of hatching under continuous darkness was confirmed in Chapter I and II, then the manifestation of the endogenous character has been analyzed from several points of view in the present chapter.

(1) The rhythmicity of hatching, which is almost perfectly checked by continuous illumination, is repossessed within some duration after the eggs are put in dark condition. The light stimulation must be the principal factor to control the hatching. Proceeding treatment with alternation of light and dark acts as an after-effect upon hatching.

(2) The chilling treatments to the eggs at various stages does not bring any appreciable effect upon the expression of the endogenous rhythmicity of hatching. In this case each modal peak in the hatching phases tends to keep the definite position. This means that the physiological activity in the eggs has certainly been maintained even under the critical low temperature less than 5°C, in which morphological development of the embryo could not occur. However an extraordinary chilliness such as -7°C has completely suppressed the egg vitality.

(3) In the same population the difference of the ovipositing time affects on the occurrence of the modal peaks of hatchability. The number of the peaks is decided by variance of the egg ages; the more the egg ages are varied, the more the peaks appear.

(4) The eggs just before hatching have latent activity of hatching which is stimulated when subjected to light. Occurrence of hatching under continuous darkness seems to be induced, instead of light, by the physiological activity in the

eggs. A delay of hatching under continuous darkness may be caused by some duration (14~19 hours) requested for recovering of the activity.

(5) Under continuous darkness a faint rhythmic phase is detectable in the activity of succinic dehydrogenase through whole incubation duration. Interval between each rhythmical peak of the activity is approximately 20 hours, which is supposed to correlate closely to the cyclic interval in the endogenous rhythmicity of hatching.

CHAPTER IV EXPERIMENTAL ANALYSIS OF THE EFFECTS OF ENVIRONMENTAL FACTORS UPON THE EGGS JUST BEFORE HATCHING

It has already been demonstrated in Chapters I to III that the rhythmic hatching of silkworm eggs occurs endogenously under continuous darkness and the rhythmic phase is largely checked by continuous illumination. The light, however, is certainly the principal factor making the eggs hatch when cast on the eggs at just hatching stage, as already seen in many papers and in the present study. Of course, the regular daily rhythmicity of hatching has been exposed in the natural condition, but the interval between the endogenous cycles takes usually about 18~20 hours, a little less than diel periodicity. Therefore, the endogenous rhythmicity is to be completely controlled by the diel change of the natural condition.

Then the author took up the light and the thermal actions which seemed to affect greatly on the hatching and analyzed the effects of these environmental factors upon hatching, using various kinds of silkworm eggs.

1. Photosensitivity of the Eggs just before Hatching

As mentioned in Section 4, Chapter III, there is the latent activity of hatching in the eggs, the embryos of which have reached to full growth. In this stage the eggs can immediately hatch when subjected to light, but considerable differences of the responsibilities likely exist among the strains. In the present experiment such differences will mainly be dealt with.

MATERIALS AND METHODS

Each group of 2000 eggs was pasted on a thick paper and incubated under the constant conditions of light (30 lux), temperature (25°C) and humidity (R. H. 80%). When the eggs grew to the blue colour stage namely full grown stage, they were treated by alternation of light and dark as in the followings,

	daytime	night
Experiment I	light (100 lux), 12 hr	dark, 12 hr;
	dark, 12 hr;	light (100 lux), 12 hr
materials :	hydrochlorized eggs of <i>Shuka</i> , <i>Daizo</i> , <i>Choan</i> × <i>Taihei</i>	
Experiment II	light (30 lux), 6hr	dark, 6 hr
	(regardless chronological time)	
materials :	hydrochlorized eggs of w_1 , b_1 3-moulter, b_2 , <i>re</i> , <i>Mysore</i> , <i>Cambodge</i>	

RESULTS

Experiment I (Table 6, Fig. 26)

In the two strains excepting *Daizo*, the modal peaks of hatching arose almost simultaneously with illumining presenting the diel rhythmicity. This tendency was not different between each opposite régime as to light.

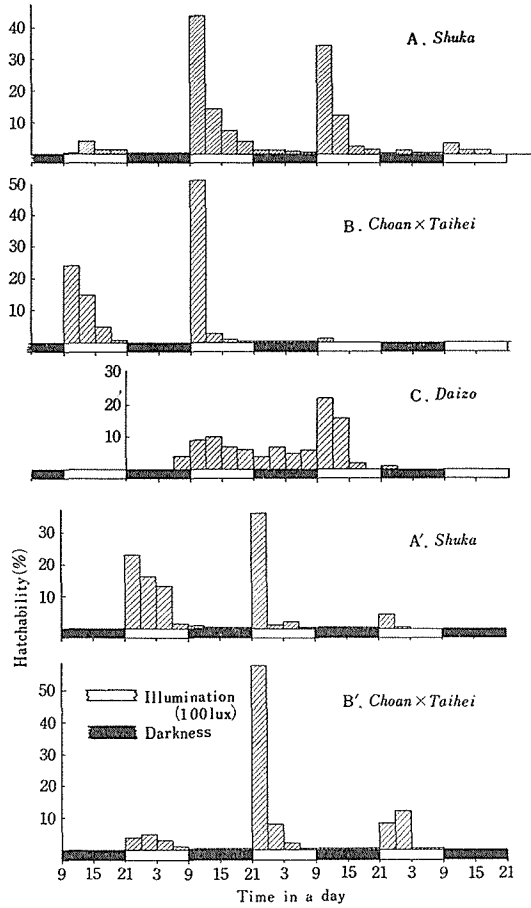


Fig. 26 Alternation of light (12 hr) and dark (12 hr) and hatching phases in normal strains

Table 6 Hatchability in Experiment I

	Strains	In light	In dark
Daytime light	A. <i>Shuka</i>	95.5%	4.5%
: night dark	B. <i>Choan x Taihei</i>	98.5	1.5
	C. <i>Daizo</i>	72.0	28.0
Daytime dark	A'. <i>Shuka</i>	99.0	1.0
: night light	B'. <i>Choan x Taihei</i>	100.0	0.0

Remarks : Light—12hr, Dark—12hr

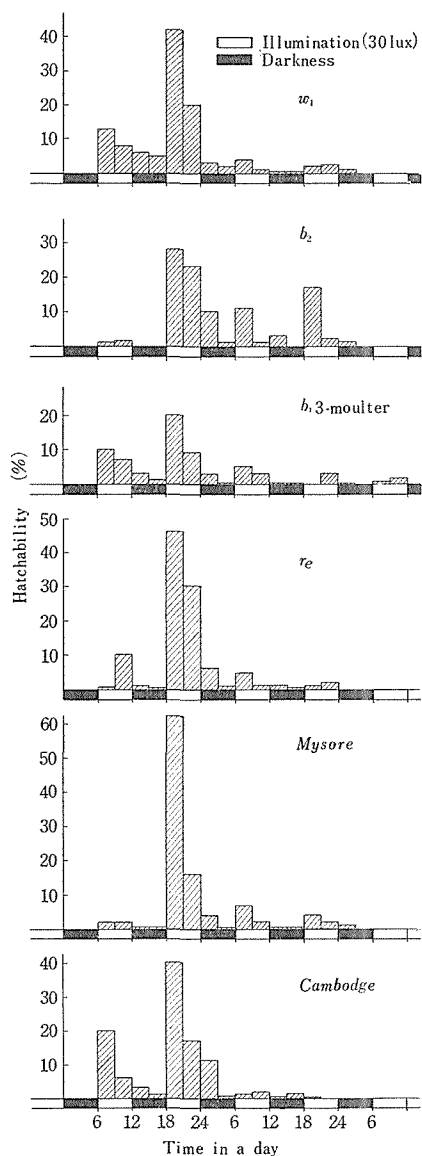


Fig. 27 Alternation of light (6 hr) and dark (6hr) and hatching phases in egg colour mutants and multivoltine strains

Therefore it is sure that the rhythmicity of hatching was strongly controlled by the light. *Daizo*, however, was not so sensitive that about 30% of the eggs hatched under the darkness. Such an insensibility in *Daizo* seems to have some relation to the arrhythmic phase of hatching which was pointed out in Chapter I.

Experiment II (Table 7, Fig. 27)

Most of the strains hatched soon when illuminated. *Mysore* and *re* were rather susceptible to the light stimulus, showing more than 90% hatchability.

Table 7 Hatchability in Experiment II

Strains	Characters	In light	In dark
w_1	white egg	85.5%	14.5%
b_2	brown egg	85.5	14.5
$b_{1,3}$ -moulter	"	89.5	10.5
r_e	red egg	92.5	7.5
<i>Mysore</i>	multivoltine	95.0	5.0
<i>Cambodge</i>	"	83.5	16.5

The other strains with exception of *Mysore* and *re* showed more than 10% hatchability under absent of light, so that they are assumed to have comparatively lower photosensitivity.

2. Thermosensitivity of the Eggs just before Hatching

In this experiment the thermosensitivity of the eggs just before hatching was studied by using alternative régime of high temperature and low one.

MATERIALS AND METHODS

The materials were treated with the same method as in the previous experiment. Each group of the eggs was subjected to alternation of high temperature and low one under light and under dark.

Experiment I Warm (12 hr) : cool (12 hr) alternation

Materials—Hydrochlorized eggs of *Choan* × *Taihei*, *Shuka* and *Daizo*

Régimes— Daytime Night
 Warm (25°C), 12hr : cool (12°C), 12 hr
 Cool (12°C), 12 hr : warm (25°C), 12 hr

The above alternation was given to the eggs just before hatching under illumination (100 lux) and under darkness, and the hatching phase was observed.

Experiment II Warm (6 hr) : cool (6 hr) alternation

Materials — Hydrochlorized eggs of *w₁*, *b₁₃-moulter*, *b₂*, *re*, *Mysore*, *Cambodge* and *Shi-No. 122*

Régimes— Daytime Night
 Warm (25°C), 6 hr : cool (12°C), 6 hr

The above alternation was given to the eggs just before hatching under illumination (30 lux).

The hatchability in the high temperature was used as an indicator of thermo-sensibility of the eggs.

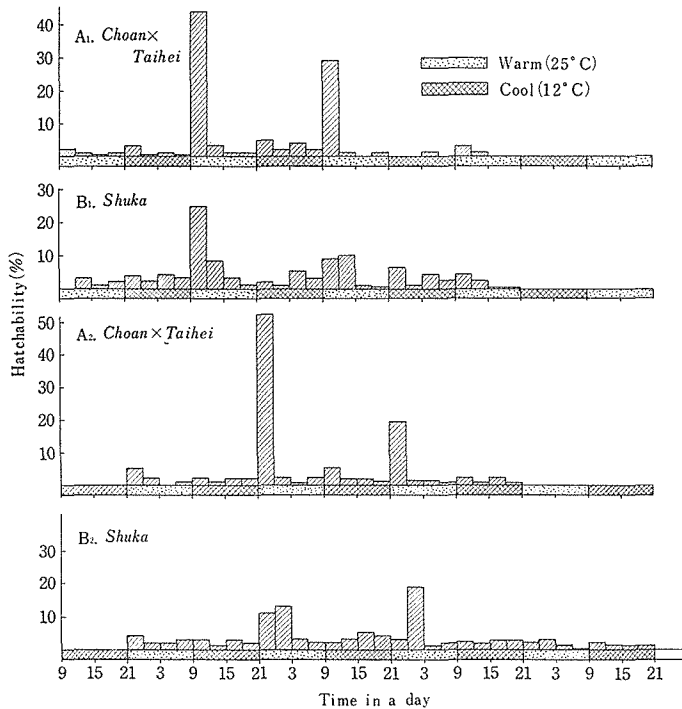


Fig. 28 Alternation of warm (12 hr) and cool (12 hr) under illumination and hatching phases in normal strains

RESULTS

Experiment I (Table 8, Fig. 28, 29)

Either under light or under dark, there appeared the rhythmic signs of hatching being almost left to the mercy of the thermal alternation.

Table 8 Hatchability in Experiment I

Régimes	Strains	In warm (25°C)	In cool (12°C)
Light (100 lux)	Daytime warm (A ₁ <i>Choan</i> × <i>Taihei</i>)	83%	17%
	: night cool (B ₁ <i>Shuka</i>)	66	34
	Daytime cool (A ₂ <i>Choan</i> × <i>Taihei</i>)	80	20
	: night warm (B ₂ <i>Shuka</i>)	66	34
Dark	Daytime warm (A ₃ <i>Choan</i> × <i>Taihei</i>)	94	6
	: night cool (B ₃ <i>Shuka</i>)	92	8
	Daytime cool (A ₄ <i>Choan</i> × <i>Taihei</i>)	98	2
	: night warm (B ₄ <i>Shuka</i>)	84	16

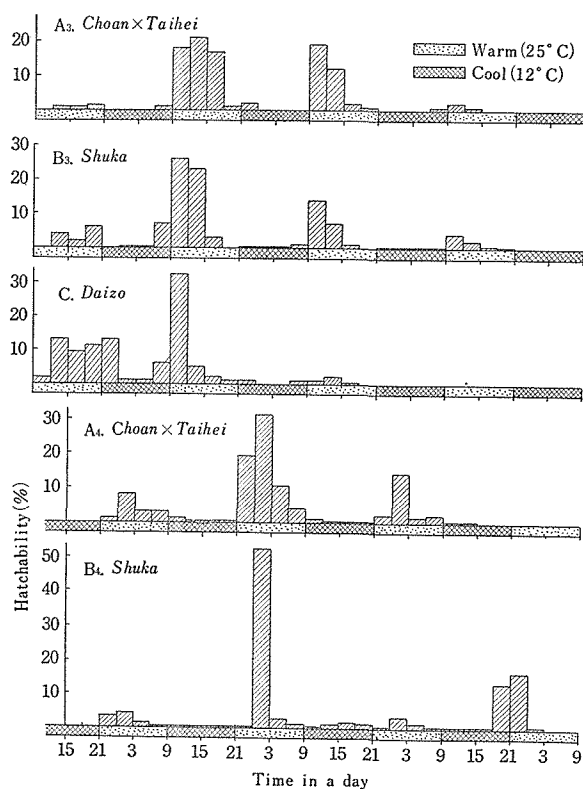


Fig. 29 Alternation of warm (12 hr) and cool (12 hr) under darkness and hatching phases in normal strains

From the results it is conspicuous that in the normal strains the thermal stimulation is also an important factor to control the hatching phase.

Now, let us make a detail observation on the two strains, *Choan* × *Taihei* and *Shuka*, and we can find out in them the phenomenon that under illumination considerable numbers of individuals hatched when removed to coolness. Such a phenomenon means that the very thermal difference (+13°C), even if negative (-13°C), viz. decrease from warm to cool, stimulates the eggs to hatch. Under darkness, however, the effect of the negative thermal difference can hardly be detectable and the hatching occurs only in the positive one (+13°C) from cold to warm. Notwith-

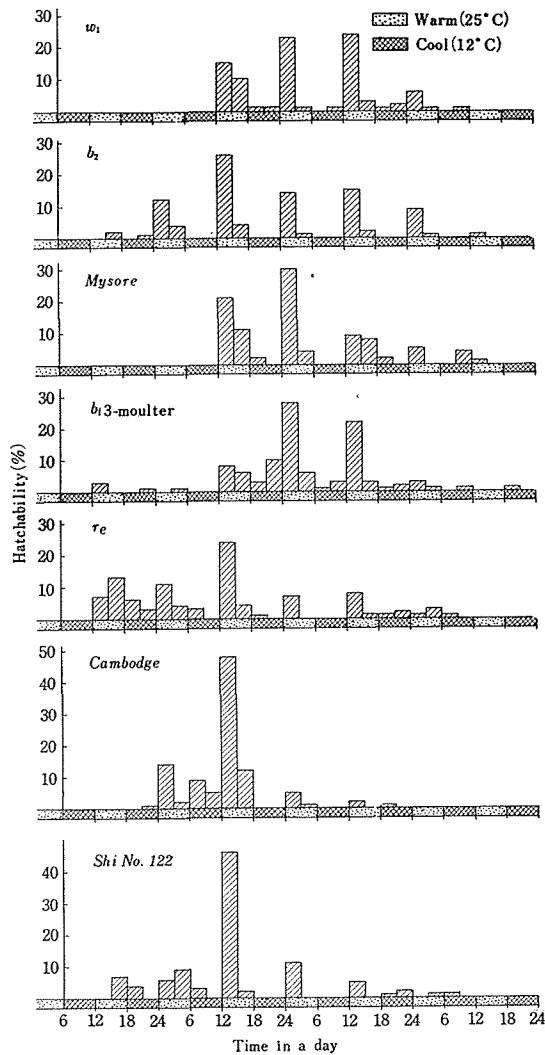


Fig. 30 Alternation of warm (6 hr) and cool (6 hr) under darkness and hatching phases in various strains

standing, the eggs of *Daizo* hatch not always in the positive difference, but can hatch even in the negative one, in which the hatchability shows 23%. The thermo-sensitivity of *Daizo* is presumed to be lower than that of the other strains. And *Choan* × *Taihei* seems to have higher sensitivity to the upward shift of temperature than *Shuka*.

Experiment II (Table 9, Fig. 30)

It is recognized that w_1 , b_2 , *Mysore* and *Shi-No. 122* have stronger sensitivities to the positive thermal difference than the other strains; b_1 3-moulter, *re* and *Cambodge* are weaker in the downward shift of temperature, viz. the negative thermal difference. Consequently the racial differences exist certainly in the thermo-sensitivity of the eggs just before hatching.

Table 9 Hatchability in Experiment II

Strains	In warm (25°C)	In cool (12°C)
w_1	94.3%	5.7%
b_1 3-moulter	79.5	20.5
b_2	97.5	2.5
<i>re</i>	84.5	15.5
<i>Mysore</i>	94.5	5.5
<i>Cambodge</i>	85.5	14.5
<i>Shi-No. 122</i>	94.0	6.0

Remarks : Light condition—30 lux

3. Effect of Combination of Light and Temperature upon the Eggs just before Hatching

In the previous experiments (Section 1, 2), the sensitivities to shift of light and of temperature were dealt with as to various kinds of eggs. And it has been ascertained that either light or temperature is an essential factor to control the hatching phase. Further studies are given in this section of environmental conditions, combined light with temperature, affecting the hatching phase.

MATERIALS AND METHODS

Each group of about 2000 eggs were subjected to the undermentioned régimes from the just before hatching to the end of hatching.

Experiment I (Light and warm : Dark and cool)

- | | Daytime (12 hours) | Night (12 hours) |
|----|---------------------------------|---------------------------------|
| a. | Light (100 lux) and warm (25°C) | Dark (0 lux) and cool (12°C) |
| b. | Dark (0 lux) and cool (12°C) | Light (100 lux) and warm (25°C) |

Materials : *Choan* × *Taihei*, *Shuka* and *Daizo*

Experiment II (Light and cool : Dark and warm)

- | | | |
|----|---------------------------------------|---------------------------------------|
| | Daytime (12 hours) | Night (12 hours) |
| a. | Light (10 or 100 lux) and cool (12°C) | Dark (0 lux) and warm (25°C) |
| b. | Dark (0 lux) and warm (25°C) | Light (10 or 100 lux) and cool (12°C) |

Materials : *Choan* × *Taihei*, *Shuka*, *w₁*, *w₂ch*, *re*, *b₂*, *b₁* 4-moulter, *b₁* 3-moulter, *Kyokko* (3-moulter), *Mysore*, *Cambodge*, *Daizo*, *Nichi-No. 1* × *Daizo* *F₂*, *Ô-No. 18*, *Shi-No. 16*

The hatchabilities (%) were measured every 3 or 6 hours.

RESULTS

Experiment I (a, b)

The results are shown in Table 10 and Fig. 31.

The rhythmic phase of hatching was presented very regularly under the combined condition of light and temperature, similar to the diel change of environments.

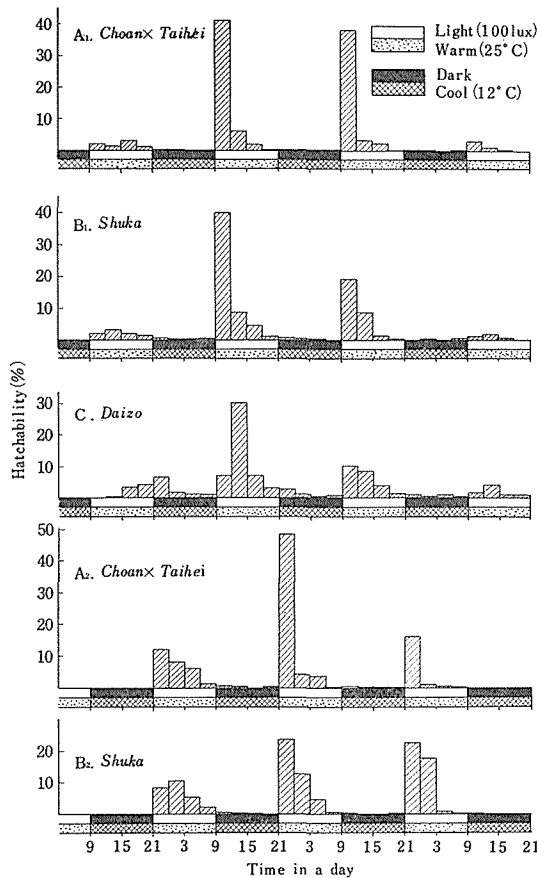


Fig. 31 Alternation of light & warm (12 hr) and dark & cool (12 hr) and hatching phases

Table 10 Hatchability in Experiment I (a, b)

Régimes	Strains	In light and warm	In dark and cool
Daytime Light and warm Night Dark and cool	A ₁ <i>Choan</i> × <i>Taihei</i>	99.5%	0.5%
	B ₁ <i>Shuka</i>	97.0	3.0
	C <i>Daizo</i>	85.0	15.0
Daytime Dark and cool Night Light and warm	A ₂ <i>Choan</i> × <i>Taihei</i>	100.0	0.0
	B ₂ <i>Shuka</i>	99.0	1.0

These results have already been expected from the data on single action of light or temperature. Especially in *Daizo* the effect of such a condition was very significant; the strain could hardly be controlled by single action of light or temperature. It should be an additional effect of light and warm, either of which stimulates the eggs hatch as previously pointed out.

The adversal treatment (Experiment I-b) as to the chronological time scarcely caused appreciable different on the hatching phase though the modal peaks decrease to three from four.

Experiment II (a, b)

The eggs of two strains hatched rhythmically with alternation of light and dark without relation to the thermal shift; in absence of light the hatching hardly occurred even if put in warmness, on the contrary in presence of light it evidently occurred even if in coolness (Fig. 32).

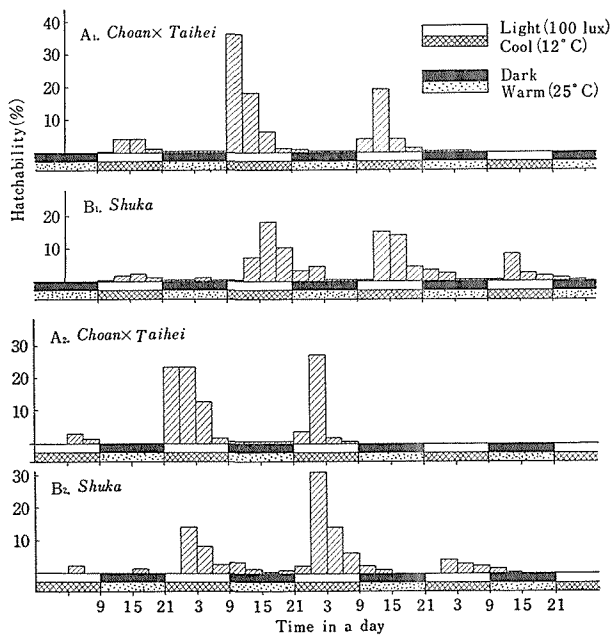


Fig. 32 Alternation of light & cool (12 hr) and dark & warm (12 hr) and hatching phases in normal strains

Table II Hatchability in Experiment II (two strains)

Régimes	Strains	In light and cool	In dark and warm
Daytime Light and cool	(A ₁ <i>Choan</i> × <i>Taihei</i>)	100%	0%
Night Dark and warm	(B ₁ <i>Shuka</i>)	88	12
Daytime Dark and warm	(A ₂ <i>Choan</i> × <i>Taihei</i>)	100	0
Night Light and cool	(B ₂ <i>Shuka</i>)	89	11

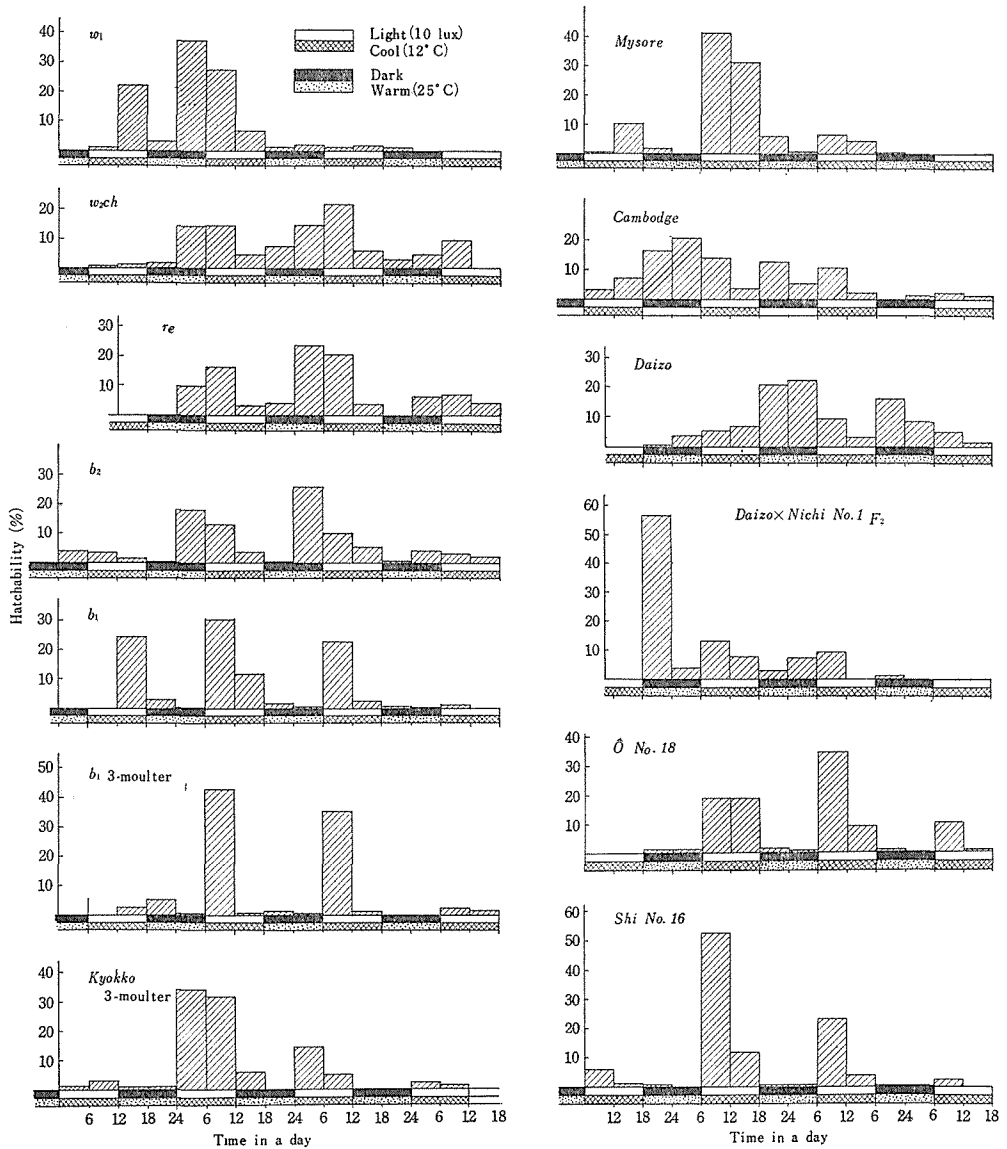


Fig. 33 Alternation of light & cool (12 hr) and dark & warm (12 hr) and hatching phases in egg colour mutants

It means that the light should be the essential factor to control the hatching in the normal black egg races.

Further, observing Table 12 and Fig. 33 we can recognize the fact that *Mysore*, *Kyokko*(3-moulter), *b*₁3-moulter, *b*₂ and the black egg strains *Ô-No. 18*, *Shi-No. 16*) should very high hatchability more than 90 % in light and cool condition.

Table 12 Hatchability in Experiment II

Varieties	In light and cool	In dark and warm
<i>w</i> ₁	57.0%	43.0%
<i>w</i> ₂ <i>ch</i>	56.2	43.8
<i>re</i>	55.0	45.0
<i>b</i> ₂	45.2	54.8
<i>b</i> ₁ 4-moulter	92.0	8.0
<i>b</i> ¹ 3-moulter	92.5	7.5
<i>Kyokko</i> (3-moulter)	90.4	9.6
<i>Mysore</i>	93.5	6.5
<i>Cambodge</i>	42.7	57.3
<i>Daizo</i>	29.5	70.5
<i>Nichi-No. 1</i> × <i>Daizo F</i> ₂	30.0	70.0
<i>Ô-No. 18</i>	93.5	6.5
<i>Shi-No. 16</i>	99.5	0.5

Daizo and its hybrid, however, showed considerable high hatchability (about 70 %) in dark and warm treatment, and in *w*₁, *w*₂*ch*, *re*, *b*₂ and *Cambodge* about a half number of the eggs hatched either in each condition, light and cool or dark and warm.

From the above facts it can be stated that the normal black egg races have stronger sensitivities to light. Such a tendency is also seen in the 3-moulter strain, *b*₁ and *Mysore* (multivoltine). On the other hand, *Daizo* (sub-multivoltine) has stronger thermosensitivity, and the egg colour mutants (white egg, red egg, brown egg excepting *b*₁) and *Cambodge* intermediate in the both sensitivities.

4. Consideration and Conclusion

It has already been known in silkworm eggs that the sensitivity to environmental factors differ according to the races (TAKEUCHI 1956, KOIZUMI 1961). But detailed researches have been remained to be resolved.

In the present chapter the sensitivities to light and temperature were fundamentally studied by using the eggs just before hatching in various varieties as the materials, and then effects of combinations of light and temperature upon the hatching phase were analyzed.

(1) When alternation of light and dark is given to the eggs just before hatching, the occurrence of hatching almost limited at presence of light. Therefore the endo-

genous character in the rhythmicity of hatching seems to be rather weaker than that in activities of other insects*. Nevertheless *Daizo* (sub-multivoltine) is not perfectly controlled by the light stimulus. Under darkness the hatchability is 28%. So the strain can be said to be comparatively irresponsible to light, besides the egg colour mutants such as w_1 , b_2 and the multivoltine strains show similar tendency. As these strains scarcely have the regular rhythmicity of hatching, the sensitivity to light seems to have close relation to the occurrence of the endogenous rhythmicity of hatching.

(2) When the eggs just before hatching are subjected to alternation of warm and cool under continuous light or under continuous dark, the significant modal peaks of hatchability appear limited at every warmness. The thermal stimulation, accordingly, is also a controlling factor on the hatching. But it has been recognized that under illumination considerable number of the eggs go to hatch when moved to coolness. This fact implies that the thermal difference, either positive or negative, acts absolutely as a factor to induce the hatching; in general, increasing stimulation (the positive thermal difference) is more strongly effective than decreasing one (the negative thermal difference). The eggs of *Daizo* can hatch not always in the warmness, but can do even in the coolness, so that they seem to be less sensitive to the positive thermal difference than the normal black eggs. The similar tendency is also detectable in b_{13} -moulter and *Cambodge*. That these strains having weaker sensitivity to the positive thermal difference show scarcely the endogenous rhythmicity of hatching under continuous darkness is assumed to have some relation to such a character as well as the photosensitivity.

(3) Putting in the combination of light and warm, the eggs of *Daizo* can hatch very well. This may be dependent upon an additional effect of light and warm. When the normal black eggs were subjected to alternation of light & cool and dark & warm, they would hatch almost limited in the light & cool. This will afford proof of the fact that the light is the essential factor to induce the hatching. *Daizo* and its hybrid tend to hatch when put in the dark & warm. In these strains the positive thermal difference may act more strongly on hatching rather than the light stimulus. Considerable number of the eggs of w_1 , w_2 *ch*, *re*, b_2 and *Cambodge* can hatch in either condition, light & cool or dark & warm. The endogenous character of hatching seems to be closely related to the photosensitivity of the eggs because these mutants and *Daizo* have hardly the regular endogenous rhythmicity of hatching.

By the way the endogenous character of hatching rhythm in silkworm eggs has supposedly been acquired by their adaptability to the natural environments, viz. daily periodic change, as proposed by MORI (1948). In case, weaker sensitivity to the environmental factors is considered to be connected with the reason why *Daizo* and

* 11~13, 42, 47, 48, 59, 62, 64, 65)

the other mutants have hardly exposed the endogenous rhythmicity of hatching under continuous darkness.

Furthermore it is quite obvious that there exist the racial differences in the responsibility to the environmental factors or in the appearances of the endogenous rhythmicity of hatching, and it is plausible that the differences are closely related especially with qualitative and quantitative differences in the serosal pigments of silkworm eggs.

The problem has been left in the future investigation.

CHAPTER V DISCUSSION ON THE OCCURRENCE OF THE RHYTHMICITY OF HATCHING IN SILKWORM EGGS

Considerable numbers of studies have already been reported on the endogenous activities of Arthropods; for example on the diel rhythmicity of the retinal pigments in Arthropod eyes (CONGDON 1907, DEMOLL 1911, WELSH 1930-51, BENNETT 1932, BENNETT & MERRICK 1932, NAGANO 1943, 1950, WEBB & BROWN 1953, KOYAMA 1955, YAGI et al. 1956) and on those of the behavior in insects (GUNN 1940, MELLANBY 1940, SUGIYAMA & MATSUMOTO 1955). Most of them, however, are treated on the rhythmicity which occurs repeatedly in the same individual for a long time. As previously described, the rhythmic character of hatching seen in silkworm eggs appears surely as mass and could not be repeated in an individual. The rhythmic manifestation of the imaginal emergence in silkworm (KIMURA 1952) will belong to such a rhythmicity, which was observed in several moths of the other Lepidoptera (PICTET 1918, SCOTT 1936, MORIARTY 1959). The rhythmicity is assumed to have endogenous character because it happened under the constant condition with darkness. Then the differences in the rhythmic phases between the eggs of normal race and of egg colour mutant, between the hibernated and the hydrochlorized eggs, between less and more moultine eggs, and lower and higher voltine eggs, etc. were examined. Consequently it has become clear that there is, more or less, racial differences in the rhythmicity. Especially the fact is noteworthy that even in the same strain the rhythmic appearance differs always between the hibernated eggs and the hydrochlorized eggs; the latter seems to have stronger character of the endogenous rhythmicity than the former. The cause depends supposedly upon the differences between the physiological activities in both the eggs.

Based on the above facts, the author measured changes of the succinic dehydrogenase activity through embryonic stages under the constant condition. And it was ascertained that the rhythmic sign of hatching occurred almost coincided with that of the enzyme activity.

Now, speaking on expression of the rhythmicity, we can recognize the existence of some latent period in hatchability when the embryos have finished their growth.

Namely, within this period the eggs are provided with the internal condition to cause hatching whenever subjected to some stimulus. Accordingly, under constant darkness the eggs can be hatched probably by the physiological rhythm persisted in themselves, unless any other stimuli are given. Each interval between rhythmical cycles is usually about 18 hours. According to F. TANAKA (1951, 1956) it is said that a similar rhythmicity to the hatching's is detectable in the manifestation of larval moulting, additionally 18-hr rhythmicity is endogenously exposed in the imaginal emergence (KIMURA 1952). It is, therefore, plausible that the endogenous rhythmicity would be kept throughout whole stages in silkworms.

Further we can take up the colouration of the eggs as a factor relating closely to the rhythmicity of hatching. The colouration of silkworm eggs is mainly decided by quantity or quality of tryptophan pigments of the serosa. In fact colourless or slightly coloured eggs were insensible to light, besides hardly exposed the rhythmic phase of hatching, as previously described. As the serosal pigments are eaten by the full-grown embryo and the egg colour changes to blue, the serosal pigments are to relate to the rhythmic character till just before hatching, and after this time tryptophan origin pigments such as the retinal pigments, the nervous pigments and the hypodermal pigments would act as the receptor of environmental factors. As aforementioned, the light is truly the essential factor making the eggs hatch at the latent period of hatchability inhibiting almost completely the endogenous rhythmicity of hatching. On the other hand the temperature is also surely a controlling factor for hatching under darkness though in this case the positive thermal difference acts as stimulation.

Therefore it can be stated that the occurrence of the rhythmicity in silkworm eggs is not caused perfectly by endogenous stimulation but caused considerably exogenous one. Such a case has also been reported on the other insects (GUNN 1940, MELLANBY 1940, MATSUMOTO • SUGIYAMA 1955).

It has been considered that the endogenous rhythmicity of activity would be the character acquired by continuous adaptation to change of the natural environments. The consideration is well coincided with arrhythmicity seen in several strains of silkworms, because they are insensitive to environmental factors, so to speak, weaker in the adaptability to the factors than the normal strains.

Additionally the author must mention on the strains of multivoltine and three-moultine, the hatching phases of which are not so rhythmical. Then the question why these black egg strains could not display the same regular rhythmicity of hatching as in the other normal strains of black eggs, will be proposed here. For the reason the author wants to support such a presumption that growth pace is abnormally fast in these strains, so that the regular rhythmicity could not be manifested.

In the eggs of Genus *Bombyx* the interval between the modal peaks of hatching is

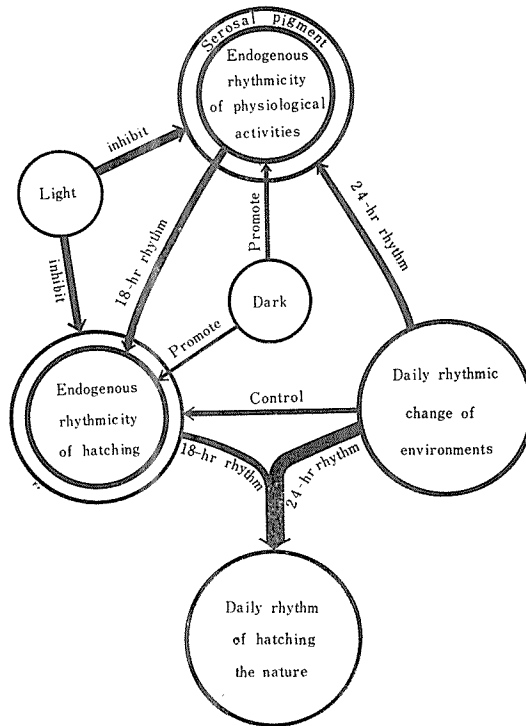


Fig. 34 Diagrammatic explanation on occurrence of rhythmicity in hatching

generally about 18 hours and not 24 hours as in the other insects. This shortened type of the daily rhythmicity must be a special character which has hereditarily been maintained in *Bombyx* eggs.

Now the occurrence of the diel periodicity of hatching in the natural condition will diagrammatically be explained in Fig. 34.

SUMMARY

The hatching phase of silkworm eggs with special concern to the rhythmic character were investigated by the following experiments.

i) The eggs of various races were incubated under the constant conditions for detection of racial differences among rhythmic phases of hatching.

ii) Effects of different temperatures, relative humidities and lights upon manifestation of the rhythmicity were studied.

iii) The expression of the rhythmicity was analyzed by chilling and by alternative treatment of light and dark for the eggs at various embryonic stages.

iv) Various conditions such as light, dark, warm and cool were given, singly or combined with each other, to the eggs just before hatching, and the exogenous

character in the rhythmicity was investigated.

The results obtained are summarized as in the followings.

I. RACIAL DIFFERENCE AND RHYTHMICITY OF HATCHING

(1) In black coloured eggs of normal strains the rhythmicity of hatching is exposed under the constant condition with darkness. The rhythmicity, therefore, can be said to be endogenous in absence of light. There is, more or less, racial differences in the manifestation of the rhythmicity.

(2) Generally long illumination causes promotion of the egg growth, while inhibits the occurrence of the rhythmicity ; the more the light intensity is strengthened, the more the rhythmicity becomes faint.

(3) The rhythmicity of hatching is indistinguishable in *Kyokko* (3-moulter), *Daizo* (sub-multivoltine), *Ô-No. 19* (European race), *b₂* (brown egg), *w₁* (white egg), *re* (red egg) and *Mysore* (multivoltine). It is indicated that the rhythmicity has close relation to egg colour which is decided quantity or quality of the serosal pigments, multism and voltinism.

(4) Each interval between modal peaks of hatching is about 18 hours, differing according to the strains.

(5) The eggs of *Bombyx mandarina* considered as an ancient species of *B. mori* has also 18-hr rhythmicity of hatching.

II. ENVIRONMENTAL FACTOR AND RHYTHMICITY OF HATCHING

(1) Under continous darkness the rhythmicity with 18-hr interval is presented either in 18°C constancy or in 25°C one, but the interval is rather shortened under 30°C. Further the rhythmicity is disturbed and becomes obscure.

(2) The regular rhythmicity is observed either under high humidity or under low one, being more or less disturbed under extraordinary low temperature.

(3) The inhibitory action to the occurrence of the rhythmicity is stronger in the fluorescent light than in the incandescent light.

III. EXPERIMENTAL ANALYSIS ON THE ENDOGENOUS RHYTHMICITY IN HATCHING

(1) The inhibitory action of light to the occurrence of the rhythmicity is proportional to duration of illumination. But they can repossess the rhythmicity before long when put in darkness. Therefore the rhythmicity is not completely ruined but is only suppressed, by light. When the eggs were subjected to artificial alternation of light (12 hr) and dark (12 hr) for considerable long time, the rhythmic sign appears in the hatching phase even if the eggs are removed to illumination ; the longer the conditioning is, the clearer the rhythmicity displays. This phenomenon is assumed to be arisen by an after-effect of the conditioned environment. The photoperiodical treatments hardly give influences upon the rhythmic appearance of hatching, which persists about 18-hr cycle.

(2) Each modal peak of hatchability occurs almost at the definite time even

though the eggs kept under warmth (25°C) after experienced chilliness (about 5°C) for 6~24 hours, and the effect of the chilliness is hardly recognizable. The fact suggests that the rhythmicity of hatching is largely controlled by the physiological activity in the eggs, which has been maintained with considerable stability even under the very low temperature near to the critical low temperature of growth of the eggs.

(3) In the same population the manifestation of modal peaks of hatchability takes always a similar formality with about 18-hr cycle even in the eggs differing in oviposited time.

(4) Number of modal peaks of hatchability is related to variance of the embryonic growth; the more the growth varies, the more the number increases. This must be caused by the rhythmic character, which divides the hatchability into 18-hr interval.

(5) Under darkness the full-grown embryos are provided with the latent activity of hatching, by which the eggs can hatch when put in some stimuli. The rhythmic sign of hatching appears under such a background as this latent period.

(6) The activity of succinic dehydrogenase was measured to know the physiological activity in the eggs. Consequently the rhythmic change with about 18-hr cycle was detected in it. The rhythmicity in the activity is plausible to be connected with the occurrence of the rhythmicity in hatching. Therefore the eggs at the latent period of hatchability can probably hatch without light stimulus by the endogenous stimulus such as the change of the enzyme activity in themselves.

IV. EXPERIMENTAL ANALYSIS OF THE EFFECTS OF ENVIRONMENTAL FACTORS UPON THE EGGS JUST BEFORE HATCHING.

(1) When alternation of light and dark is given to various eggs at just before hatching, black eggs of normal strain hatch only in light without racial differences. On the other hand, *Daizo*, w_1 , b_2 and *Cambodge* hatch not only in light but also in dark. Then, either difference of light intensity, positive or negative, seems to be a stimulation to cause hatching. These strains can be said to have comparatively weaker sensitivity to the positive difference of light intensity.

(2) When various eggs are subjected to alternation of warm and cool, black eggs of normal strains hatch almost only in warm, while *Daizo*, b_1 (3-moulter), *re* and *Cambodge* hatch not only in warm, but also in cool. This hatching seen in cool is exposed under illumination, but not under darkness. This implies that either thermal difference, positive or negative, is a stimulation to cause hatching. These strains can be said to have rather weaker sensitivity to the positive difference of temperature.

(3) If alternation of light & warm and dark & cool is given to various eggs, the rhythmic phase of hatching comes out even in the eggs of *Daizo*, which has

stability in arrhythmicity. This will depend upon the additional effect of environmental factors.

(4) If alternation of light & cool and warm & dark is given to various eggs, black eggs of normal strains hatch only in light & cool, indicating that the light is the essential factors to cause hatching. On the contrary, the eggs of *Daizo* and its hybrid hatch rather more in warm & dark than in light & cool. From the fact it has become clear that these eggs have different characteristics from normal eggs in the photo- and thermo-sensitivities. The eggs of w_1 , w_2ch , re , b_2 and *Cambodge* hatch samely in either condition.

(5) As abovementioned, the photo- and thermo-sensitivities are different according to the strains in silkworm eggs. Black eggs of normal strains which have distinct rhythmicity of hatching are sensitive to both the positive differences of light intensity and of temperature, while the eggs of *Daizo* and egg colour mutants which hardly have rhythmicity of hatching are not so sensitive to both the stimuli.

As the rhythmicity of hatching has been considered to be a character acquired by adaptability to diel change of environmental conditions, the differences in the rhythmicity of hatching in silkworm eggs may be caused mainly by the differences in triptophan pigments of the eggs closely relating with the adaptability to environmental factor. However, that the eggs of multivoltine and 3-moultine strains have faint rhythmicity of hatching is probably due to another reason that their growth pace is abnormally faster than the other strains.

(6) The rhythmicity of hatching in silkworm eggs is not only dependent upon the endogenous character, but also upon the exogenous one. An 18-hr rhythmicity is presumed to be a hereditary character acquired in the eggs of *Bombyx mori* and *B. mandarina*.

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要 約

蚕卵を自然状態に保護すると、毎日早朝に孵化がおこなわれる。蚕品種によつても異なるが、この状態は通常2日ないし3日、日週期的にくりかえされる。このような孵化の日週周期性は経験的にも知られていることであるが、これを生起させる要因としては当然光の刺激が考えられる。しかし蚕卵を恒温、恒湿かつ恒暗状態で催青した場合も、ある時期にいたると、卵は日週期的に孵化をくりかえす(田中1957)。竹内(1956, 1957)は蚕卵の発育後期における明暗が、孵化の早晚斉否にどのような影響をおよぼすかについて研究し、蚕卵の孵化には一般に定時間隔をもつた孵化階調があることを観察した。同様な現象は家蚕幼虫の脱皮現象(田中深1956)にも、成虫の羽化現象(坪井1910, 木村1952)にも知られている。これらはいずれも同じ個体でくりかえされる週周期性ではないから、いわば群週周期性といつてよいものであろう。

さて孵化に関する研究は基礎と応用とをとわずすこぶる多いが、これを日週周期性という見地から検討したものはほとんどなかつた。

そこで著者は日週周期性生起の様相を究明するために、その強弱を品種的に調べさらに種々な環境条件における週周期性の発現状態を研究した結果、蚕卵の孵化は一般に、環境に依存しておこなわれる反面、恒暗下で遺伝的な約18時間々隔の自律的週周期性が発現されることを知つた。そしてこの自律的週周期性の発現機構を実験的に解析し、その過程を明らかにした。本論文の要旨をまとめると次のようである。

1 蚕品種と孵化の自律的週周期性

(1) 黒卵系の普通品種においては恒温湿、恒暗の条件下で孵化の週周期性が認められる。その発現の程度は品種によつて異なる。(Fig. 1, 2, 3)。

(2) 恒明環境では孵化の週周期性は抑制される。そして照度が強い程その傾向が強い。(Fig. 同上)。

(3) 自律的孵化週周期性は3眠蚕(旭光)、欧州種(欧19)、多化系(大造、マイソール、カンボージュ)および卵色突然変異系(w_1 , r_e , b_1)等では不明瞭かあるいはほとんど現われない。(Fig. 4, 5, 6)。

(4) 自律的孵化週期のピークの間隔は品種によつても多少異なるが、およそ18時間である。

(5) 桑蚕の自律的孵化週期は恒明下(30lux)でも発現され、週期ピークの間隔は恒暗下で明確な18時間を示す(Fig. 7)。

2 種々な恒定環境要因と孵化の自律週周期性

(1) 恒暗状態では温度の高、低いずれのばあいにも孵化週周期性が発現する。このばあい18°Cではきわめて明瞭な18時間週期であり、30°Cでは間隔はそれより短縮される傾向がある。(Fig. 8, 10)。

(2) 恒暗状態では湿度の高(100%)低(30%)いずれのばあいにも孵化週周期性がみられる。(Fig. 11)。

(3) 光線は前述のように孵化週周期性を抑制するが、紫外線は電灯光線よりもその作用が強い。そして孵化も遅延する(Fig. 12)。

以上のように自律的孵化週周期性は暗状態であれば、種々な恒定環境下で普遍的に発現する。

3 孵化の自律的週周期性の発現機構に関する実験的解析

(1) 孵化週周期性におよぼす明暗作用

光線の照射は孵化週期を抑制するが、照射時間が長い程孵化週期は不明瞭となる (Fig. 13)。しかし一度光線下におかれても暗状態に入れることによつて自律的孵化週期性はすみやかに取戻される (Fig. 13)。したがつて光線は週期性を完全に消失させるものではなく、一時的に抑制するものと思われる。

催青期間中に人為的環境週期 (明暗12時間交互) を与えると、これによく順応して、孵化のさいに After-effect がみられる (Fig. 14a)。これは自然環境においても昼夜の日週期によく順応性をもつことを意味する。

また催青期間中に長日, 短日, 中日処理等を施しても、孵化週期には影響なく、恒暗下ではやはり18時間週期を示す (Fig. 14b)。

暗催青から孵化直前に明処理をおこなうと、ただちに大半が孵化する (Fig. 13 D1)。これは光線刺激が孵化に対して第1義的な要因であることを示すものであるが、そのさい蚕卵は孵化能力を潜在 (後述) していたものと解される。

反対に明催青から孵化直前に暗処理をおこなうと、孵化は遅退して (Fig. 13 Dd), その後大きな孵化ピークが現われる。この遅退時間は光線によつて抑制されていた週期性が暗状態で解放されるまでの時間に相当している。

(2) 胚子発育の各時期における低温接触と自律的孵化週期性

催青開始前に5°C以下に蚕卵を冷蔵しておき、これを数時間毎に取り出して催青 (25°C) を開始していくと、恒明催青では催青開始時刻の早晩に比例して孵化が始まり (Fig. 16), ほぼ正規分布になるが、恒暗催青では前述のように孵化は週期的に現われて、しかもそのピークの現われる時刻は、催青開始時刻に関係なくほぼ一定である。そして催青をおくらせて開始したもので、孵化は1週期 (18時間) 後方のピークに次第に移行していく (Fig. 15, 17, 20)。

また催青中期および後期に蚕卵を低温 (3°~5°C) に7~9時間接触させても孵化週期の現われる状態は、無処理のものとはほとんど同一である (Fig. 18, 19)。それ以上の接触時間を与えると1週期後方のピークに移行する (Fig. 19)。

したがつて低温中 (5°C) においても週期的な生理活動は停止することなく持続されていることが示唆される。すなわち胚子の生長は低温によつて抑制されても、卵内の生理的週期性は依然として持続されるものと推定される。

(3) 産卵時刻の差と自律的孵化週期

産卵時刻の異なる卵 (ただし同一集団) でも恒暗下では孵化ピークは一定の時刻に集まり、同一週期型となる (Fig. 21)。しかし産卵時刻の差がいちじるしければ発育遅速の巾を広くするので、18時間の週期間隔によつていくつかに分割されて、孵化ピークの出現数を増す (Fig. 22)。

(4) 孵化週期のピークと性比

孵化ピークを構成する群の性比には差を認めなかつたので (Table 4), 週期的ピークの形成は卵の性的差異に基づくものではない。

(5) 孵化能力の潜在性と自律的孵化週期

暗催青において孵化直前の卵は刺激によつてただちに孵化しうる能力を持っている。すなわち胚子が完成されてもしばらくはそのままの状態に留まり、孵化の潜在性を保持する期間が存在する (Fig. 23)。

もしこの期間に環境要因の刺激が作用しないならば、卵内生理の変化が誘因となつて、孵化を生起せしめるものと考えられる。したがつてこの期間の存在は孵化の週期的発現を助長させ

るものと思われる。

(6) 孵化の自律日週期性と卵内生理活動

呼吸酵素系の Krebs cycleの一環であるコハク酸脱水素酵素の Activity を催青卵について時間を追って測定し、そこにほぼ20時間を間隔とする週期性の存在することを確めた(Fig. 24, 25)。この酵素活動は卵内生理活動の1指標と考えられ、しかもこれが孵化の週期性によく関連している。すなわち孵化の自律週期性発現は卵内生理の週期に原因があるものとする。

4 孵化に対する環境要因の作用解析(孵化週期の環境依存性)

(1) 孵化における光感性

孵化直前から明と暗の処理を交互におこなうと、普通黒卵系の品種ではほとんど明状態のときだけに孵化がなされる(Fig. 26)。一方大造や w_1 , b_2 , カンボージュ等の品種では暗状態のときにも或る程度孵化がなされた(Fig. 27)。すなわち蚕卵は照度上昇のみならず、照度下降にも感応する。したがって照度隔差が孵化刺激となるものと思われる。前者は照度隔差に感受性強く、後者はいずれもさほど強くはない。

(2) 孵化における温感性

同様に孵化のさいに高温と低温の交互処理をおこなうと、普通黒卵系の品種ではほとんど高温接触時に孵化がなされる(Fig. 28, 29)。しかし大造をはじめ b_1 3眼, r_e , カンボージュ等は低温時でもある程度孵化がなされた(Fig. 29, 30)。ここでも温度隔差が孵化刺激となつていくことが証明された。すなわち前者は温度隔差に感受性強く、後者はさほど強くはない。

(3) 孵化における光と温度の組合せ効果

明で高温、暗で低温の交互処理を孵化時におこなうと、比較的環境作用に影響をうけなかつた大造も明・高温時に多く孵化した(Fig. 31)。これは環境の相加的効果と思われる。

次に明で低温、暗で高温の交互処理(光と温度の相反作用)を同様におこなうと、普通黒卵系ではほとんど前者(明冷時)に孵化がなされた(Fig. 32, 33)。これは光が絶対的な孵化の支配因子であることの証左である。これに反して大造およびその交雑種は後者(暗温時)にやや多く孵化がなされた(Fig. 33)。また w_1 , w_2 , ch , r_e , b_2 , カンボージュ等の系統は両者に約半数宛孵化がなされた(Fig. 33)。

以上のことから自律週期性を有する普通黒卵系の品種と、それを有しないかまたは弱い多化系および卵色変異系とでは、孵化活動の際の光感性、温感性およびこれの組合せ効果(相反作用反応)等において明らかな差異を有し、前者は環境依存性が強く、後者の大部分は完全には依存しないことがわかった。

5 孵化の週期性発現に関する論議

孵化の自律的週期性発現の機構は極めて複雑であり、様々な要因が関与していることは前述してきた通りである。

まず、普通黒卵系品種に孵化週期がみられ、白卵系にはみられないことから、卵色素がこれと密接な関係にあるものと思われる。トリプトファン系色素が光線に全く平行的に感応する事実が複眼等に多くみられるので*、蚕卵の漿液膜色素(トリプトファン系)も鋭敏に光に感応するであろう。すなわちこの光感受性を通して自然環境ではその日週期によく同調し、生理リズムの調和が保たれる結果となる。週期活動には一部自律性、一部他律性という例(GUN 1940, MELLANBY 1940, 杉山・松本 1955)が多くみられ、また自律週期性は自然環境の日変(屋

* 3), 4), 5), 6), 7), 37), 52), 80), 84), 85)

夜)に適應した形で長い間に獲得された形質であると考えられるので(森 1948), 家蚕の孵化のばあいも環境要因に対する感受性の強弱が自律週期性の存否につながるように思われる。前述の白卵系, 赤卵系等の卵色変異系において孵化週期が不明瞭なのは, 色素欠乏による光感受性の鈍さに由来し, 大造品種においては環境要因に対する感受性の鈍さが自律性を示さない理由と考えられる。

孵化週期性を不明瞭にする Factor として, 多化性因子と3眠性因子がある。これらの系統ではホルモン分泌のバランスが發育を促進する方向に強くはたらくために(諸星 1949), 卵内生理状態も正常品種と異なることが考えられ, このようなことが群週期的な歩調を乱すものと思われる。ゴキブリの週期的活動には食道下神経球から分泌されるホルモンが支配していることが知られていることから(HARKER 1958), 發育に関するホルモンが孵化の週期性に当然関連を有するものと思われる。なお多化性系統は卵色の面からも白卵系(w_8)に属するので(吉武 1956), 週期性発現はいつそう困難になるものと思われる。

次に孵化の週期間隔についてみると, 自律的発現においては18~24時間で, 18時間が典型的であつた。この18時間週期の原因については不明であるが, 桑蚕においても同様であることは興味深い。今後さらに研究が進められねばならない。今恒定条件(暗中)では自律的な18時間週期を示し, 自然環境ではその24時間週期に同調する孵化の週期性を, Fig. 34に示し, 相互の関係を模式的に現わした。

次に自律週期性の根源が生理活動にあることは前述したが(森 1948等), 蚕においてもこの生理性が全期にわたつて一貫しているものと思われる。すなわち卵内の生理リズムが孵化週期性として現われ, その後幼虫期に持続し, 各令の脱皮時に再びみられる。田中深(1951, 1956)が幼虫の脱皮機能の研究で2山型について述べていることは, これに該当するものであろう。

このリズムは更に蛹期に連続している。木村(1952)は蚕蛾の羽化においてやはり18時間の週期性を認めている。したがつてこれらの事柄は卵—幼虫—蛹—蛾のいずれの時期にも共通な遺伝形質に由来するものと解される。孵化, 脱皮, 羽化等のさいにみられる週期現象は, 体内生理週期の顕著な指標点であろう。