CHANGE OF PHOTOREFLECTION FOLLOWING WITH DEVELOPMENT OF THE SILKWORM EGG, *BOMBYX MORI* L.

1. REFLECTION PROPERTIES FROM THE EGG UNDER DIFFERENT PHOTIC CONDITIONS

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A superficial coloration of the silkworm egg is generally whitish or yellowish just after oviposition, but later it changes, except in non-diapause egg, to the specific color of race, which is originated from the serosal pigments of the egg. The hereditary mechanisms on manifestation of such pigment color were investigated biochemically by many workers (TOYAMA 1913, UDA 1932, UMEYA 1938, FUKUDA 1940・'41, HARIZUKA 1943, KIKKAWA 1943, SUZUKI 1943, etc.).

As is well known, the silkworm eggs of normal races to be hibernated take dark purple, under natural condition, in summer and change gradually to lighter color from autumn to winter. The hibernated eggs, when subjected to warm temperature in spring, become again darker color, which is caused by a migration of the pigments in the serosal cell (WATANABE 1919, TAKAHASHI 1920, etc.).

Thus many investigations were already carried out on the superficial coloration of the silkworm egg, but it was observed only by naked eyes without using a definite index. Then, tried to measure exactly the egg coloration and structural character, the authors studied it photophysically using photoreflection indicators from egg mass or from single egg, which change following with development of the egg.

MATERIALS AND METHODS

Expt. I

The hibernating eggs of a hybrid between *Nichi-131* and *Shi-131* were used for the materials. These eggs were kept under a constant condition of temperature 25°C with relative humidity 80% since just oviposited. The experimental regimes are as follows.

1. **24L** The eggs were constantly exposed to a light intensity of 100 lux throughout the experiment.
2. **18L6D** The eggs were subjected to 18-hours light (100 lux) and 6-hours dark every day.
3. **24D** The eggs were kept under a constant darkness throughout the

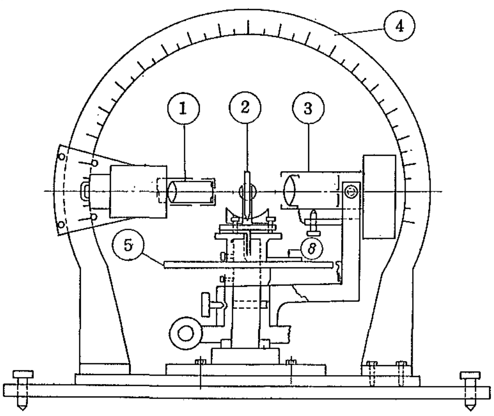

Fig. 1 Lateral view of 3-Dimentional Goniophotometer (GP-meter)
1) Light source, 2) Sample holder, 3) Light receptor 4) Scaled arch, 5) Angular disk

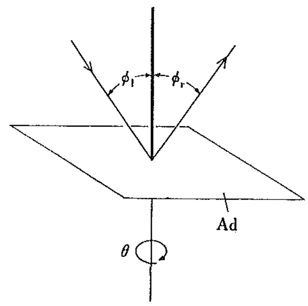

Fig. 2 Relation of incidence angle (ϕ_i) and reflection angle (ϕ_r) with rotation of angular disk (Ad)

observation.

a) Measurement by Three-Dimentional Goniophotometer (GP-meter)

Fig. 1 shows a lateral view of GP-meter.

Each group of the eggs pasted on a black paper was taken out and measured the photoreflection intensity from the egg mass. In this case JEFFRIE'S method (SAWAJI 1965) was applied, of which the procedure is shortly shown in the following. The eggs supported with the sample holder ② are exposed to the light ① (12 V, 30 W) rotating the angular disk ⑤, next the reflected light is accepted into the light receptor ③ and its relative intensity is recorded on the autorecorder through an amplifier. Here the rotation speed and angle (θ) of the angular disk are 120°/min and 360° respectively, and the incidence angle (ϕ_i) is constantly 45°, the reflection angle (ϕ_r) is taken 45°, and 90° (Fig. 2).

b) Measurement by Automatic Micro-luster Meter (ML-meter)

A lateral aspect of ML-meter is denoted in Fig. 3.

Each one of the eggs attatched on a black paper was used for the measurement. First the egg set on the sample holder ② makes it focused into an enlarged image on the pint glass of the camera box ③, next it is illuminated by the lamp light ①, and the reflected light from the egg is catched on the pinted image by the light receptor ④, its relative intensity being recorded on the self-recorder through an amplifier. Here, the incidence angle (ϕ_i) is 45°, magnification of the object 20 times and the light receptor moves horizontally 2 cm to receive the image.

In the above two measuremental methods the reflection index (gloss) was calculated from a ratio between the maximum value and the minimum value in the reflection intensity curve, which is called I-θ curve. Additionally

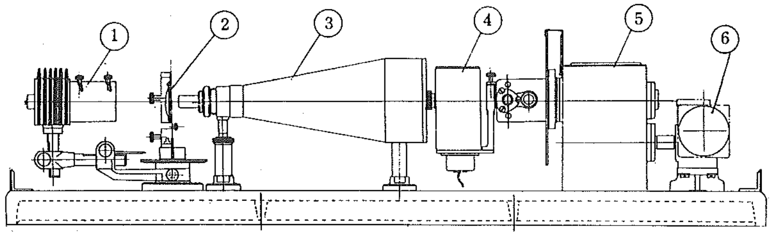

Fig. 3 Lateral view of Automatic Micro-luster Meter (ML-meter)
1) Light source, 2) Sample holder, 3) Camera box
4) Light receptor, 5) Gear box, 6) Motor

the eggs of each group were stained with CARNOY'S solution and observed their pigmentation.

Expt. II

The overwintered eggs of a hybrid between *Nichi-122•124* and *Shi-115•124* were used for the materials. These eggs were subjected to the same condition as in Expt. I, and the reflection indicator from the egg mass or from the single egg was measured respectively by GP-meter and ML-meter until hatching.

EXPT. I CHANGES OF THE PHOTOREFLECTION FOLLOWING WITH THE DEVELOPMENT OF THE HIBERNATING EGGS AFTER OVIPOSITION

A. REFLECTION INTENSITY

1. Measurement by GP-meter

a) 24L (Fig. 4)

Observing I-θ curve, the reflection intensity is as large as 3.5~4.0 in the 1st and the 2nd days after oviposition, but decreases greatly in the 3rd day (2.0~2.5). Thenceforth the intensity falls gradually and takes almost a constant value of 1.5~2.0.

The pigmentation of the serosal cell in each day is as follows.

1st day: pale yellow, not yet pigmented.
2nd day: slightly reddish yellow, pigment formation starts.
3rd day: purplish red, pigment formation is clearly observable.
4th day: brownish purple, all serosal cells entirely pigmented.
5th day: from this time pigmentation unchangeable.

In the light of the above observation, it is clear that changes of the reflection intensity are closely coincided with that of the pigmentation in the egg.

After the 3rd day three cyclic peaks which appear every 90° rotation of the sample are seen in each I-θ curve. Such a pattern is believed to indicate the characteristic feature of the egg shape, because the reflection intensities from any direction of the egg should be all equalized and so the curve be somewhat linear, if the egg surface would take a sphere shape. The first intensity peak

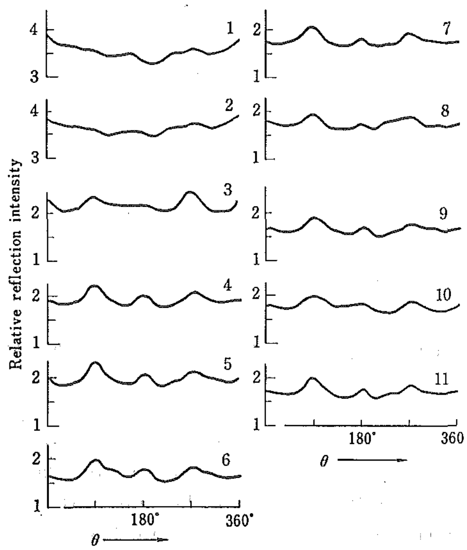

Fig. 4 I-θ curve under 24L in hibernating eggs ($\phi_r=90°$)

(90°) and the third one (270°) will be caused samely by the reflection from the longitudinal direction of the egg, while the second one (180°) brought by that from the transverse direction. Supposedly this means that a flattened part or a slightly cave-in part exists in the center of the egg which passed more than 3 days after oviposition.

b) **18L6D** (Fig. 5)

The change of the reflection intensity takes almost the same tendency as in 24L, though the rhythmical peak of the intensity is more faint than in 24L. It is, however, recognizable that the structural change occurs on the egg surface from the 3rd day after oviposition.

c) **24D** (Fig. 6)

The reflection intensity is as large as 3.5~4.0 till the 2nd day after oviposition, and later falls to 2.0~2.5 in the 3rd day. Subsequently it keeps a constant value of 1.5~2.0. The intensity decrement in the 3rd day is almost the same as that in 18L6D. The

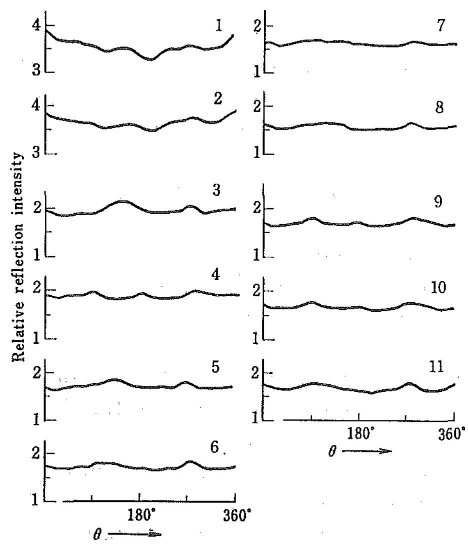

Fig. 5 I-θ curve under 18L6D in hibernating eggs ($\phi_r=90°$)

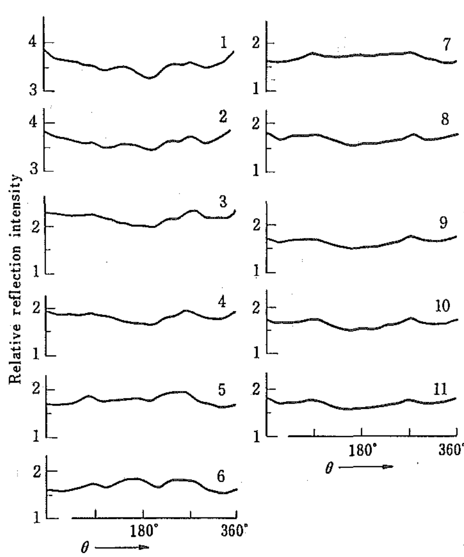

Fig. 6 I-θ curve under 24D in hibernating eggs ($\phi_r=90°$)

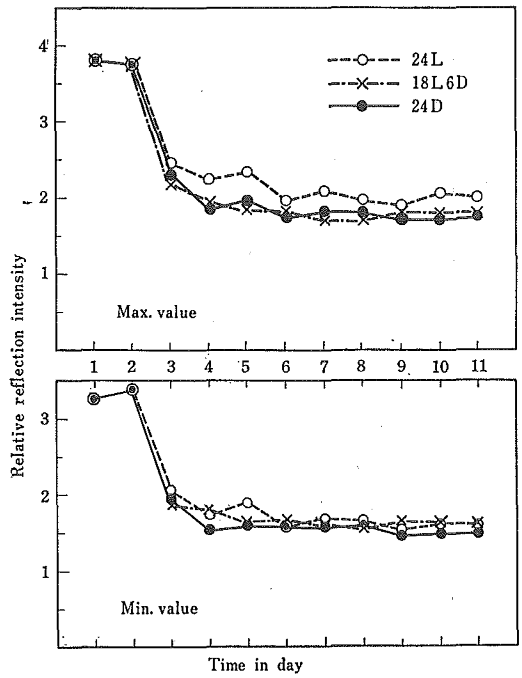

Fig. 7 Changes of the maximum and the minimum values in each regime ($\phi_r=90°$)

three cyclic peaks observed in I-θ curves of the above two regimes are too faint to be detectable except in the 6th day.

Fig. 7 shows changes of the maximum value and the minimum value in each day. The maximum value of the reflection intensity is a little higher in 24L than in the other regimes, though the fluctuation of the value resembles closely to each other. Distinct differences are difficult to see between the changing phases of the minimum value among each regime. A sudden fall of the intensity value in the 3rd day indicates well that in this time the serosal cells are almost entirely pigmented.

2. Measurement by ML-meter

a) 24L (Fig. 8)

I-θ curve of the reflection intensity from the single egg is clearly different in shape from that by GP-meter. From the 3rd day a depressed part appears at the central part of the curve. It becomes more remarkable with the development of the egg. The depressed part is fairly asserted to be resulted by the structural change of the egg, in which the flattened or the slightly cave-in part, as mentioned above, is produced in the central part of the egg, because the periphery of this part forms a pale shadow by the oblique illumination ($\phi_i=45°$). Accordingly we can easily know the surface structure of the egg by I-θ curve.

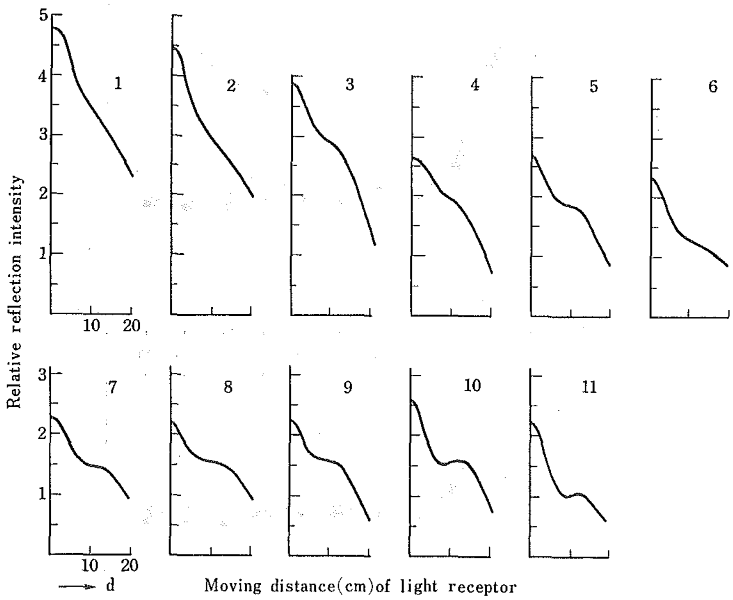

Fig. 8 I-θ curve under 24L in hibernating egg ($\phi_r=45°$)

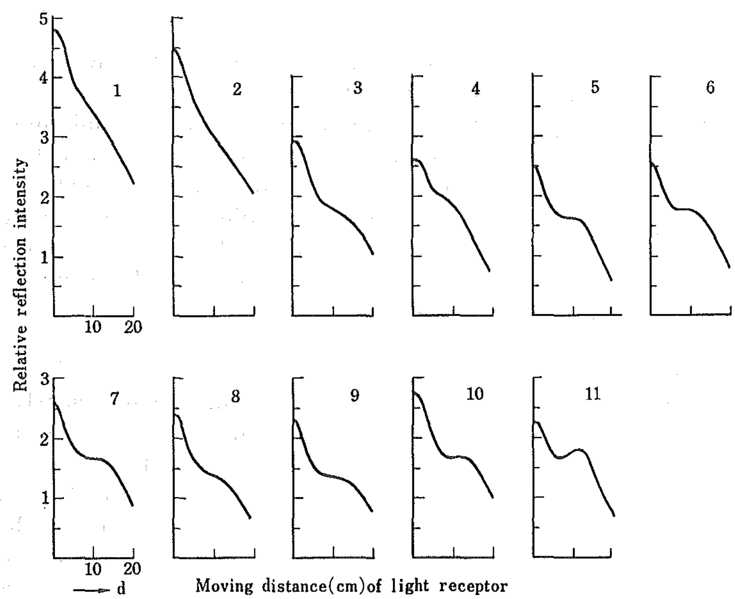

Fig. 9 I-θ curve under 18L6D in hibernating egg ($\phi_r=45°$)

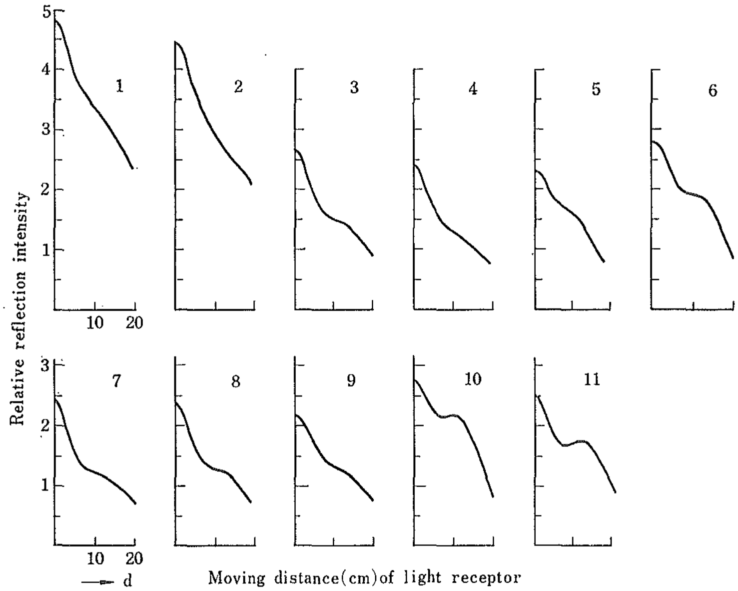

Fig. 10 I-θ curve under 24D in hibernating egg ($\phi_r=45°$)

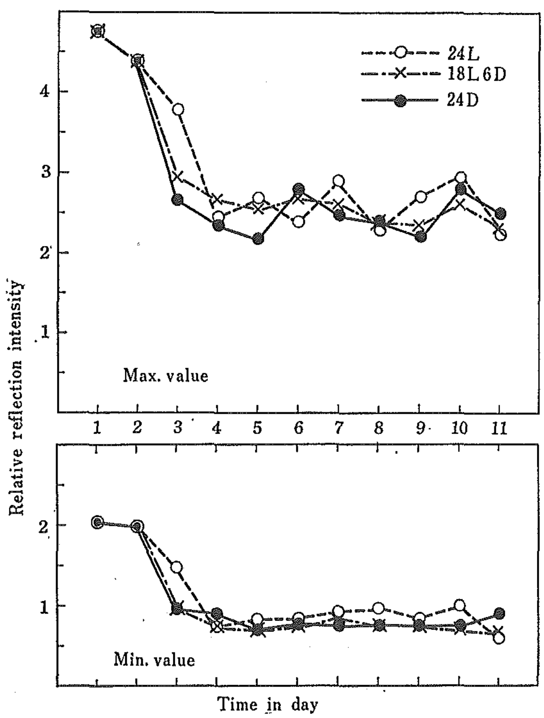

Fig. 11 Changes of the maximum and the minimum values in each regime ($\phi_r=45°$)

b) **18L6D** (Fig. 9)

The intensity value is largest in the 1st day after oviposition, and afterwards decreases gradually. After the 3rd day, when a heavy decrement happens in the intensity, the value is almost unchangeable. The depressed part is seen in I-θ curve from the 3rd day as in the case of 24L.

c) **24D** (Fig. 10)

The reflection intensity is very large until the 2nd day after oviposition, and afterwards it decreases greatly persisting almost a constant value. The depressed part in I-θ curve appears in the 3rd day and later.

As shown in Fig. 11, each maximum value of the intensity is considerably larger than that by GP-meter, while each minimum value smaller than that by GP-meter (Fig. 7). The former value more fluctuates than the latter. The firstly appeared most decreased value among the maximum intensities appears in the 4th day in 24L, but in 18L6D and 24D one day later, though significant difference hardly detectable among the changing phases of the values.

B. REFLECTION INDEX

1. Measurement by GP-meter

a) **24L** (Fig. 12)

The change of the reflection index (gloss) calculated from I-θ curve is shown in Fig. 12. The index value is lowest (1.09) just after oviposition, later raised suddenly in the 3rd day and reaches the maximum value (1.17) in the 4th day. Successively the index decreases gradually, persisting a constant value (about 1.10) after the 7th day. The tendency appears almost samely both in the cases of $\phi_r=90°$ and $\phi_r=45°$.

b) **18L6D** (Fig. 12)

The fluctuation curve is considerably similar to that of 24L in each case of $\phi_r=90°$ and $\phi_r=45°$. However, the maximum value (about 1.13) appears 2 days later (6th day) and smaller than in 24L. In $\phi_r=90°$, the difference in the above value is very significant between 18L6D and 24L.

c) **24D** (Fig. 12)

The change of the reflection index is not so evident, in which a flat peak

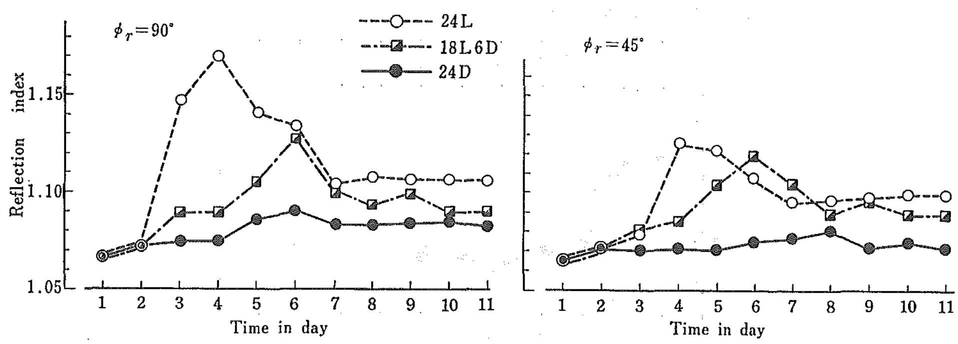

Fig. 12 Change of reflection index in each regime measured by GP-meter

can be seen in the 6th day in the case of $\phi_r=90°$. The index values are wholly smaller than in 24L and 18L6D.

As mentioned above (Expt. I-A, 24L), the pigment formation in the serosal cell is in progress in the 3rd day and almost completed in the 4th day, when the index reaches the largest value. The maximum value, therefore, seems to indicate coincidently the embryonic stage, in which all the serosal cells are pigmented entirely.

2. **Measurement by ML-meter**

a) **24L** (Fig. 13)

The changing phase of the reflection index obtained by ML-meter differs so much from that measured by GP-meter. The most characteristic feature is detectable in the 10th day, when the greatest decrement of the index occurs in the case of ML-meter, but not in the case of GP-meter (Fig. 12). The tendency seems to be caused by the fact that in that time a sudden decrease takes place in water-content of the egg (UMEYA 1946).

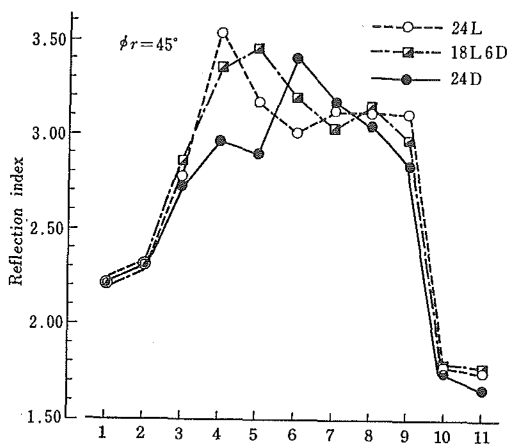

Fig. 13 Change of reflection index in each regime measured by ML-meter

b) **18L6D** (Fig. 13)

The change of the reflection index is somewhat similar to that in 24L, but the maximum value appears slightly smaller and one day later than in 24L.

c) **24D** (Fig. 13)

The change of the reflection index resembles, to some extent, that in 18L6D, but the maximum value is seen one day later and 2 days later than in 18L6D and in 24L, respectively. Each index value is a little less as compared with that in each of the other regimes.

C. CONSIDERATION

In the light of the above results, it is recognized that the changing phase of the photoreflection in each regime is coincided very well with that of the pigmentation of the hibernating egg after oviposition. The pigmentation of the overwintering egg is known to be influenced by thermal condition (WATANABE 1919, MINOYA 1926, KUTSUKAKE 1952, etc.) and photic condition (NAGASHIMA 1956). In the present paper the latter condition was taken into study. According to NAGASHIMA (1956), when the egg has been constantly illuminated since oviposition, the formation of the serosal pigments proceeds rather faster, besides they are produced more densely than in dark condition. These tendencies were indicated correspondingly with the reflection index.

The index value, however, appears to be larger in that measured by ML-meter than by GP-meter. It is note worthy that a great fall of the index value occurs in the 10th day in the case of ML-meter (Fig. 13), but any sign of such a phase is indetectable in the same day in the case of GP-meter (Fig. 12). Observing the change of water content in the egg to go hibernation, it increases markedly from the 2nd day to the 5th day after oviposition and reaches suddenly the minimum value in the 7th day or in the 10th day, thenceforth the value being kept almost constantly till winter.

This phenomenon suitably expressed by the reflection index by ML-meter. Accordingly the reflection index, especially by ML-meter, is regarded not only capable to indicate the pigmented grade but also some other changes occurring in the egg contents. GP-meter which was applied to egg mass is characterized by the ability that it can measure the reflection intensity from every direction of the object, while ML-meter by the function that catchable more detailed changes of the egg surface and contents from its definite direction.

EXPT. II CHANGES OF THE PHOTOREFLECTION FOLLOWING WITH THE DEVELOPMENT OF THE OVERWINTERED EGGS

A. REFLECTION INTENSITY

1. Measurement by GP-meter

a) 24L (Fig. 14)

The reflection intensity is 2.0~2.5 and the value decreases to 1.5~2.0 from the 2nd day to the 6th day, when the egg coloration becomes darker by migration of pigments in the serosal cells. A period from the 5th day to the

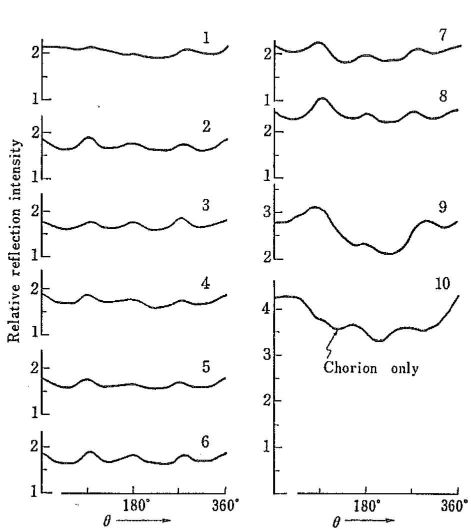

Fig. 14 I-θ curve under 24L in overwintered eggs ($\phi_r=90°$)

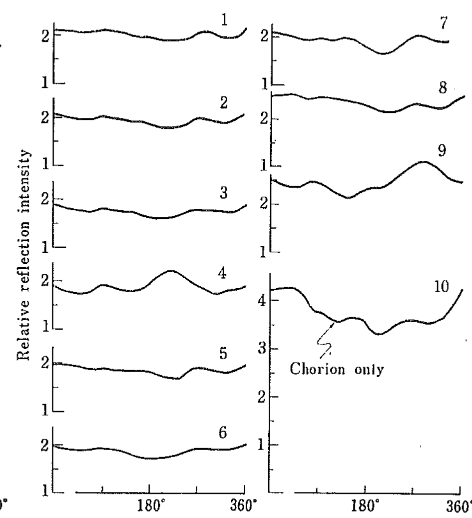

Fig. 15 I-θ curve under 18L6D in overwintered eggs ($\phi_r=90°$)

6th day corresponds to the blastokinesis of the embryonic stage, in which the intensity value is smallest. After the time, however, the intensity begins to increase and at last it reaches the highest value of 2.0~3.0 just before hatching (9th day). The reflection intensity from the chorion only (10th day) is as high as 3.5~4.5.

In *I*-*θ* curve the three rhythmic peaks with 90° intervals can be recognized as in Expt. I. Such a pattern shows the cave-in part produced at the center of the egg. Especially the intensity is lowest in $\theta \fallingdotseq 180°$. This tendency suggests that a longitudinally long shadow exists on the surface of the egg.

b) 18L6D (Fig. 15)

The change of the reflection intensity is almost the same as that in 24L. The serosal pigments migrate more slowly than those in 24L, being distributed uniformly in all serosal cells in the 3rd to the 4th days. The intensity value becomes low in the 5th to the 6th days when the blastokinesis occurs in the egg, and after the time it takes an upward tendency to reach the maximum value of 2.5~3.0 just before hatching. The three cyclic peaks with 90° intervals are not so evident compared with that in 24L.

c) 24D (Fig. 16)

The minimum value of the intensity is seen during the 3rd to 4th days. From the 6th day the intensity becomes larger increasingly, when the blastokinesis occurs in the egg. Afterwards the value gets the maximum value of

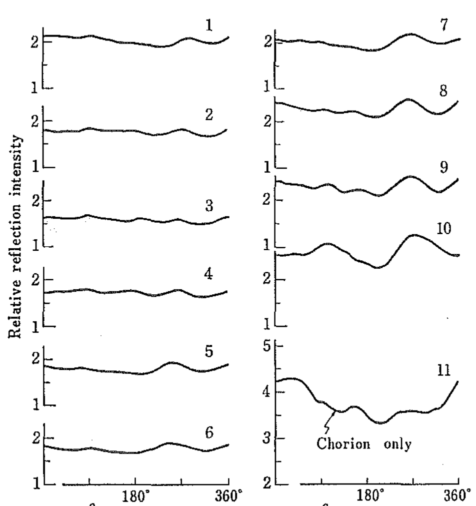

Fig. 16 *I*-*θ* curve under 24D in overwintered eggs ($\phi_r = 90°$)

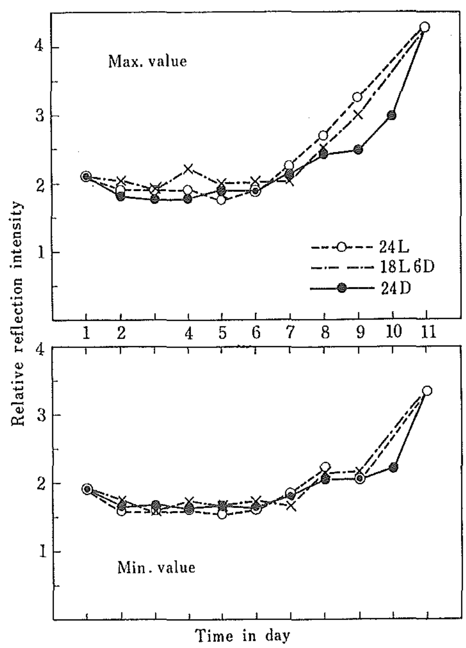

Fig. 17 Changes of the maximum and the minimum values in each regime ($\phi_r = 90°$)

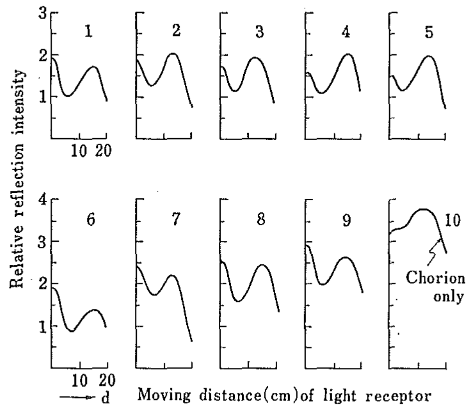

Fig. 18 I-θ curve under 24L in overwintered egg ($\phi_r=45°$)

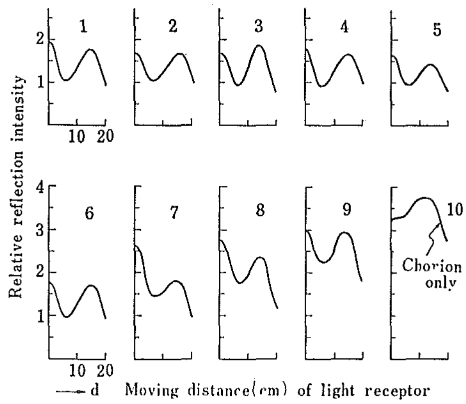

Fig. 19 I-θ curve under 18L6D in overwintered egg ($\phi_r=45°$)

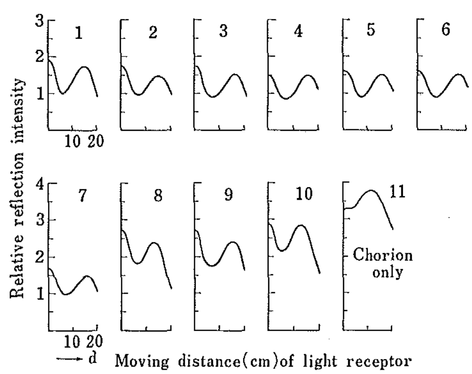

Fig. 20 I-θ curve under 24D in overwintered egg ($\phi_r=45°$)

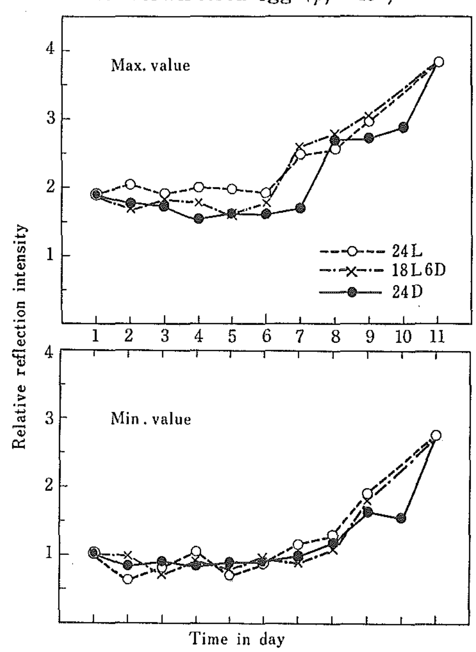

Fig. 21 Changes of the maximum and the minimum values in each regime ($\phi_r=90°$)

2.5~3.5 in the 10th day just before hatching. A manifestation of the rhythmic peak of I-θ curve which resembles that in 18L6D is more faint than in 24L.

The changing feature of the intensity following with the egg growth is indicated very well in Fig. 17. In 24D the intensity value appears less in the 8th to the 10th as compared with that in 24L and 18L6D, either in the maximum value or in the minimum value.

2. Measurement by ML-meter

a) 24L (Fig. 18)

The manifestation of I-θ curve is evidently different from that in Expt. I. The intensity decreases markedly in the 6th day and afterwards increases gradually till it becomes largest just before hatching (9th day). The existence of the cave-in part in the egg surface is very significant as compared with that recorded by GP-meter.

b) 18L6D (Fig. 19)

The changing phase of the intensity has a close resemblance to that in 24L. The intensity value decreases gradually until the 5th day, later tending to increase.

c) 24D (Fig. 20)

A slight decrease of the reflection intensity takes place in the 4th day and the 6th day. The shape of I-θ curve is similar to that in 24L and 18L6D.

The changing feature of the maximum and the minimum values in the reflection intensity is shown in Fig. 21. The value persists almost a constant grade until the 6th day, and later it increases remarkably. Each maximum value is rather smaller in 24 D than in 24L and 18L6D. The change of the minimum value in each regime resembles to each other except for a decrement of the value in the 10th day of 24D.

B. REFLECTION INDEX

1. Measurement by GP-meter

a) 24L (Fig. 22)

The reflection index (gloss) is 1.08 in the 1st day, raising in the 2nd day, and later decreases gradually reaching the lowest value of 1.09 (ϕ_r=90°) or 1.06(ϕ_r=45°) in the 5th day. The second peak (index about 1.12) appears in the 7th day and the highest peak (index 1.20) in the 9th day (just before hatching) after a great fall in the 8th day when the embryonic head is pigmented blackish. The reflection index of the egg shell is 1.11.

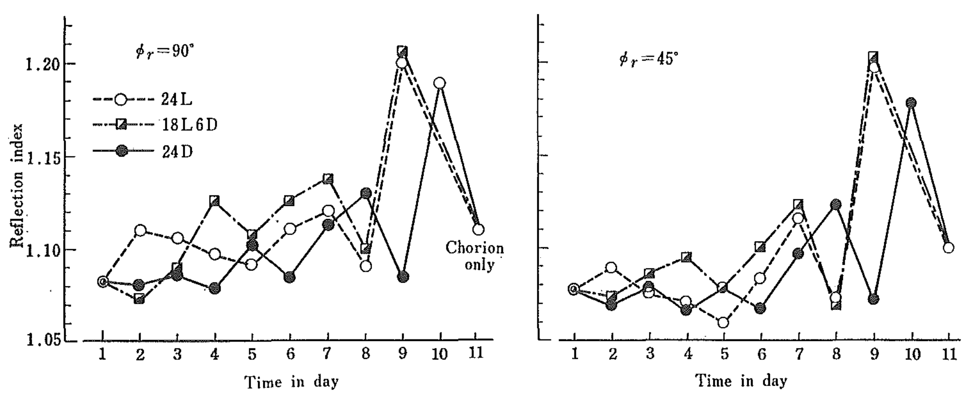

Fig. 22 Change of reflection index in each regime measured by GP-meter

b) **18L6D** (Fig. 22)

The peak of the reflection index appears in the 4th day, the 7th day and in the 9th day, respectively; the index values of the former two peaks are considerably larger than those in 24L. This phenomenon is seen samely in $\phi_r=90°$ and $\phi_r=45°$. The first peak occurs 2 days later (4th day) than that in 24L (2nd day).

c) **24D** (Fig. 22)

The changing feature of the index value is quite near to that in 18L6D, though the first peak and the second one come out one day later. The index value from the initial day to the 4th day is a little smaller than that in the other regimes.

2. **Measurement by ML-meter**

a) **24L** (Fig. 23)

As shown in Expt. I, the index value is greatly higher than that of egg mass. A fluctuation in the index curve is closely similar to that in the case of GP-meter; the first peak (index 2.00) appears in the 2nd day, the second one (index 1.75) in the 6th day and the third one (index 1.95) in the 9th day. Here it is noticeable that the dual diminished value in the 5th day and the 8th day indicate correspondingly the blastokinesis and the eye spot stage of the egg, respectively.

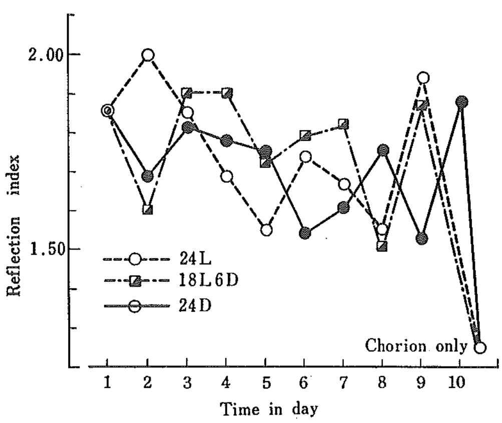

Fig. 23 Change of reflection index in each regime measured by ML-meter

b) **18L6D** (Fig. 23)

The index value is so much higher than that by GP-meter. The first peak (index 1.58) and the second one (index 1.56) appear 2~3 days later and one day later than that in 24L, respectively.

c) **24D** (Fig. 23)

The fluctuation phase of the reflection index resembles that in 18L6D, but the smallest value which appears in the 6th day and in the 9th day is delayed more 1~2 days than in the other regimes.

C. CONSIDERATION

As is well known, the overwintered egg, when subjected to warm temperature in spring, becomes increasingly darker in coloration with migratory expansion of pigments contained in the serosal cell (WATANABE 1919, MINOYA 1926, KOGURE 1930, KUTSUKAKE 1952, TSUTSUMI et al. 1959, etc.). Such a pigment migration terminates at the blastokinesis stage of embryo (MINOYA 1926, TSUTSUMI et al. 1959). The changing phase of the egg coloration mentioned above is expressed well coincidentally with the reflection intensity, viz. the intensity is high in the initial day, later decreasing gradually till the 5th day or the 6th day when the blastokinesis occurs. After the blastokinesis the reflection intensity tends to increase remarkably. The tendency is assumed to be dependent relatively upon the substantial change in the serosal pigments which takes place successively to the blastokinesis (MINOYA 1926). Further the fact that the pigment migration goes faster under illumination than under darkness (NAGASHIMA 1956) is not so adequately expressed by the reflection intensity, although this inclination is seen in the minimum value of the 2nd day (Fig. 17•21). In each regime there are two peaks and troughs in the reflection index from incubation to hatching. The first trough corresponds to the blastokinesis stage and the second to the eye-spot stage just before hatching. However, each trough of 24D appears about one day later than that of 24L and 18L6D, which exists almost in the same day, respectively. It tells us that the light accelerates the growth of the silkworm egg (NAGASHIMA 1956, TAKEUCHI 1956•'57, KOIZUMI 1958).

In any way, it can be stated that the photoreflection adequately indicates the coloration, the surface structure and some other patterns in the silkworm egg.

SUMMARY

In the present paper, an account is given of the changing phase of the photoreflection following with the development of the silkworm egg under different photic conditions. The results obtained are summarized as in the followings.

1. Hibernating egg just after oviposition

a) The reflection intensity decreases greatly the 3rd day, and later it persists almost a constant value.

b) In *I-θ* curve (reflection intensity) measured by 3-Dimentional Goniophotometer (GP-meter), three rhythmic peaks with 90° intervals are recognizable from the 3rd day after oviposition. Such patterns are considered to show that a flattened part or a slightly cave-in part is produced at the center of the egg in that time. Each peak is remarkable in 24L, but faint in 18L6D and 24D.

c) In *I-θ* curve by Automatic Micro-luster Meter (ML-meter), a depressed part appears from the 3rd day after oviposition. The part is quite significant in each regime and believed to indicate the existence of the cave-in part in the center of the egg. ML-meter works more usefully to catch the structural change of the egg than GP-meter.

d) The developmental differences of the eggs among the photic conditions are hardly distinguishable in *I-θ* curve.

e) The fluctuation phase of the intensity index in each regime differs evidently from each other; especially in 24L the index value is quite large in the 3rd to the 4th days, though in 24D being not so fluctuated and also small through development.

f) The situation of the maximum value in the reflection index is well

coincided with the time when all the serosal cells are pigmented entirely.

g) The changing feature of the reflection index by ML-meter resembles closely that of water-content in the hibernating egg.

h) The difference in the growth speed such as 24L>18L6D>24D is adequately expressed in the reflection index either by GP-meter or by ML-meter.

2. *Ovewintered egg from incubation to hatching*

a) The reflection intensity decreases gradually until the 6th day when the blastokinesis occurs, and afterwards tends to increase greatly. The tendency corresponds to the migratory phase of the serosal pigments.

b) The difference in the developmental speed among the three regimes is also difficult to detect from I-θ curve as in the case of the hibernating egg.

c) It is asserted by I-θ curve that the cave-in part exists in the overwintered egg.

d) The reflection index is considerably fluctuated. The first peak of the index curve suggests the start of the pigment migration in the serosal cells and its position shows that the egg growth proceeds with such an order as 24L>18L6D>24D.

e) There are two peaks and two troughs in the index curve; the first trough corresponds to the blastokinesis stage and the second to the eye-spot stage of the egg. Then we can know the embryonic stage superficially by the reflection indicator from the egg.

In any way, it can be stated that the photoreflection from the egg indicates adequately the coloration, the surface structure and some other patterns in the silkworm egg.

LITERATURES CITED

FUKUDA, S. (1940) Zool. Mag. Japan, 52 (11) : 415-429.
——— (1941) J. Genet. Japan, 17 : 97-108.
HARIZUKA, M. (1943) Bull. Sericul. Exp. Stat., 11 (2) : 197-210.
KIKKAWA, H. (1943) Ibid., 11 (3) : 311-345.
KOGURE, M. (1930) Res. Rep. Nagano Sericul. Exp. Stat., 11 : 1-152.
KOIZUMI, J. (1958) Sanshi-kenkyu, 25 : 36-49.
KUTSUKAKE, H. (1952) Kanebo-sanshikenkyu, 1-86.
MINOYA, K. (1926) Bull. Saitama Sericul. Exp. Stat., 13 : 1-45.
NAGASHIMA, E. (1956) J. Sericult. Sci., 25 (4) : 288-294.
SAWAJI, M. et al. (1965) J. Cotton Tech. Inst., 62 : 1-86.
SUZUKI, K. (1943) Bull. Sericul. Exp. Stat., 11 (2) : 125-196.
TAKAHASHI, S. (1920) Dainippon-sanshiho, 29 (340) : 9-12.
TAKEUCHI, K. (1956) Sanshi-kenkyu, 18 : 12-16.
——— (1957) Ibid., 19 : 27-32, 22 : 1-18.
TOYAMA, K. (1913) J. Genet. Japan, 17 : 97-108.
TSUTSUMI, Y. et al. (1959) Sanshi-kenkyu, 29 : 21-25.
UDA, H. (1932) Memor. Publ. 10th Anniv., Mie Agricul. & Forest. Col., 1-42.
UMEYA, Y. (1938) J. Genet. Japan, 14 (6) : 278-288.
——— (1946) Sanshi-ho, 12 (4) : 393-468.
WATANABE, K. (1919) Bull. Sericul. Exp. Stat., 4 (2) : 107-116.