Acrylamide Gel Electrophoresis of Blood Protein During the Moulting and the Metamorphosis in the Silkworm, *Bombyx mori* L.

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It is well known by means of paper electrophoresis that blood protein of the silkworm consists of three kinds of component, even though pattern of the blood proteins differs from the developing stage of the silkworm (Aizawa, 1955; Kobayashi and Komatsu, 1956; Aizawa and Murai, 1958; Komatsu, 1958, 1959; Shigematsu, 1958; Aizawa, Kobayashi and Abe, 1960). On the other hand, Inagami (1954) had reported by means of Tiselius' electrophoresis that blood protein in the silkworm consisted of three components, albumin, α-, and β-globulin. Sasaki and Oda (1955) claimed that blood protein consisted of five components.

This paper deals with changes of the pattern of blood protein in the silkworm with the progress of the age, using acrylamide gel electrophoresis following the method of Raymond and Weintraub (1959).

**MATERIALS AND METHODS**

The larvae, pupae, and moths of the F1 hybrid between two races, J. 124 and C. 124, were used as specimens of this work. Blood was collected from the insects that had been sterilized on their surfaces by immersion for approximately three minutes in 75 per cent ethanol. The insects were then rinsed in distilled water and dried with absorbent paper. Samples of blood were obtained from specimens in each stage from the 3rd instar larva to moth and centrifuged by 3,000 rpm for 10 minutes and/or stored in refrigerator at −20°C, respectively.

Zone electrophoresis was carried out in acrylamide gels using micro-free boundary electrophoretic apparatus, following the method Moriya (1964), with some modifications.

Preparations of acrylamide gel: One part of 10 per cent of aqueous solution of Cyanogum 41 which was produced by American Cyanamid Co. mixed with the same part of veronal buffer of pH 8.6 and ionic strength of 0.01. β-dimethylamino-propini-
trile (DMAPN) was added into the mixed solution mentioned above with stirring until final concentration of DMAPN reached at 0.3–0.4 per cent. Thereafter, the solution was gelatinated in a box after the same part of 10 per cent solution of ammonium persulfate as that of DMAPN was added in the mixed solution. The size of gels as a supporting medium was 12.0×7.5×0.5 cm.

Electrophoresis: A content of the blood protein at each stage of the silkworm was detected by means of Lowry's method (LOWRY et al., 1959), for the detection of amount of sample prior to the experiment (Fig. 1). A slit, 5 cm in width, was made in the gel with small knife. Then small piece of filter paper in which definite volume of the sample was absorbed inserted in the slit.

The electrophoresis on acrylamide gel was carried out with a sample of blood for 120 minutes at 5°C. in refrigerator with a current at 250-300 V. using veronal buffer of pH 8.6 and ionic strength of 0.05.

The dye employed in the present work was 0.5 per cent solution of Amido Black 10 B in solvent of following composition: The solvent was methanol (4 parts), distilled water (5 parts) and acetic acid (1 part). About 1 minute was sufficient to stain the proteins in gel. Excess dye on the gel was washed by the solvent mentioned above in several times. The stained gel was cleared by 2 per cent solution of glycerol and enveloped in Kre Wrap and stored in dark box.

**RESULTS AND DISCUSSION**

For convenience the 7 major constituents have been named A, C, D, E, F, G, and
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**Fig. 2** Diagramatic representation of electrophoretic patterns in blood protein of the silkworm in the 3rd and 4th instar larva. 1: 2-day-old larva in the 3rd instar, 2: Larva at the 3rd moulting stage, 3: 2-day-old larva in the 4th instar, 4: Larva at the 4th moulting stage.

H, starting with the designation of A for the most rapidly migrating component. Traces of other minor constituents are also presented as part of the patterns, indicated by the fainter bands at certain stages.

Electrophoretic pattern which consisted of six components, such as A, C, D, E, F, and G were seen in the blood obtaining from the 3rd and 4th instar larvae, even though protein content of the blood was less than that of the 5th instar larvae.

As shown in Figure 2 and Plate-1, four components named as A, C, F, and G were separated from the blood in 1-day-old or 2-day-old 3rd instar larva. Thereafter, six components mentioned above were observed in the blood from larvae on one day before the 3rd moulting and at the 4th moulting stage, showing that amount of component F became larger at the moulting stage.

There were ten components, such as A, B, C, D, D', E, E', F, G', and H' in the blood derived from the 5th instar larva (Fig. 3, P1.-2): New components B, D', and E' appeared clearly in the blood from 1- and 3-day-old larvae, even though no those components were seen in the blood from the 3rd and 4th instar larva. Moreover, both components G' and H' instead of component G shown in previous stage were appeared in this stage. Amount of component F became larger in the blood from 5-day-old larva of the 5th instar and a tailing between components C and F was seen in the blood.

As presented in Figure 3 and Plate-2, eight components were seen in the larval blood at the mounting stage for both components, D' and E' disappeared. Separation of C and F was very hard, for a tailing between afore-mentioned two components became remarkable and the tailing of female blood was more visible than that of male blood.

As shown in Figure 3, component B' appeared at the middle time of the spinning period. Subsequently, nine components, such as A, B, B', C, D, E, F, G',
and H' were shown as blood pattern. KOBAYASHI and KOMATSU (1956) and AIZAWA et al. (1960) had been reported that paper electrophoretic patterns of the blood protein showed as three components in larvae from the 4th to the 5th instar and component M appeared in mounting period. From the results mentioned above, we are inclined to believe that each of three components, C, F, and G may be coincided with three components, b3, b2, and b1 respectively, and that a tailing between C and F also coincided with component M, even though no electrophoretic method was similar.

From one day before pupation to the 3rd day in pupal period, electrophoretic patterns of the blood differed from those in the previous period: Both components, H and G appeared as bands instead of components H' and G'. Consequently, nine components, such as A, B, B', C, D, E, F, G, and H were seen in blood proteins, showing that the amounts of three components, F, G, and H became larger.

New component G' appeared in the blood of 5-day-old pupae and amount of component B' decreased on the 7th day of pupal period (Fig. 4, Pl.-3).

Both components D and G decreased on one or two days before imagination. Just after imagination, nine components, such as A, B, B', C, E, F, G', H, and I were observed in blood proteins without any tailing but only component I appeared on the cathodal side, while two components, D and G disappeared, and both F and H decreased rapidly (Fig. 4).

The results obtained from the present work differ from those of the previous authors, even though different method was used for the separation of blood proteins. From foregoing results, it is concluded that patterns of blood protein change with the lapse of time in both post embryonic development and imaginal differentiation.

**SUMMARY**

The changes of blood protein have been studied by means of acrylamide gel electrophoresis. Electrophoresis followed by staining for protein with Amido Black 10 B reveals a series of protein pattern in the blood of the silkworm, which appears to change in number as well as in concentration, throughout the life cycle (Figs. 2,3 and 4). This fact suggests that electrophoretic patterns of the blood protein change according to developing stages derived from the moulting and metamorphosis.
REFERENCES

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摘 要

カイコの体液蛋白のアクリルアミドゲル電気泳動
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カイコの脱皮・変態に伴なう体液蛋白成分の変化をアクリルアミドゲル電気泳動法で調べ、つきの結果を得た。

1. 3〜4齢期の幼虫体液には、移動度の大きい方から A, C, D, E, F および G の 6 成分の蛋白が存在し、中には F 成分の量が多い。

2. 5齢期には、A, B, C, D', E', F, G' および H' の 10 成分の蛋白が存在し、孵化期には、D' および E' が消失して A, B, C, D, E, F, G' および H' の 8 成分となるが、5齢盛期期ころに出現した C, F 間のテーリングが著しい。吐糸中期ころには、A, B, B', C, D, E, F, G' および H' の 9 成分が存在する。

3. 吐糸終了から孵化に至るまでには明瞭な変化が現われ、A, B, B', C, D, E, F, G および H の 9 成分となり、F, G および H 成分がそれぞれ増加する。その後、孵化 3 日目ころまでは変化しない。

4. 孵化 5 日目ころに G' 成分が現われ、10成分となる。

5. 羽化 1〜2日前の蛹では、D, G 両成分が減少し、羽化後直後の体液蛋白は A, B, B', C, E, F, G', H および I の 9 成分となり、蛹期のそれとは明らかに異なることが知られた。

6. 以上により、カイコの体液蛋白成分は後胚子発育および成虫化に伴なって変化するものといえる。

Explanation of Plate

1. Electrophoretic patterns of the blood protein in the 3 rd and 4 th instar larva. 1, 2, and 3: Larvae on the 1 st, 2 nd, and 3 rd day of the 3 rd instar, 4: Larva at the 3 rd moulting stage, 5, 6, 7, and 8: Larvae on the 1 st, 2 nd, 3 rd, and 4 th day of the 4 th instar, 9: Larva at the 4 th moulting stage.

2. Electrophoretic patterns of the blood protein in the 5 th instar larva. 1, 2, 3, and 4: Larvae
抄

ハチミツガにおける核多角体病ウイルスの接種量と死亡率との関係

ハチミツガにおける核多角体病ウイルスの接種量と死亡率の関係をプロビット法によって分析した。
4齢の幼虫に多角体を食下させると幼虫、蛹、蛾の各期にわたって感染死する。この場合接種した各ウイルス量の対数と幼虫死亡率のプロビットは明らかに直線関係を示すが、接種ウイルス量の対数と幼虫、蛹および蛾での死亡率をこみにしたプロビットの間には直接関係が存在しない。このことは変態現象がウイルス感染に影響を及ぼすことを示している。幼虫にウイルスを皮下注射した場合の感染率は、同じウイルス量を縫口接種した場合に比較してかなり高いので、縫口接種されたウイルスは感受性組織に到達できないものと解釈される。

（渡部 仁）

録

カイコの細胞質多角体病の発病に伴なう
血液および中腸における核酸ならびに
蛋白量の変化

5齢幼虫に細胞質多核体病ウイルスを添食し、感染発病に伴なう血液および中腸細胞における蛋白、RNAおよびDNAの変化を調べた。感染個体においては血液中の蛋白はいわじろしく減少するが、中腸における蛋白は健康巣とほとんど変わらない。また中腸細胞のRNA、DNAは健康巣に比べると増加する。健康巣、病巣の中腸および多角体から抽出されたRNAの塩基組成はそれぞれ異なっており、多角体のRNAの（G+C）/（A+U）[(Guanyllic acid+Cytidyllic acid)/(Adenylic acid+Uridylic acid)]は0.89できわめて低く、これに対して健康巣および病巣の中腸ではそれぞれ1.18, 1.12であった。

（渡部 仁）