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Flow Mechanism for Fluidity of Silkworm (*Bombyx mori*) Blood in a Capillary

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Flow property and dispersal state of silkworm (*Bombyx mori*) blood in a capillary were studied using various bore-sizes of a low shear capillary viscometer combined with photomicroscopy. The viscosity of blood showed the characteristic shear rate dependence and viscosity value influenced by the capillary bore-size. This dependence was affected by the change in dispersal state of particles in the silkworm blood and also affected by the feeding. These effects can be attributed to the formation change of the aggregates of dispersed blood cell particle and particle number distribution in a capillary. The change in flow mechanism of the silkworm blood obtained from the feed of mulberry leaves and artificial feed was elucidated by the blood cell particle distribution in a capillary. From this experimental result, a flow model of the blood was derived and the wall layer was determined to be composed of double layers in the flowing liquid of a capillary.

Keywords : silkworm, blood, flow property, capillary flow, flow model, *Bombyx mori*

Silkworm blood is a very important bio-fluid which supplies various nutrients for its organ and removes unneeded substances from the body to the outside through the dorsal vessels . It is also important as a regulator of physiological functions such as respiration and growth in the silkworm metamorphosis. The blood therefore affects the quality of the product during metamorphosis such as the cocoon. Thus, knowledge of the flow behavior of silkworm blood is valuable in investigating the flow mechanism of blood cell particles in a capillary.

Earlier papers (Mitsuhashi, 1997; Watanabe, 1982) showed that the silkworm product in various processes during metamorphosis was a food of superior quality. In some countries, silkworms have been used as very valuable medicinal or pharmaceutical material to prevent disease such as hypertension (Ryu, 1999), because they include a high quality of proteins and many other nutrients. The quality of the silkworm in various stages of metamorphosis depends on its physiological function. This function is regulated by the blood and physicochemical property of the blood varies with the change in feed. Silkworm blood viscosity depends on the dispersal state of particles in the blood, making this flow property of importance. The only paper on this subject of silkworm blood, however, is an earlier report by the authors (Nakamura & Mineshita, 1999a). In that study, the flow property of silkworm blood obtained from various species and periods was determined by the measurements with a LS-40 cone-and-plate viscometer, scanning electron microscope, G.C. mass spectrogram and HPLC. It was reported that the primary silkworm blood cell particle was composed of two main parts of a peculiar particle: one was fibrous and included the fibrous protein part and the other was a spherical part including the fat globule particle. These particles joined together and formed the aggregate structure, and this structure could be redispersed and easily became the primary particle. This process was reversible by the shear rate or shear stress. The flow behavior of silkworm blood was found easier by the photomicroscopic observation of flowing blood in a narrow capillary tube. Consequently, in the present paper, the effect of various bore-sizes of a glass capillary tube on the flow property of silkworm blood was investigated, a theoretical treatment applied, and the flow mechanism of the blood in the tube was elucidated.

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Experimental

Samples and Materials Two silkworm blood sources were used as experimental samples throughout this experiment. One was obtained from fifth instar larvae of a hybrid race silkworm (Kinshu×Showa) grown under ordinary circumstances and fed on mulberry leaves. The other was from the same race of silkworm grown under the same conditions except that this sample was fed artificial feed. Results of the G.C.mass spectrogram showed a difference of chemical component between the feeds: the artificial feed contained a large amount of cholesterol (Ito,1984; Nakamura *et al*., 1999). Viscosity measurement with LS-40 viscometer of blood in silkworms fed the artificial feed was reported previously (Nakamura *et al*., 1999). To obtain blood from the larvae, the legs were cut off to about 1mm length by anatomizing scissors. Further, details of this method were reported in the previous paper (Nakamura & Mineshita,1999a).

Measurements The aggregate structure of silkworm blood gathered and mixed together makes coagulates of a large cluster of blood cell particles over five hour period, and this process is irreversible by shear rate or shear stress. Therefore, for the viscosity measurement, the experiment was carried out within five hours to prevent coagulation and, a capillary viscometer combined with a photomicroscope designed by the authors (Mineshita, 1970; Nakamura *et al*., 1997) was used. Figure 1 shows a schematic diagram of the vertical section of the viscometer with the photomicroscope. The silkworm blood viscosity was measured at 37±0.1°C with shear rate of 50–1200 s^{-1}. Capillary dia-

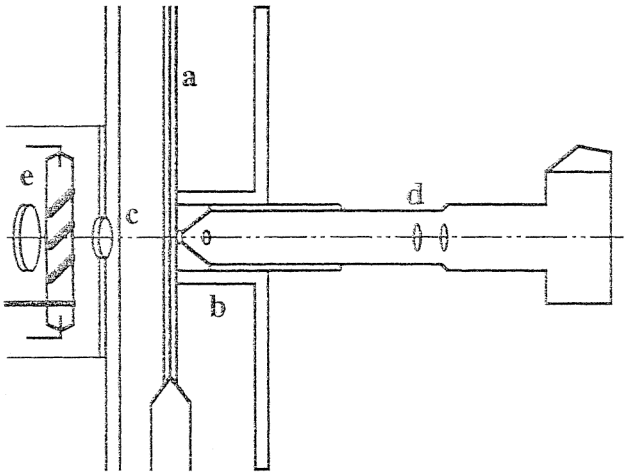

Fig. 1. Schematic view of the experimental method to determine viscosity and dispersal state of silkworm blood in a capillary. (a) Capillary portion of viscometer, (b) Water bath, (c) Condenser lens, (d) Photomicroscope, (e) Xe fish tube.

Table 1. The dimensions of the viscometers

Radius of capillary $R_c\times10^2$ (cm)	Radius of bulb R_b (cm)	Length of capillary L (cm)	Radius of manometer $R_m\times10^2$ (cm)
7.61	1.095	27.7	3.79
5.02	1.260	28.7	4.00
3.20	1.187	24.5	1.27
3.08	1.245	28.7	1.42
3.02	1.270	28.9	1.44
2.38	1.250	28.6	1.06
2.28	1.261	28.8	1.44
1.90	1.260	29.0	1.07

Table 2. The B values of the visscometers.

$R_c\times10^2$ (cm)	B_{exp} ($\times10^2$)	B_{cal} ($\times10^2$)	%-Deviation
7.61	45.28	45.58	–0.66
5.02	8.43	8.55	–1.40
3.20	13.75	13.77	–0.15
3.08	8.78	8.76	+0.23
3.02	7.49	7.46	+0.40
2.38	5.22	5.18	+0.77
2.28	2.45	2.44	+0.41
1.90	2.44	2.43	+0.41

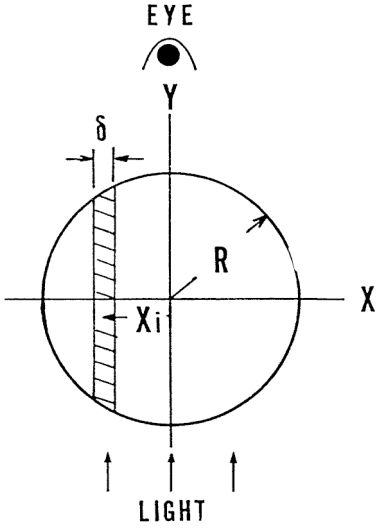

Fig. 2. Schematic representation of theoretical calculation of particle number distribution in a capillary.

meters and dimensions of the viscometer used throughout the experiment are shown in Table 1 and Table 2. A capillary diameter of 20–90 μm was applied to count the smaller particles of blood obtained from the photo micrograph of the image of the flowing particle in a capillary. Other details of the viscometer have been reported elsewhere (Mineshita, 1976)

Theoretical treatment These experimental results were determined by the following theoretical treatment. The physical function Q (density or flow rate) due to the distribution number of dispersed particles of the silkworm blood cell changes with each position of the vertical section in a capillary tube. Therefore, Q is expressed a function of x and y in Fig. 2. Function x is expressed as the distance toward the axis of the abscissa having a thickness (δ) and y is expressed as the distance toward the vertical section from light source to eye looking continuously set at the opposite side, respectively. Under photo-microscopic observation, the experimentally observed value Q^* depends on the function of x, and the relationship between Q and Q^* is expressed as follows:

$$Q^*(x) = \int_{-\sqrt{R^2-x^2}}^{\sqrt{R^2-x^2}} Q(x,y)dy, \qquad (1)$$

where R is the radius of the capillary tube. To obtain the average value divided by the number of K layers in x cross section of the tube, Equation (1) becomes Equation (2).

$$Q^*_i(x) = \int_{x_i-\frac{\delta}{2}}^{x_i+\frac{\delta}{2}} Q^*(x)dy, \delta = \frac{2R}{K}. \qquad (2)$$

If total distribution of the dispersed particles in whole and cross section in a tube is a constant value of n_0, Equation (2) is calculated, and distribution the particles divided by the K layer is expressed as follows,

$$N_i/N = y_{i+1} - y_i, y_i = \frac{x_i}{R}\sqrt{1-\left(\frac{x_i}{R}\right)} + \sin^{-1}\frac{x_i}{R}. \qquad (3)$$

The value of N represents the total number of particles at the cross section in a capillary tube, and is expressed as follows:

$$N = n_0 \cdot \pi \cdot R^2. \qquad (4)$$

This value of N is also expressed as the ratio of flux and flow rate:

$$N = f/v, \qquad (5)$$

where f is the flux and v is the flow rate of dispersed particles in a capillary. To obtain N value experimentally, total amounts of dispersed particles at the cross section were obtained by photomicroscopic observation.

Thus, schematic representation of the theoretical calculation of particle number distribution flowing in a capillary is shown in Fig. 2. This figure shows that total numbers (N) and partial numbers (ΔN) in unit area (δ) are counted in a capillary radius (R).

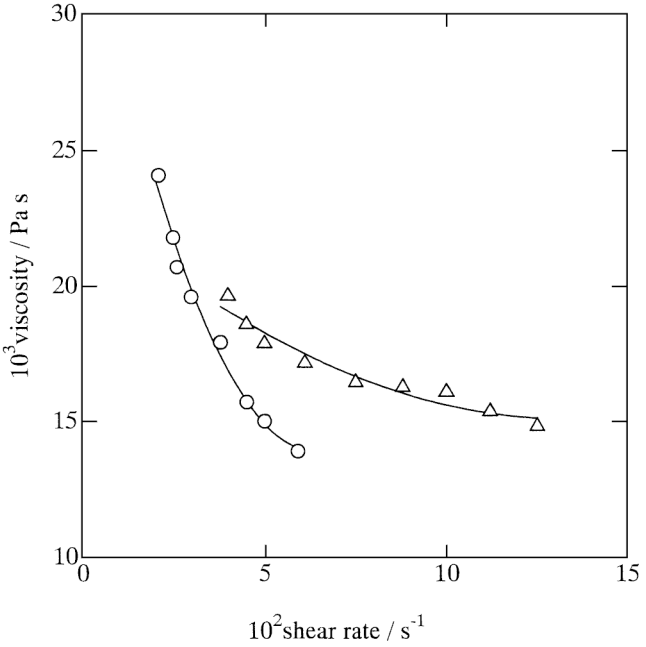

Fig. 3. Viscosity (η)versus shear rate (1/s) plots of silkworm blood measured with various bore-sizes of capillary viscometer (blood from silkworm fed mulberry leaves) (○ R_c=1.90×10^{-2} cm, △ R_c=3.20×10^{-2} cm).

From the experimental results of flow curve and photomicrograph of flowing silkworm blood cell particles in a capillary, the characteristic flow behavior of the blood can be represented as the formation change in aggregates of dispersed particles in the capillary.

Results

Figure 3 shows the viscosity (η) versus shear rate (1/s) plot of the blood obtained from fifth instar of the hybrid race silkworm fed mulberry lreves as measured by various bore sizes of the capillary viscometer. Two characteristic flow behavior were noted, one was a non-Newtonian flow behavior at all ranges of shear rate, and the other was a viscosity decreasing effect with the decreasing of capillary bore size of the viscometer. The viscosity decreased 10–30% when the capillary bore was changed from the largest to the smallest at the same shear rate. The shear dependence of the viscosity was more markedly observed at a lower shear rate than at a higher one, and the difference in viscosity due to the size of the capillary bore was also notable at a lower shear rate. Similar results of viscosity decrease in a small-sized capillary tube were observed with mammalian milk and blood (Schmid-Schonbein, 1981; Barnes *et al*., 1989). Both of these disperse particles in milk and blood is homogenized with uniform spherical particles; however, the dispersal of particles in silkworm blood is heterogeneous and in a complicated form composed of two types of particles. It was determined that the blood in a narrower capillary tube flowed more readily than in a wider tube because of the change in a concentration distribution due to the radial displacement of red and white corpuscles in the tube.

Nakamura and Mineshita (1999c) earlier reported that a primary blood cell particle of silkworm blood was composed of two parts, based on a natural scanning electron microgram: a spherical part containing a large amount of fat globules, and a fibrous part containing a large amount of fibrous protein.

In this case of the silkworm blood, such a radial displacement of individual blood cell particles in a capillary tube could not be expected because of a formation change in the complicated structure of these particles. Moreover, the structure of these blood cell particles composed a weak network structure. The network structure formed aggregates at a low shear rate, and aggregates were redispersed and became primary particles at an applied high shear rate, and this change was reversible with shear rate. Therefore, the change in the aggregate structure was caused by shear dependence and capillary bore size dependence on the viscosity of the silkworm blood cell particles.

Other plots of viscosity (η) versus shear rate (1/s) of the blood obtained from fifth instar larvae of hybrid race silkworm fed artificial feed are shown in Fig. 4, and were measured using various bore sizes of a capillary viscometer. Viscosity value of this blood at a certain shear rate was larger than that of the blood obtained from silkworm fed mulberry leaves. Non-Newtonian flow behavior was observed remarkably at a low shear rate and a viscosity decreasing effect depending on the bore size of the capillary tube was also seen. Shear dependence on the viscosity was more marked at a low shear rate than at a higher rate, but the difference in viscosity between bore size of the viscometer was more marked at a high shear rate than at a lower rate. The difference in flow behavior between blood obtained from silkworms fed mulberry leaves and those fed artificial feed thus depends on the formation change in aggregations of cectuparticlere Nakamura *et al*. (1999) found earlier that primary blood cell particles of silkworm blood formed aggregates, then easily coagulated, so that anti-coagulant was necessary to keep them stable. (Nakamura & Mineshita, 2001)

To clarify the process of the formation change in aggregates in

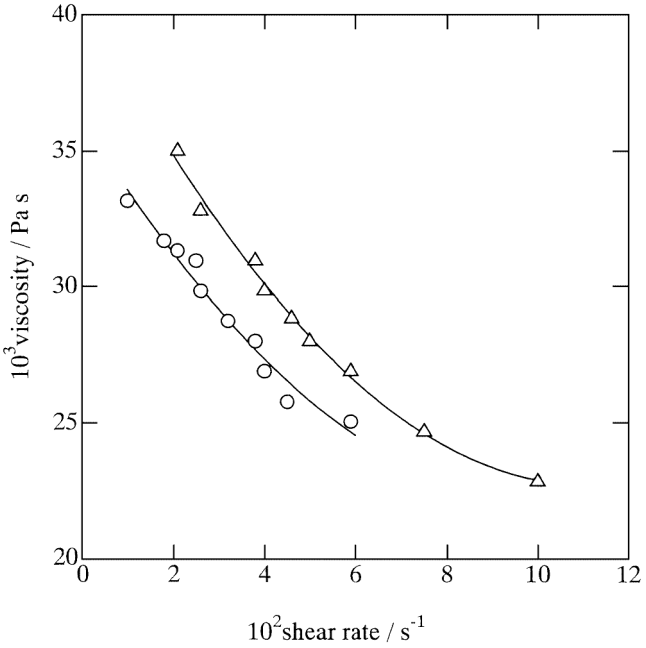

Fig. 4. Viscosity (η)versus shear rate (1/s) plots of silkworm blood measured with various bore-size of capillary viscometer (blood from silkworm fed artificial feed) (○ R_c=1.90×10^{-2} cm, △ R_c=3.20×10^{-2} cm).

each blood source, it is necessary to compare the difference between the primary blood cell particles and the difference between aggregates formed by these particles.

A scanning electron micrograph of various forms of blood obtained from the fifth instar larvae of hybrid race silkworm fed mulberry is shown in Fig. 5. Figure 5(a) shows the primary blood cell particles in a dispersed state and the aggregates constituted of these particles are shown in Fig. 5(b). Primary blood cell particles are seen to be composed mainly from the more even surface of spherical part including the fat globules and fibrous part like a fine needle including fibrous protein surrounding this spherical part. Aggregates of blood cell particles were composed with some groups gathering with the primary blood cell particle. In this case of the silkworm blood, aggregates were composed mainly from a large amount of spherical part. The aggregates were redispersed and separated each other by a shear and was recognized as a reversible change.

Another scanning electron micrograph of blood cell particles obtained from the blood source fed artificial feed is shown in Fig. 6. The primary blood cell particles were composed from the more rugged surface of the spherical part including fat globules and the hazy fibrous part including fibrous protein (Fig. 6(a)). In this case, the spherical part of the aggregates was covered by the hazy fibrous part, and aggregate formation was caused mainly by a tangle of the fibrous part as shown in Fig. 6(b).

A photomicrograph shows the image of dispersed particles in continuously flowing blood from fifth instar larvae of hybrid race silkworm fed mulberry leaves when it was flowing in the capillary with various bore sizes (Fig. 7). To calculate the particle numbers of blood flowing in a capillary, a photomicrograph of the dispersed state of the blood taken using a tube 20 μm of

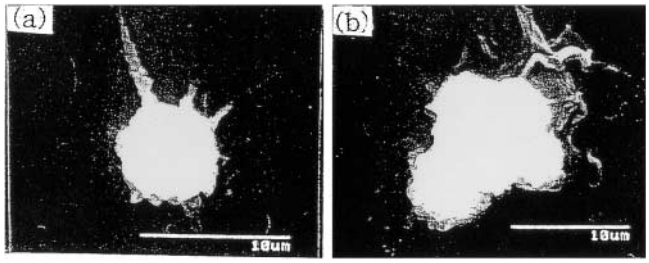

Fig. 5. Scanning electron micrograph of the structure of blood from fifth instar larvae of hybrid race silkworm fed mulberry feed. (a) Primary blood cell particles, (b) Aggregate structure of blood cell particles.

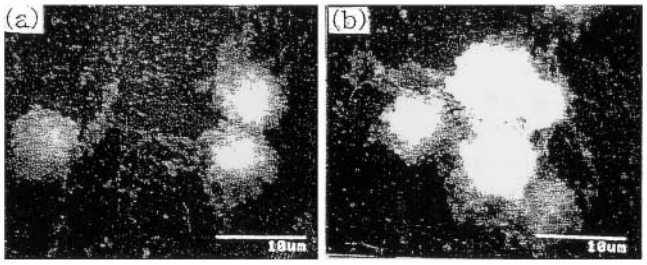

Fig. 6. Scanning electron micrograph of the structure of blood from fifth instar larvae of hybrid race silkworm fed artificial feed. (a) Primary blood cell particles, (b) Aggregate structure of blood cell particles.

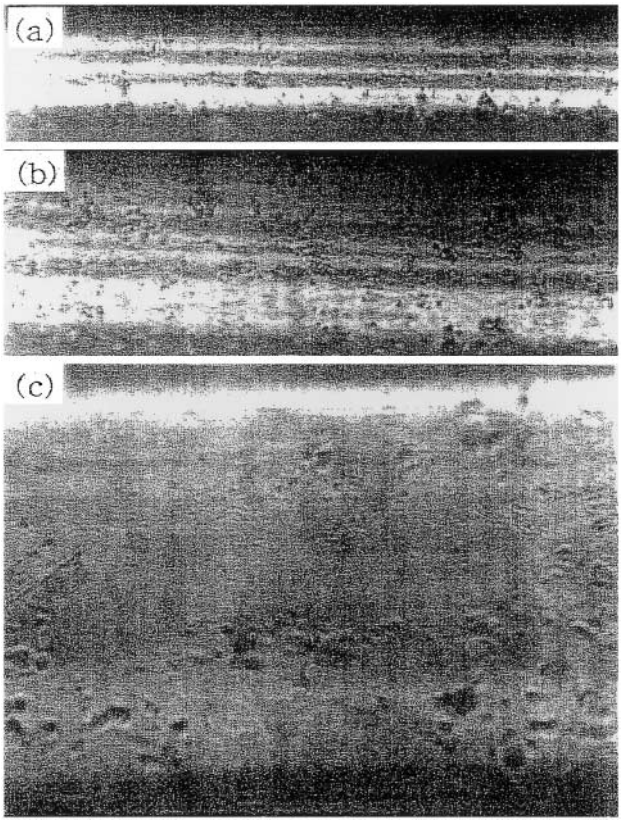

Fig. 7. Photomicrograph of the image of dispersed particles in blood flowing in a capillary (blood from silkworm fed mulberry leaves). (a) Capillary diameter=20 μm, (b) Capillary diameter=40 μm, (c) Capillary diameter=90 μm.

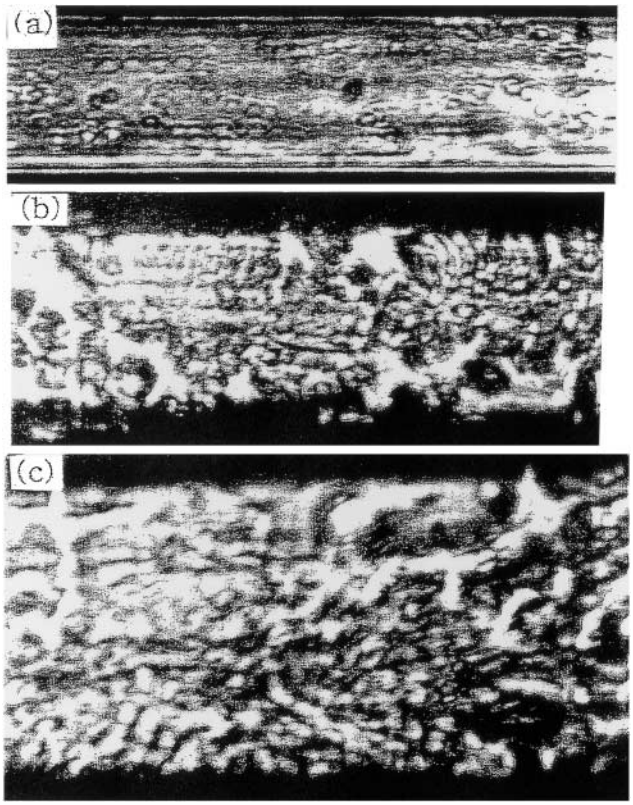

Fig. 8. Photomicrograph of the image of dispersed particles in blood flowing in a capillary (blood from silkworm fed artificial feed). (a) Capillary diameter=20 μm, (b) Capillary diameter=40 μm, (c) Capillary diameter=80 μm.

diameter is shown in Fig. 7(a). A photomicrograph of a tube with the diameter of 40 μm is shown in Fig. 7(b), and that with a tube diameter of 90 μm in Fig. 7(c). These figures show that the flowing particles are small and separated from each other, and the aggregates mainly gathered at the center of the tube.

Contrary to what these photomicrographs show, different results were obtained from the same race of silkworm fed artificial feed (Fig. 8). The photomicrograph of the flowing blood in the capillary tube was taken with the tube diameter of 20 μm, 40 μm and 80 μm, respectively (Fig.8 (a), (b) and (c)). In this case, almost all flowing particles aggregated but did not separate as a primary particle. This means that the primary particle gathered and formed aggregates easily, and these aggregates composed of the fibrous part were flowing rather near the capillary wall. From this photomicrograph, the number of blood particles was counted at the cross section and represented as the total number *N*. The ratio of $\Delta N_i/N$ could be represented as the number of dispersed particles occupied by a certain area against the total number in a unit area at the cross section in a capillary. This ratio shows the blood particle number distribution in a capillary tube.

To identify this difference between the dispersed state of blood from each source in a capillary, the ratio ($\Delta N_i/N$) of distribution number of dispersed particles against relative position of blood in various capillaries was calculated. The plots of the ratio versus relative position of blood cell particles in various capillaries from the hybrid race silkworm fed mulberry are shown in Fig. 9. There was a large amount of spherical part of dispersed particles in the blood, collected mainly near the side of capillary wall (Fig. 9 (a), and (b)). In the smallest capillary bore size, the large dispersed particles gathered and aggregates were formed closely to the wall (Fig. 9(a)). In the largest capillary bore size, large aggregate formation occurred at the center of the capillary tube (Fig. 9 (c)). In the blood from silkworm fed mulberry leaves, fat globules of spherical part are shown surrounded by a thin layer of fibrous protein molecules and, as the result, an attractive force occurs between the hydrophilic binding of the blood particle surface and the capillary wall. In this case, some of the fat globules formed the network structure of aggregates at the capillary wall.

The blood from silkworm fed artificial feed, a large amount of fibrous part of dispersed particles was located closely to the center of the capillary tube (Fig. 10(a), and (b)). In this case, a large amount of aggregates was also observed closely to the capillary wall. In the smallest capillary bore size also, large dispersed particles gathered and aggregates were formed closely to the capillary wall (Fig. 10(a)). In the largest capillary bore size, the large aggregates were observed on both sides of the capillary wall and in the center of the tube (Fig. 10(b)). This means that the fibrous protein layer surrounded by fat globules in the blood might be untangled and adsorbed at the capillary wall. Therefore, the attraction force between the hydrophilic binding of fibrous protein and capillary wall will occur and, as a result, fat globule-globule interaction will increase and the flow will become easier at the center of the capillary tube. In this case, some of the fibrous protein formed the network structure on both sides of the center of the capillary tube and capillary wall.

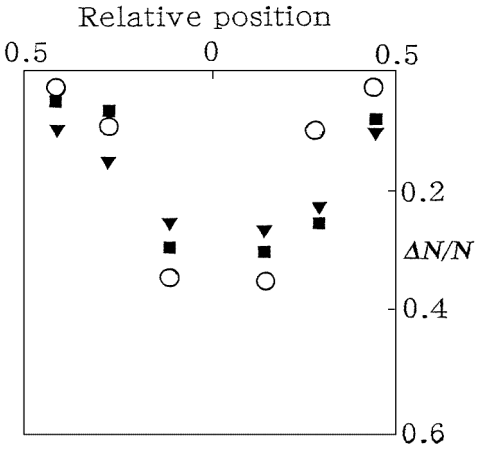

Fig. 9. Ratio($\Delta N_i/N$) versus relative position of blood cell particles flowing in a capillary (blood from the silkworm fed mulberry leaves). (a) ▼ Capillary diameter=20 μm, (b) ■ Capillary diameter=40 μm, (c) ○ Capillary diameter=90 μm.

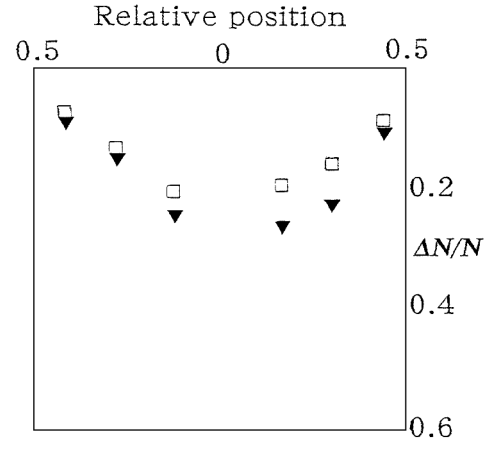

Fig. 10. Ratio($\Delta N_i/N$) versus relative position of blood cell particle flowing in a capillary (blood from different silkworm sources, 2R=20 μm). (a) ▼ fed mulberry leaves, (b) □ fed artificial feed.

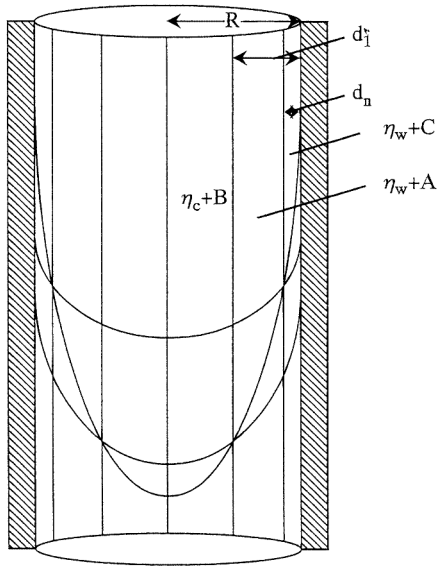

Fig. 11. Flow model of double layers of silkworm blood cell particles flowing in a capillary.

Discussion

From this particle distribution profile of silkworm blood in various capillary bore sizes, silkworm blood in the widest capillary bore size showed the largest amount of fat globules gathered at the center of a capillary tube. The particle distribution curve in the narrowest capillary bore size showed the smallest amount of fat globules flowing at the center in a capillary tube; the largest amount of fat globules gathered and formed aggregates at the near side of the wall in the narrowest capillary. This means that fat globules of hybrid race silkworm blood in the widest capillary bore size are scarcely influenced by the steric hindrance of the capillary wall, and therefore will form a large aggregate structure easier near the center of the tube. However, when the spherical fat globule part flows in the narrowest capillary bore size, it is influenced by the steric hindrance of the capillary wall, and then an aggregate structure is formed more easily near the capillary wall. Thus, the flow property of silkworm blood changes with the formation change of aggregates of blood cell particles in a capillary.

In blood from hybrid race silkworm fed artificial feed, large particles conjugated continuously were also observed at the center of the capillary tube, even in the smallest capillary bore size. In this case, aggregates of blood cell particles were formed with two spherical particles and one fibrous particle, and other aggregates were formed with three or more spherical parts and fibrous parts. These were observed on both sides of the capillary wall and in the center of the tube, since various structures of aggregate were found. Thus, non-Newtonian flow behavior and the dependence of capillary bore-size on viscosity of the blood cell particles in a capillary were caused by the formation change of aggregates of silkworm blood cell particles in the capillary.

The difference in formation of aggregates between blood obtained from silkworm fed the two diets is caused by the change in chemical component of their blood. Nakamura and Mineshita (1999d), previously reported that linoleic acid, linolenic acid, oleic acid and palmitic acid were main components of fatty acid in the blood of silkworm fed mulberry leaves. While only, linolenic acid, palmitic acid, stearic acid, and oleic acid were found in blood of those fed artificial feed. Moreover, it was found that the sialic acid (N-acetyl neuraminic acid) content in silkworm blood was important in the formation of aggregates as a hydrophilic component, and its amount varied with the process of metamorphosis of the silkworm. These chemical components cause changes in the formation of aggregates of the silkworm blood cell particles (Nakamura, 1999).

From the above result, a flow model of silkworm blood in a capillary is shown in Fig. 11. The flow has three flow layers: the layer composed of a layer with (thickness; d_n) at the nearest side of a capillary wall; a flow layer (thickness; d_i) at near side of this wall and a certain distance far from the wall and which is influenced by the wall; and a flow layer (thickness; $R–d_i$) at the center of the capillary tube. The wall was assumed to be composed of double layers with viscosity values of η_w+C, η_w+A, respectively, and the viscosity of the center of the tube to be η_c+B. Hence, non-Newtonian flow behavior and dependence of the capillary bore size on viscosity of silkworm blood were caused by the change in formation of aggregates in a capillary.

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