tion of protein synthesis is indicated by the formation of lamellated ER. Disappearance of fibroin globules is accompanied by formation of many lysosomes and large vacuoles. Protein synthesis is promptly resumed after ecdysis and within a few hours newly secreted silk materials appear already in the gland lumen.

* Acknowledgments. The author thanks Dr Frantisek Sehnal for valuable advices and comments on the manuscript.

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III. The physico-chemical properties of silk fibers and the fiber spinning process

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1. Species specificity of the conformation of silk fibroins

Arthropods produce every variety of silk fibers. Amino acid compositions and the primary structure of silk fibroins have been extensively studied and found specific to the species they belong. The molecular conformation of polypeptides including proteins is dependent on physical (shape, size, rigidity) and chemical (hydrophobic, hydrophilic, etc.) properties of the side-chains of the constituent residues that characterize the amino acids. Therefore, the conformation of silk fibroins is species specific.

a) Fiber

Glycine is the only amino acid having no geometrical isomers and proline (and hydroxyproline) is the only one having no peptide group, $-NH \cdot CO-$. A sequence of glycine residues prescribes a special helix called polyglycine II and that of poly-L-proline residues, poly-L-proline I and poly-L-proline II. Most of the other sequences of L-residues prescribe a helical conformation called α-helix, of the right-handed type[1] and the rest, an extended conformation called β-form. Those ordered conformations except poly-L-proline I and II can be transformed to the β-form, like the unordered conformation, when stretched as in fiber.

So far, the α-helix, the β-form, polyglycine II and collagen-like coiled coil have been distinguished in the structure of silk fibers[2]. It should be noted, however, that the principal conformation is that of (parallel) β silk even when specified as others. In the β-form, each extended chain is hydrogen-bonded, through $-C=O$ and $-NH$ radicals, between 2 neighboring chains to form a pleated sheet which is piled up one by one forming a so-called antiparallel-chain pleated sheet structure[3]. The side-chains are accomodated between the sheets. Thus, the c-dimension of a unit cell, the minimum unit of the crystal structure, becomes smaller with more glycine residues, which have the shortest side chain, $-H$, in the molecular chains. The β silk is further classified into 6 groups based on this c-dimension[2,4]. These are summarized in table 1.

b) Aqueous silk

It would appear that there is no felicitous experimental means to study the conformation of silk proteins as they are found in the silk gland. Silk fibroins are

regenerated from de-gummed silk using reagents, or taken straight from matured silk glands. In any case, they have to be dispersed in water prior to measurements.

The Cotton effect characteristic of the β-form of polypeptides was first measured with *Bombyx mori* fibroin[5]. This enabled optical rotatory dispersion (ORD) and circular dichroism (CD) techniques to be used to analyze the β-form as well as the α-helix of polypeptides. Using these techniques, the conformation of various silk fibroins was analyzed (table 2)[6]. Subfamilies Bombycinae and Thaumetopoeinae fibroins are known not to form any established, ordered conformation. This is certainly because 2 constituents (glycine and alanine) appearing alternately in the sequence prescribe different helices having almost equal stabilities. However, some of the alanine residues in the former fibroins are substituted by serine residues that favor the β-form and the latter ones contain more than 50 mol% alanine residues that favor the α-helix. These cause a subtle difference between the two. The optical rotatory power is far weaker in *B. mori* fibroin than in unordered poly(glutamic acid) even considering high content of optically inactive glycine. This may suggest the presence of some compact (and even loosely ordered) conformation in solution[7]. Saturniinae fibroins, whose main primary structure is consecutive alanine residues, contain 15–20% α-helix (but basically unordered). *B. mori* sericin containing 28 mol% serine was interpreted to have the β-form content of 5–10%[8]. Silk fibroin from caddisworms, containing 24 mol% glycine, 10 mol% serine and 9 mol% proline[9], show very similar ORD and CD as *B. mori* sericine does[10].

Some years ago, the author proposed a molecular model for *B. mori* fibroin in aqueous silk (fig. 1)[11], in which the fibroin molecule is composed of 3 subunits and each unit chain is folded back along itself many times to form a rod-like particle. More recently, Lotz and Keith[12] proposed a crankshaft conformation with poly(L-Ala-Gly)II lamellar crystals prepared in the manner described by Brack and Spach. This conformation is a model for Silk I[13], or the α-form (not α-helix) of silk fibroin[14], known as a quasi-stable crystalline structure and has dimensions (a = 4.72 Å; b = 14.4 Å, traverse to the lamellae; c = 9.6 Å, along the polymer axis) in an orthorhombic unit cell containing 2 polymer chains. The direction of hydrogen bonds is in the a-axis (that is, perpendicular to the lamellae). The molecular model proposed by the author can be understood to be a kind of folded-lamellar structure of this type.

2. *Properties of silk fibers*

Comparative studies were carried out on silk fibers of various species and races with stress placed on the dynamic elastic modulus and degree of crystallization

Table 1. Species specificity of the fiber structure of arthropod silks (based on the data by Rudall et al.[2])

Example	Glycine value*	Group	Type of fiber	Characteristic of the sequence
Phymatocera aterrima (Fam. Blennocampinae)	660/1000	0	Polyglycine II	Extremely high Gly content
Bombyx mori	430/1000	1	β (c = 9.2 Å)	Contains $[Gly\text{-}(Ala, Ser)]_n$ sequence
Anaphe moloneyi	370/1000	2	β (c = 10.0 Å)	High Gly and Ala content
Antheraea mylitta	250/1000	3	β (c = 10.6 Å)	Contains $[Ala]_n$ sequence
Thaumetopoea pictyocampa		4	β (c = 15.0 Å)	Residues with bulky side chains, rich
Nephila senegalensis		5	β (c = 15.7 Å)	Same as above
Digelansinus diversipes	20/1000	6	β (c = 13.8 Å)	Rich in Ala and Gln, low in Gly
Arge usterata	60/1000	7	α-helix	Gln, high; rich in Lys, Asp
Nematus ribesii	360/1000		Collagen	Rich in Ala, Ser, Gln, Pro
Chrysopa species			Cross β	Ser, high; rich in Gly and Ala
Olinga feredayi (caddis worm)	240/1000		β	Ser, Pro, high; rich in Tyr, acidic and basic residues**

*Some of the values are only very approximates. **Data on another caddis fly, *Stenopsyche griseipennis*.

Table 2. Species specificity of the conformation of silk fibroin in solution

Family	Subfamily	Genus	Species	Type of conformation
Bombycidae	Bombycinae	*Bombyx*	*mori*	Unordered, easy to become β
		Bombyx	*mandalina*	
	Thaumetopoeinae	*Anaphe*	*moloneyi*	Unordered, slightly α-helical (A. r.)
		Anaphe	*reticulatae*	
	Saturniinae	*Antheraea*	*pernyi*	
		Philosamia	*cynthia ricini*	α-helix
Stenopsychidae (caddis worm)		*Stenopsyche*	*griseipennis*	β

as determined by the X-ray technique. The data obtained with all the races were put together and treated statistically[15].

a) Bombyx mori fiber

Cocoons were collected among 15 Japanese races (F_1 hybrids). Baves were reeled from them, with extreme care taken not to cause plastic deformation, and tested. Elastic modulus of the bave, E, is linearly related to the size of the bave, d (in denier), almost regardless of the portion of cocoon layer from which the specimens were gathered. The correlation coefficient, r, is negative and the regression line is given by

$E' = 1.92 - 0.11\,d$ ($\sigma = 0.062$, $r = -0.82$)

where E' and σ (standard deviation) are expressed in $\times 10^{11}$ dyn/cm^2, assuming that density of the silk fibroin is 1.36 g/cm^3. Monofilaments of the silk fibroin show similar relationship in the degree of crystallization, C, as well as in the elastic modulus (fig. 2). The regression line is given by

$C = 0.50 - 0.070\,d$ ($\sigma = 0.008$; $r = -0.96$)

The elastic modulus of silk sericin, calculated by comparing those of the bave and of the fibroin monofilament, is independent of the size (and also of the portion of cocoon layer) and shows values of 0.41, 0.71 and 0.90 $\times 10^{11}$ dyn/cm^2 when measured at frequencies of 0.028, 170 and 5000 Hz, respectively. Thus, the linear relationship shown by the baves is due to silk fibroin, and not to silk sericin which cements twin fibroin monofilaments. The tenacity and elongation of baves also show linear relationships; however, the correlation coefficient of the elongation is, as might be expected, positive.

There must be many factors affecting these linear relationships; however, the size alone accounts for up to 75% (this is calculated as 100 r^2) in the case of the elastic modulus of the bave. Thus, the fibrous structure and its physical (and also its chemical) properties are prescribed by the size of the bave; this size is determined to an important extent by the silkworm race.

b) Other silk fibers

Such linear relationship in the elastic modulus was also found in *Antheraea pernyi* fibers and seemed to be common to the silk fibers produced by any kind of silk spinning worm by a similar process[16]. The value of 100 LC/SC, the ratio of long-side chain residues to short side-chain residues multiplied by 100, is specific. For the Anaphe group the mean value is 5.5, for the Bombyx group it is 13.5, and for the Tussah group the

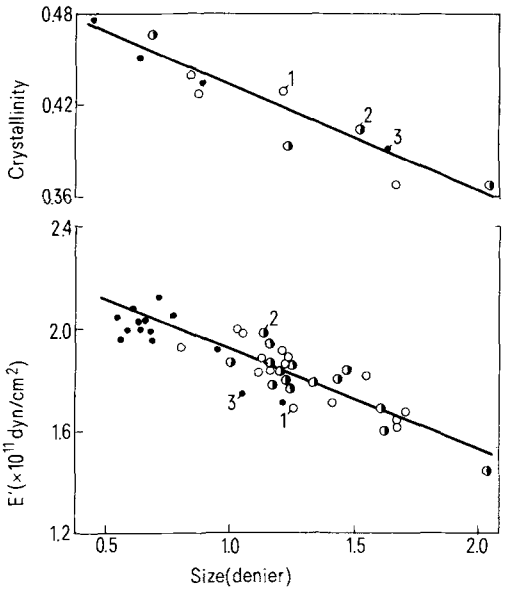

Figure 2. Degree of crystallization and elastic modulus *vs* size for monofilament of *Bombyx mori* fibroin (20 °C, 60% relative humidity). Specimens, taken from: 1, outer; 2, middle; 3, inner portions of the cocoon layers. Elastic modulus was measured at 170 Hz.

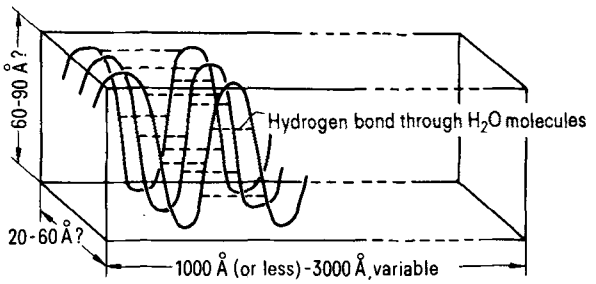

Figure 1. A molecular model for *Bombyx mori* fibroin in aqueous silk. The neighboring segments of each subunit are hydrogen bonded between the peptide groups through water molecules whose number is fluctuating and decreases with increasing polymer concentration, making the molecule shorter and the folding structure more stable. Dimensions of the cross section are given based on measurements of the low-angle X-ray scattering by O. Kratky et al. and the length, on measurements of the light scattering and flow birefringence (see Iizuka[11]). Certain minor changes are made to the earlier model to establish this new model.

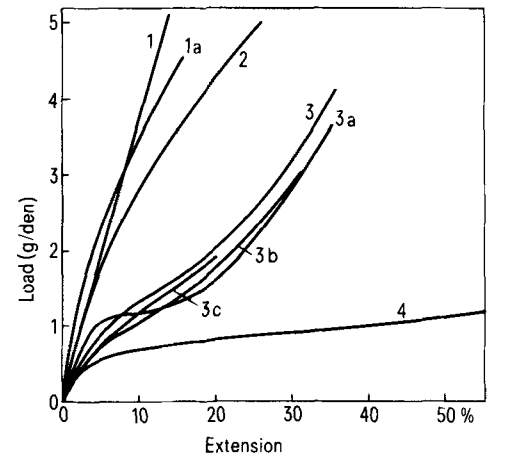

Figure 3. Load-extension curves of various fibroin fibers and of Byssus fiber (20 °C, 65% relative humidity). (Reproduced from Lucas et al.[17]), 1, *Anaphe moloneyi*; 1a, *Anaphe infracta;* 2, *Bombyx mori;* 3, *Antheraea mylitta;* 3a, *Antheraea pernyi;* 3b, *Nephila madagascariensis;* 3c, *Caligula japonica;* 4, *Pinna nobilis.*

mean is 26.6. Sea silk *(Pinna nobilis)* has a very high value (156)[17]. Silk fibers in which residues with longer side-chains are abundant are less crystalline, which is reflected in their load-extension curve (fig. 3).

3. *Fiber spinning process*

Foà and Hiratsuka were the first to demonstrate that mechanical stresses accompanying extrusion of aqueous silk cause the fibroin molecules to coagulate[18]. Many years have passed since then and the spinning process is still not well understood. However, it is at least evident that an 'α-to-β transition' of silk fibroin occurs under shearing stresses, α representing an unordered conformation.

a) Regenerated fibroin

De-gummed *B. mori* silk was dissolved with a 9.3 M LiBr solution, dialyzed with tap water for 3–4 days followed by overnight dialysis with deionized water which was clarified prior to measurements by centrifugation for 30 min at 56,000 × g. Using a cone-plate viscometer, the shearing stress was measured at predetermined shear rates between $6 \cdot 10^{-3}$ and $2 \cdot 10^{2}$ sec^{-1}, the shear rate being raised by regular consecutive steps.

Concentrated fibroin solutions apparently show a Newtonian behavior (fig. 4). A tremendous increase in the shear stress occurs suddenly at a certain shear rate, at and above which the shear stress no longer vanishes promptly when rotation of the plate of viscometer is stopped. This reveals the formation of some structure in solution. At the same time, a number of tiny water-insoluble clots having a crystalline structure similar to that of silk fiber are found in solution. Thus, it is possible to determine the critical shear rate, $\dot{\gamma}_c$, at and above which silk fibroin form fibrous structure[19]. When the polymer concentration is increased, the critical shear rate decreases in a range below 0.5 g/100 ml and increases in a range above this concentration (fig. 5). While it is very high when the dialysis is carried out only with deionized water, it can be lowered by the addition of Ca^{++} or Mg^{++} ions to solution, indicating that these divalent cations promote mechanical denaturation. Addition of urea to solution makes the critical shear rate higher.

Flow birefringence measurements show that even at the highest shear rate covered by the viscometer used ($2 \cdot 10^{2}$ sec^{-1}) the fibroin molecules remain almost unoriented (fig. 6)[11]. This suggests that the crystallization of silk fibroin is not brought about merely by ordering of the fibroin molecules, but by unfolding of the molecular chains under shearing stresses. This conclusion is reasonable judging from several theoretical calculations[20] on the flow behavior of macromolecules that suggest roles of mechanical stresses in extending molecular chains.

Two particles located at slightly different distances from the plate (but at the same distance from the center line of the plate) flow at different velocities parallel to the plate. This is due to the velocity gradient perpendicular to the plate in which they collide and join. The doublet of 2 solid spheres so formed was observed to rotate as a rigid dumbbell for some time and then separate in a capillary tube[21]. Glutamic acid and lysine residues contained in fibroin molecule by a few mol% are ionized at physiological pHs. It is natural to consider that the collision gives a chance for chelate-type junctions to be made between

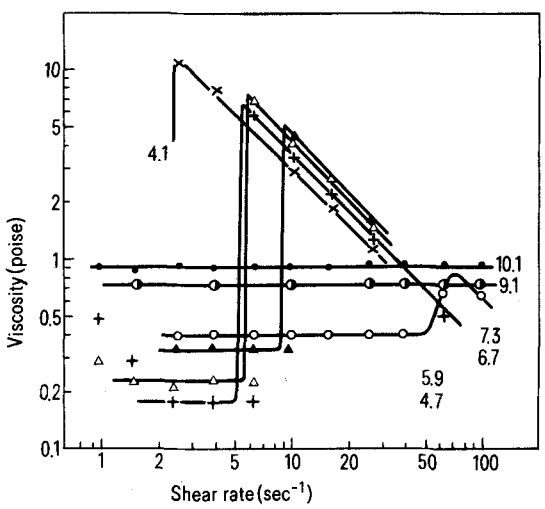

Figure 4. Viscosity vs shear rate for regenerated silk fibroin. The numbers on the lines represent polymer concentration in g/100 ml.

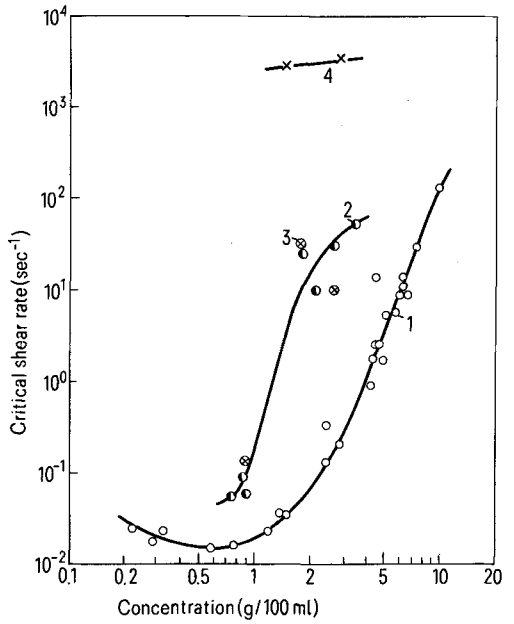

Figure 5. Critical shear rate vs concentration for regenerated silk fibroin dialyzed in various ways. 1, 3–4 days with tap water followed by overnight dialysis with deionized water; 2, 2 days with tap water – 2 days with deionized water; 3, 2 days with a 0.01 M $CaCl_2$ solution – 2 days with deionized water; 4, 3–4 days only with deionized water.

the ionized carboxyl groups, $-COO^-$, of these residues, through Ca^{++} or Mg^{++} ions. Such junctions will be more durable against shearing stresses. Collision frequencies increase with increasing shear rate, eventually causing the formation of some three-dimensional structure in solution, in which the viscous drags surpass forces keeping the hydrogen-bonded molecular foldings (fig. 1), and open them up to make the molecular chains ready to form the extended β-form. The hydrogen bonds through water molecules must be readily broken. When the polymer concentration is increased, the fibroin molecules become shorter (the collision frequencies not increasing in a way as might be expected) and more stable against shearing stresses. This explains the behavior of the critical shear rate especially above 0.5 g/100 ml.

The critical shear rate becomes higher with the increase of K^+ and Na^+ ions. These monovalent cations may extract some of the water molecules in question as the result of modifying solvent structure[22], stabilizing the molecular foldings. The critical shear rate is constant in a pH range between 6.0 and 8.5 where proton exchange of this amphorite is expected to be negligible.

b) Silk fibroin taken straight from the silk gland

The fibroin solution is no longer Newtonian, showing relaxation stresses even at the initial state. De-gummed silk contains a lot of divalent metal compounds. (5–10 metal atoms[16] for each fibroin molecule of mol.wt 300,000. Some of them may be extracted when dissolved and dialyzed.) In native fibroin solution, ions of these metals are considered to be plentiful enough to form solution structure of the chelate-type without intentional application of shearing stresses. This also explains why aqueous silk is gel-like in the silk gland. The shearing stress increases at 2 or 3 shear rates. When the last increase appears, mechanical denaturation is already achieved. The (pseudo) critical shear rate is about $5 \cdot 10^1$ sec^{-1} and almost independent of the polymer concentration (0.5–4.0 g/100 ml).

Kataoka[23] has tested round slices of the middle division of matured silk gland with its content, its cellular membrane stripped off and the sericin washed away in advance, using a parallel-plate plastometer. The critical shear rate (for fiber formation) is determined by observing transparency of the circumference of the cylindrical specimen where the maximum shear rate is acting under a constant-rate compression. He has proposed another critical shear rate, the shear rate for β-nuclei formation, at which the specimen, while not becoming opaque immediately, does so 20 h after deformation. This is of course lower than the original critical shear rate. Both become lower with increasing polymer concentration. His measurements may be worthwhile if performed on aqueous silk (but they are not as exact as with the silk gland); however, there seem to be many uncertainties involved. Hirabayashi[24] has shown that the critical shear rate decreases in solutions of *Antheraea pernyi* fibroin with increasing polymer concentration.

c) Fiber spinning by the silkworm

It is known that the bave is spun by joint forces of drawing (from outside, by the motion of a silkworm's head) and ejection (from inside) of aqueous silk

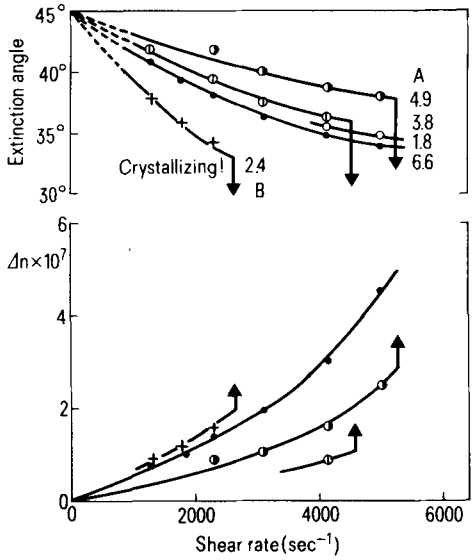

Figure 6. Flow birefringence vs shear rate for regenerated silk fibroin. The numbers on the lines represent polymer concentration in g/100 ml. Solvent: A, 4 M urea solution; B, H_2O (fibroin, dialyzed only with deionized water).

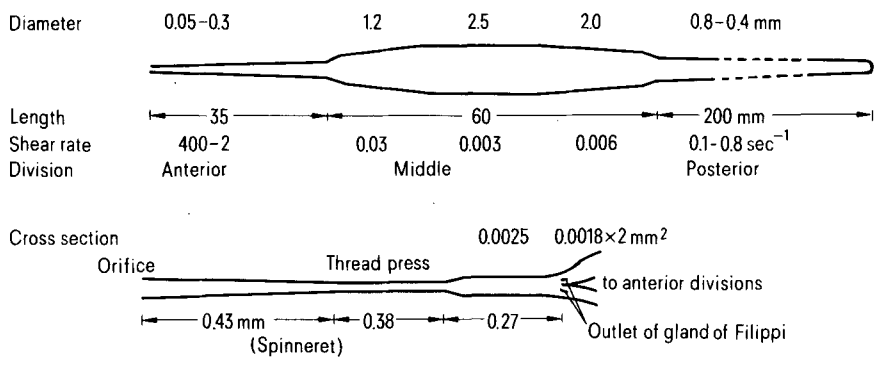

Figure 7. Profile of the dimension and the distribution of shear rate for the silk gland and the spinneret. Following assumptions were made: aqueous silk is uniform in the property, the spinning speed is 1 cm/sec, and the nozzle is round having an uniform diameter. The shear rates were averaged in each cross section from the center of the tube toward its wall only about the portion supposed to be occupied by the fibroin solution.

through the spinneret, the load of drawing being less than 0.01 g/den and that the spinning speed ranges from 0.4 to 1.5 cm/sec. If the bave is drawn out artificially at higher speeds, aqueous silk becomes coagulated even far inside the anterior division of silk gland. At a speed of 2.0 cm/sec, the birefringence of aqueous silk in that division close to the spinneret is already about 0.020[25], which is comparable to that of silk fiber, 0.057. Distribution of the shear rate in aqueous silk, which is gel-like including its outer, sericine layer, was calculated assuming that it is same as for a Newtonian fluid, as the first approximation (fig. 7).

The critical shear rate of aqueous silk, whose fibroin concentration changes from 30% to 15% (at the end of spinning), would be 10^2–10^3 sec^{-1} judging from what has been mentioned. The mechanical denaturation may start somewhere in the anterior division and numerous nuclei so formed grow into oriented microfibrils under ever-increasing shear rate toward the orifice, those microfibrils coming together in a tapered nozzle to form fibrils which are then bundled to compose a bave. It has been suggested that aqueous silk is lengthened 3 times its original length after passing through the inlet to the spinneret[26], where some organ - possibly a 'thread press' - related to a feedback mechanism controlling the quality of the bave being spun might exist. Silk sericin which wraps up silk fibroin may help the latter pass easily through the nozzle after it has hardened. It may also serve as a donar of divalent cations and an acceptor of water squeezed out from the crystalline regions formed.

When data from various silk worm races[27] are put together, one sees that the spinning speed, V, can be expressed as a function of size of bave, d, for each portion of the cocoon layer,

$V = V_o \exp[-kd]$

where V_o and k are constants (fig. 8)[15]. The biological meaning of this relationship is unknown. At all events, the spinning speed increases as the size diminishes. In the meantime, the size of bave decreases with decreasing inner size of the anterior division of silk gland. Both pieces of evidence lead to the conclusion that the thinner bave has experienced higher shear rates and has a more advanced fibrous structure, accordingly. This will explain the correlations obtained concerning the properties of silk fibers.

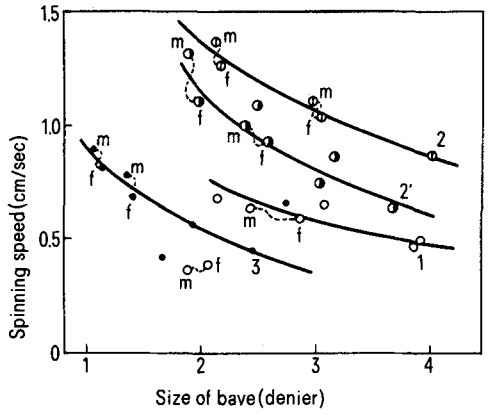

Figure 8. Spinning speed vs size for baves of various races of *Bombyx mori* silkworms (based on the data by Ogiwara and others[27]). m and f indicate that the observations were made especially on male and female silkworms, respectively. 1, outer; 2, middle (the first half); 2', middle (the latter half); 3, inner portions of the cocoon layer.

Two review articles on silk proteins by the same author have been published previons to this short contribution[28].

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