THE COLLOIDAL BEHAVIOUR OF SERICIN. V.*

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Sec. XV. The Gelatinization of the Sericin Solution.

A very striking property of certain protein solutions is that they can be transformed into gels. Bogue(49) assumes that gelatin molecules have tendency to adhere to form long chains, that hydration of these chains takes place, and that when a sufficiently high proportion of water passes from the solution into the threads, gel formation occurs.

One of the chief characteristics of sericin is its power of producing a heat-reversible gel. Silberman states in his book, "Eine sechsprozentige Lösung erstarrt beim Erkalten gallertartig, obwohl sie bereits bei einem Gehalt von 1% gelatiniert. Die Erstarrungsfähigkeit wird indessen durch langes Kochen aufgehoben."

On cooling a moderately strong sol of sericin, it sets to a jelly, which is reconverted into sol on warming. This change does not take place at a definite temperature, for the process is continuous as is shown by the cooling curve. Agglomeration is negligible in comparison with the degree of hydration of sericin micellæ, so it is apt to form a clear jelly.

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* Continued from p. 352 of this volume.

(49) Bogue, *J. Am. Chem. Soc.*, **44** (1922), 1343.

On adding some electrolytes it forms an opaque cloudy jelly, for its agglomeration is raised by it. This gelatinization may be due to increase of its hydration following the decrease of charge on sericin micellæ.

The sericin sol obtained from cocoons having good "kaijo" forms a jelly more easily than that obtained from cocoons having bad "kaijo." This may be due to the difference in hydration and form of sericin micellæ. The nonspherical colloid particles are, in general, apt to gelatinize more easily than the spherical(50).

(1) **Effect of Concentration of Sericin on Gelatinization.** A gelatin sol of 0.25 per cent. does not gelatinize at room temperature. In 1929 S. Bito and N. Oku(51) found that the sericin solution of 0.1 per cent. can form gel after a considerably long time at room temperature and that the sol-gel transformation is reversible. I measured the degree of gelatinization of sericin solutions by the angle of inclination of the test tube containing the sericin gel just when the surface was observed to move, and by the height of liquid raised in a capillary tube having an inner diameter of 0.18 mm. when the end of the capillary tube was brought in contact with the surface of the gel. The results are shown in Table 76.

Table 76. For good "kaijo" (at 15°C.)

Conc. of sericin (%)	Angle of inclination of the test tube (degree)		Height of liquid in the capillary tube (mm.)	
	2 hr. later	18 hr. later	2 hr. later	18 hr. later
0.88	91	above 90	2.0	1.8
0.73	70	,, 90	2.1	2.0
0.66	62	94	2.1	2.0
0.58	50	84	2.2	2.1
0.44	35	63	2.4	2.3
0.35	23	50	2.6	2.5
0.29	20	41	2.7	2.7
0.176	20	23	3.1	3.0
0.088	20	20	3.3	3.2

The rate of gelatinization of the sericin solution depends on the pH value of the medium. At the isoelectric point of sericin, the solution tends to set into a gel easily, for the charge on the sericin particle is the least. The influence of pH on the gelatinization of the sericin sol is shown in Fig. 34.

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(50) S. Praksh, *J. Ind. Chem. Soc.*, **9** (1932), 193.

(51) S. Bito and M. Oku, *Reports Gunze Phys. Chem. Lab.*, 1929, No. 4, 10.

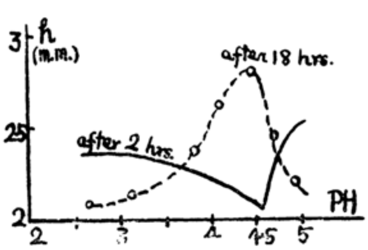

Fig. 34.

The height of liquid raised in the capillary tube was minimum near pH 4.5 after two hours; so pH 4.5 may correspond to the isoelectric point of sericin. When the rate of gelatinization becomes greater, the cloudy, milky gel sets and easily a syneresis takes place in the range of pH 3.5–4.8. So the height of liquid had a maximum value near the isoelectric point of sericin because of syneresis developed after 18 hours.

(2) **The Change in pH during the Gelatinization.** During gelatinization, the pH of the sericin solution generally increased at first, reaching a maximum point after a few hours, then fell gradually, and a syneresis took place (Table 77 and Fig. 35).

Table 77.

1.18% (Good "kaijo"), 18°C.

Time	pH
30 min.	5.61
60 ,,	5.71
90 ,,	5.80 (greatly gelatinizes)
120 ,,	5.84
180 ,,	5.67
300 ,,	5.16
360 ,,	5.10
30 hr.	4.41 (syneresis)
48 ,,	4.19

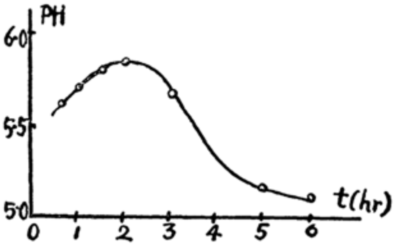

Fig. 35.

(3) **Effect of Time of Heating on Gelatinization.** Prolonged heating at 100°C. caused lowering of viscosity, gradual loss of sensitiveness to electrolytes, and an increase in cataphoretic velocity. At the same time the solution became clear gradually and refused to gelatinize again at room temperature. These may be due to the disintegration of sericin micellæ into smaller units and the decrease in their hydration.

It is obvious that pH of the sericin gel increases with increasing time of heating, and that longer time is necessary to form a stiff gel.

(4) **Effect of Ultra-Violet Ray on the Sericin Gel.** On exposing a sericin solution to ultra-violet ray radiated from a mercury lamp, it became remarkably clear, and its viscosity diminished, and further difficulty in gelatinization was caused by heat. The cause may be attributed to the decrease of the size and hydration of sericin particles.

(5) **Cooling Curve of the Sericin Gel.** I cooled down the sericin sol from 80°C. in a cooling apparatus and obtained a cooling curve following Fisher's formula:

$$\log (T-T_e) = \log (T_a-T_e)-at,$$

where T_a, T_e and T are respectively initial, end, and arbitry temperatures, and a is constant for sol or gel.

It is clear that $\log (T-T_e)$ is a linear function of time t. But in the case of transformation from sol into gel, the heat of gelatinization is evolved, so the cooling curve shows a sudden change at about 50°C. (Table 78 and Fig. 36).

Table 78.

Conc. %	Temp. at B
0.80	43.2
0.85	46.6
0.91	50.1
1.02	55.6

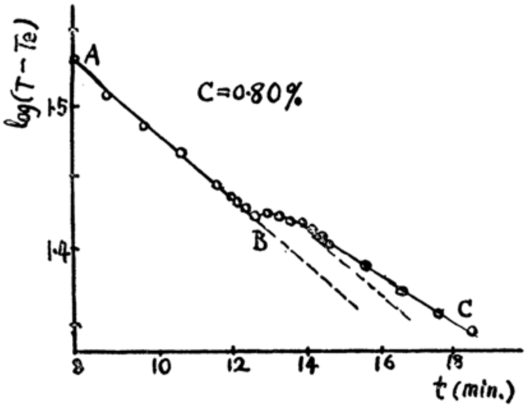

Fig. 36.

The temperature of the sudden change was raised with increasing concentration of the sericin solution. And in low concentrations the temperature, at which the cooling curve bent, was proportional to the concentration of the sericin sol, and the sericin solution might be able to set to a gel at temperatures from 40°C. to 50°C. after a long time. Near this point the variation of the surface tension of the sericin solution with time of standing was very remarkable as already stated.

The values of constant a are on an average 0.022 for the sericin sol and 0.0195 for the sericin gel. The constant a is equal to $E/C\rho$, where E is a cooling factor, C and ρ are respectively the specific heat and the density of sericin.

So I can presume that the conductivity of heat in the sericin sol is greater than that in the sericin gel. If the specific heat of the sericin gel be 0.389, that of the sericin sol may be 0.350.

(6) **Effect of Temperature on the Setting of Gel.** On standing at various temperatures for two hours, sericin solutions gave the results shown in Table 79 in gelatinization.

Table 79. [Cf. (1) of this Section.]

Temp. (C.)	19	30	40	50	60	70
Angle of inclination of the test tube (degree)	180	180	180	165	90	70
Height of liquid in the capillary tube (mm.)	2.0	2.05	2.05	2.15	2.5	2.6

Near the temperature 55°C., the angle of inclination and the height of water raised in a capillary tube showed remarkable changes, and it is considered to be due to sol-gel transformation which can be found in most organic gels and in some inorganic gels, such as thorium arsenate, V_2O_5, mercurisulpho-salicylic acid. The time of setting is generally decreased as the temperature is raised. At low temperatures sericin micellæ can form larger molecular aggeregates and their hydration is increased.

(7) **Softening Point of the Sericin Gel.** With respect to sol-gel reversibility, it must be remarked that the temperature of initial gelatinization and that of melting are never quite the same. Some examples are given in Table 80.

Table 80.

	Setting-temp.	Melting-temp.
4% Gelatin sol	28°	31°
4% Agar-agar sol	40°	85°

The test tube, containing a stiff sericin gel and having a connecting narrow glass tube through the stopper, was inverted vertically in water and then it was heated, the temperature being raised at the rate of 0.5° per minute. The temperature at which the gel is softened and falls down is called the softening point of the gel. The results are given in Table 81.

Table 81.

Good "kaijo"		Mid. "kaijo"		Bad "kaijo"	
Conc. (%)	Soft. p. (C.)	C (%)	S. p. (C.)	C (%)	S. p. (C.)
0.915	89.1	0.84	83.2	0.77	74.1
0.85	85.5	0.72	76.1	0.70	70.0
0.78	83.0	0.615	69.0	0.62	64.5
0.60	75.0	0.54	65.5	0.51	59.0
0.40	60.1	—	—	—	—

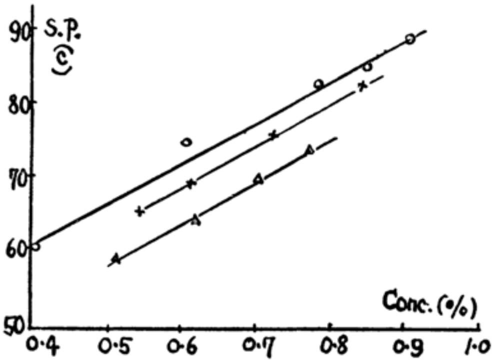

Fig. 37.

As shown in Fig. 37, the softening point of the gel was proportional to the concentration of the gel in such low concentrations.

The softening temperature varied with different "kaijo" of cocoons. The sericin gel obtained from cocoons having good "kaijo" had a higher softening point than that obtained from cocoons having bad "kaijo".

(8) **Reversible Sol-gel Transformation.** On cooling the sericin solution, the sol-form sets to a gel-form and the latter was reconverted into the sol-form on warming.

(a) **Effect of Redissolution.** The sericin gel became reversible to the sol on warming above 60°C. Such a re-dissolved sol sets again to a gel on cooling after a considerable time.

(b) **Effect of Shaking of the Gel.** When the sericin gel was shaken strongly, it transformed imperfectly into a sol which was reconverted into a gel after a long time. That was caused by a thixotropic property of the sericin gel.

(9) **Effect of the Other Substances on Gelatinization.** In general, the rate of gelatinization is accelerated by the addition of a small amount of acids or salts, and retarded by the addition of bases (Table 82). The action of acids and alkalis is the most important factor.

Table 82.

	Gelatinatization	
	accelerated by	retarded by
Acids	HCl, H_2SO_4, CH_3CO_2H, Tartaric acid, Malic acid. Citric acid, Succinic acid. Phthalic acid, etc.	Carbonic acid. Oxalic acid, Glycocoll,
Bases	—	NaOH, KOH, NH_4OH
Salts	$(NH_4)_2CO_3$, Na salts	—
Organic Matters	Glycerin, Mannite, Glucose, Cane sugar, etc.	Alcohols (in very low conc.)

From the obtained results it can be noticed that the sodium salts accelerate gelatinization in accordance with Hofmeister's lyotropic series in low concentration: $I > NO_3 >$ tatrate $> Cl >$ acetate $> PO_4 > SO_4 >$ oxalate. The salts which lead to swelling and hydration of the sericin micellæ, affected the rate of gelatinization acceleratively.

From stabilizing electrolytes, the ions carrying the same charge are adsorbed. By increasing the concentration of such electrolytes, the time of setting of the gel increases, and agglomeration tendency decreases.

Sec. XVI. Freezing of the Sericin Solution.

On freezing a sericin solution, sericin precipitated in form of fibers which were only incompletely soluble on thawing. In the case of a certain protein, the dispersed phase, protein particles, changes its colloidal porperty on freezing and can not be converted again into colloidal sol state by melting. The sericin solution was frozen by cooling in the freezing mixture at −6°C. for two hours and then left to stand at room temperature overnight. The two components of sericin behaved differently on thawing: the fibrous precipitate which consisted probably of sericin A did not re-dissolve while the other did. This difference may be employed for the separation of sericin A and sericin B in low concentration. The proportion of these two components of sericin separated by freezing are shown in Table 83.

From Table 83 it can be seen that the proportion between irreversible and reversible sericins varies remarkably with " kaijo " of cocoons and that these results are nearly coincident with those obtained by the precipitation with ammonium sulphate. The amounts of irreversible sericin, sericin A,

Table 83.

"Kaijo"	Conc. of sericin (%)	Quantity of solution taken (g.)	Irreversible sericin		Reversible sericin	
			(g.)	(%)	(g.)	(%)
Good	0.958	9.883	0.075	78.12	0.021	21.87
	0.710	9.912	0.057	79.16	0.015	20.83
	0.500	9.967	0.037	75.51	0.012	24.49
Middle	1.10	9.984	0.066	58.92	0.046	41.07
	0.750	9.932	0.042	56.00	0.033	44.00
	0.605	9.950	0.032	53.33	0.028	46.66
	0.483	9.879	0.025	51.02	0.024	48.97
Bad	1.076	9.982	0.046	42.59	0.062	57.40
	0.880	9.968	0.036	40.90	0.052	59.09
	0.590	9.985	0.023	38.98	0.035	59.32

increases with increasing concentration of sericin, but in high concentrations it tends to coagulate together with a small quantity of reversible sericin.

Table 84.

Conc. of sericin (%)	Content of N (%)	
	Irrev.	Rev.
0.20	16.40	16.05
0.36	16.35	15.95
0.60	16.30	15.55
0.74	16.26	15.40

The isoelectric point of irreversible sericin in 0.1 N NaOH was nearly at *p*H 3.89.

The nitrogen contents of irreversible and reversible sericins from cocoons of good "kaijo" are shown in Table 84.

It is noticed that the nitrogen contents of irreversible and reversible sericins obtained from the dilute solution are approximately equal to those of sericin A (16.36%) and sericin B (16.11%) respectively. At high concentration separation of the two components is incomplete as already described, and at the same time the decomposition of sericin micellæ may probably occur on freezing. T. Tadokoro found that the nitrogen content of frozen orizenin was less than that of the original substance.

(1) **The Frozen Sericin and Bound Water.** The coagulated irreversible sericin contains a small amount of bound and capillary water. The sericin was separated from the liquid by filtration and, after adhering water was removed with dry filter paper, it was dried in a steam bath. The results given in Table 85 were obtained for good "kaijo".

If the percentage of the water content be denoted by P, and that of the irreversible sericin by S, then P is a linear function of S:

$$P = bS - a,$$

where a and b are constants. Constant a depends on the "kaijo" of cocoons, and the better the "kaijo," the greater its magnitude. Constant b is universal for sericin and about 0.24.

Table 85.

Conc. (%)	Water content		Irreversible sericin (%)
	(g.)	(%)	
0.958	0.650	6.64	78.12
0.71	0.607	6.17	79.16
0.50	0.510	5.14	75.51

(2) Freezing Point Depression of the Sericin Solution. **(a) Non-dialysed Sericin Solution.** Freezing point depression of sericin solutions obtained for good "kaijo" by the usual physico-chemical method are shown in Table 86. P denotes the osmotic pressure in atmospheres calculated from the following formula:

$$P = 1.25\varDelta \text{ (at } 15^\circ\text{C.)},$$

where $\varDelta$ is the freezing point depression of the sericin solution. To a sericin solution containing exactly 10 grams of water, 0.01 mol of sucrose was added, which, if all of the water were free to dissolve sucrose, should form a molar solution. The freezing point depresson ($\varDelta_a$ in Table 86) of the sericin-sucrose solution was measured, and the depression in the freezing point due to the added sucrose was thus determined. The molecular constant for the depression of the freezing point was taken as 2.10° instead of the usual value 1.86°, inasmuch as sucrose forms a hexahydrate in solution. If less than 10 grams of water is free to dissolve sucrose, the depression of the freezing point due to the addition of the sucrose will be greater than expected theoretically.

Table 86.

Conc. (%)	$\varDelta$ (C.)	P (atm.)	$\varDelta_a$	Bound water (%)
0.08	0.001	0.00125	2.188	3.55
0.23	0.005	0.00625	2.210	4.25
0.36	0.008	0.0100	2.229	4.90
0.45	0.010	0.0125	2.245	5.39
0.59	0.017	0.0212	2.265	5.87
0.67	0.022	0.0275	2.280	6.24
0.75	0.025	0.0312	2.295	6.68
0.85	0.033	0.0412	2.305	6.75
0.92	0.038	0.0475	2.315	6.93
1.06	0.052	0.0650	—	—

Accordingly, if $\varDelta_a - \varDelta$ is greater than 2.10°, a direct proof is obtained that 10 grams of water was not all free to dissolve sucrose, and the value of the excessive depression is directly related to the amount of bound water. Thus one mol of sucrose dissolved in 1000 grams of water combines with 6 mols of water, and we have, theoretically, one mol of sucrose hexahydrate dissolved in $1000-(18\times 6)$ or 892 grams of water, which gives a depression of 2.1°. Therefore, the percentage of bound water equals,

$$\frac{\varDelta_a-(\varDelta+2.10)}{\varDelta_a-\varDelta}\times 89.2.$$

The freezing point depression of the sericin solution ($\varDelta$) is approximately proportional to the concentration when the latter is small, but it increases much more rapidly than concentration when high. This may be chiefly due to the increase of electrolytes and the decrease of adsorption of sericin micellæ.

The bound water of sericin calculated from the above formula varied with the concentration of sericin and also with the *p*H of the solution.

At the same concentration, the freezing point depression of the sericin solution obtained from cocoons having bad "kaijo" is greater than that obtained from cocoons having good "kaijo." This was coincident with the experimental results of electrical conductivity.

Table 87.

Conc. (%)	$\varDelta$(C.)	P (atm.)	$\varDelta$	Bound water (%)
0.12	0.004	0.0050	2.205	4.09
0.20	0.006	0.0075	2.222	4.67
0.22	0.007	0.00875	2.225	4.74
0.27	0.009	0.01125	2.245	5.42
0.40	0.015	0.01875	2.275	6.31
0.45	0.018	0.02250	2.315	7.11
0.67	0.033	0.04125	2.425	10.89
0.80	0.046	0.0575	2.495	12.71

(b) **Dialysed Sericin Solution.** The results obtained for the sericin solution dialysed for two days are given in Table 87, For the solution containing less than 0.3 per cent. sericin the osmotic pressure was nearly proportional to the concentration of sericin. Table 88 shows the molecular weights of sericin calculated from Morse's equation :

$$M=\frac{C}{P}\times RT\,,$$

Table 88.

	Sericin (g./100c.c.)(C)	$\frac{C}{P}$	Mol. weight (M)
Nondialysed sericin	0.08	64.0	15050.24
	0.23–0.45	36.2	8935.80
Dialysed sericin	0.20	26.7	5855.0

where M is the molecular weight of dry sericin, P osmotic pressure in cm. of water of density 1, C concentration of the dry protein in g. per 100 c.c. of the solvent, R the gas constant, and T absolute temperature.

The molecular weight of sericin is really much greater than these values and it would probably be in the order of ten thousand.

Sec. XVII. Some Properties of Sericin Gel.

(1) **Precipitation of Sericin in an Electric Field.** When two platinum electrodes a few centimeters apart were dipped in sericin gel and an electrical field was established in it by connecting the electrodes with the terminals of a direct current, sericin began to coagulate in a ring form around the anode after a few minutes, and gradully larger similar zones were produced outside the first one as shown in Fig. 38. In some cases sericin coagulated in fibrous form near the middle between the two electrodes.

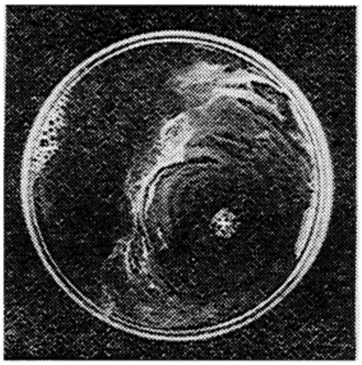

Fig. 38.

When a drop of congo red solution was added to the sericin solution before gelatinization and an electric current was passed through the gel, it turned red near the cathode and blue near the anode,

The flocculation of sericin preceded naturally the syneresis of gel zones and the flocculated sericin consisted probably of sericin A.

When small amounts of salts of alkaline earth and earth metals such as $MgCl_2$, $AlCl_3$, $Al(NO_3)_3$, or salts of heavy metals such as $NiSO_4$, $FeSO_4$, $SnCl_2$, $Pb(NO_3)_2$, $Zn(NO_3)_2$, were added to sericin gel, the rythmical rings were produced around the cathode on passing an electric current through the gel. The formation of such rings may be due to the coagulation of sericin caused by the action of the cations of these salts.

Salts of alkali metals such as NaCl, KCl, NH_4Cl, sodium acetate, $KMnO_4$, ammonium molybdate, and organic matters such as urea, glucose, resorcinol, allantoin, have no effect on the formation of rings around the anode. Considering from these facts, it is probable that salts of magnesium, aluminium, and heavy metals are contained in neither the sericin gel nor the sol.

(2) **Liesegang's Phenomena in the Sericin Gel.** Under suitable circumstances, a precipitate formed by diffusion of reacting solutions into a gel may be deposited in a number of rythmical bands or layers such phenomena were first observed by Liesegang in 1896.

Liesegang's phenomena in the sericin gel were first noticed by S. Bito and M. Oku(52) in 1929 and they investigated the rhythmical precipitation of silver chromate, magnesium hydroxide, and lead iodide.

I also studied the same problem and obtained some rhythmical precipitates. The sericin sol containing minute amount of potassium dichromate and sodium citrate was gelatinized in three hours at room temperature and a drop of a 10 per cent. silver nitrate solution was allowed to diffuse into the gel overnight. Silver chromate precipitated in a series of concentric rings. By the addition of one drop of silver nitrate solution in sericin gel, the rhythmical rings were produced in the presence of the following substances:

Inogranic substances such as $K_2Cr_2O_7$, K_2CrO_4, $AlCl_3$, $FeSO_4$, MgO, borax, ammonium molybdate, etc.;

Organic substances such as phthalic acid, glycine, pyrogallol, diphenylamine, sodium salicylate, ammonium oxalate, etc.

(3) **Diffusion in the Sericin Gel.** A test tube was filled one-third with a sericin sol. After the sol solidified to a jelly the solution (5 c.c.) to be investigated was poured upon it, and the test tube was placed in a thermostat. After a day, the supernatant fluid was decanted from the jelly. The jelly was examined on the progress of diffusion by means of a cathetometer after 6 hours and 30 hours (Table 89 and 90). In order to compare with the diffusion in other jellies, I also determined the diffusion in gelatin and agar jelly of the same concentration. In the same concentration, diffusion of dyestuffs in the sericin gel was slower than in agar or gelatin gel. Moreover, the order of velocities of diffusion was not quite the same in agar or gelatin as in sericin: Bismarck brown < malachite green < methyl violet < quinoline yellow < rhodamine G. < congo red < eosine, in agar or gelatin.

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(52) S. Bito and M. Oku, loc. cit.

Table 89.

Diffusion of inorganic salt solutions into sericin gel. (cm./day)
(Conc. of salt solutions, 1 N ; temperature, 10.0°C.)

Sericin Salt	0.45%	0.58%	0.67%	0.72%	0.84%	0.96%
$K_2Cr_2O_7$	0.35	0.33	0.32	0.33	0.21	0.20
$KMnO_4$	0.72	0.72	0.65	0.62	0.50	0.34
$CuSO_4$	0.85	0.85	0.83	0.83	0.73	0.45
K_2CrO_4	1.00	1.02	0.97	0.95	0.95	0.65
$Cu(CH_3CO_2)_2$	1.19	1.12	1.09	1.04	1.00	0.85

Table 90.

Diffusion of dyestuff solutions into sericin gel. (cm./day)
(Conc. of dyestuff solutions, 0.1% ; temperature, 10°C.)

Sericin Dyestuff		0.56%	0.58%	0.66%	0.69%	0.80%
I	Bismarck brown	0.10	0.10	0.0	0.0	0.0
	Methyl violet	0.20	0.20	0.15	0.10	0.08
	Malachite green	0.25	0.25	0.25	0.23	0.13
	Quinoline yellow	0.30	0.30	0.30	0.28	0.22
II	Congo red	0.45	0.40	0.30	0.30	0.28
	Rhodamine G.	0.60	0.45	0.35	0.33	0.30
	Eosine	0.65	0.60	0.40	0.38	0.35

(To be continued.)