THE COLLOIDAL BEHAVIOUR OF SERICIN. VI.*

By Hideo KANEKO.

Received January 26th, 1934. Published October 28th, 1934.

Part II.

COLLOIDAL BEHAVIOUR OF SERICIN A AND SERICIN B.

Sec. I. The Solubility of Sericin A and Sericin B.

Sericin A and sericin B, obtained by the fractional precipitation from the sericin solution with ammonium sulphate, differ in many properties from the natural sericin existing on the cocoon fibers. At the ordinary temperature the precipitation is accompanied by slight chemical change and is irreversible. Precipitated sericins are only very slightly soluble in cold water, and their solubilities and degrees of dispersion increase remarkably on both sides of the isoelectric point and also in dilute salt solutions. While sericin B is white, sericin A has a slightly gray colour. The solubility of sericin A is diminished more easily by heat or by the action of air than that of sericin B.

(1) **Solubility of the Precipitated Sericins in Alkalis and Acids.** The chemical constitution of the sericin molecule decides the general properties of sericin. The most active groups in the molecule are the basic amino-group and the acid carboxyl-group. So hydrogen- and hydroxyl-ions have a

---

* Continued from p. 421 of this volume.

predominating influence both on the solubility of sericin in water and on the physico-chemical properties of its solution. The solubility of proteins depends generally on the quantity present. Such an anomalous solubility of sericin was investigated by K. Kondo, and I also noticed it with sericin A and sericin B. The definite quantities of sericin A or B were taken in small conical flasks containing 100 c.c. of 0.005 mol NaOH solution, and the flasks were frequently shaken and left in a thermostat of 14°C. for 48 hours. The nitrogen contents of the solutions were determined after treating them by a centrifuge.

The solubility of precipitated sericins in dilute alkali solutions increases first with increasing quantity of sericin, reaching a maximum point, and then diminishing gradully. Sericin A is less soluble in alkali than sericin B.

(a) **Effect of *p*H on Solubility.** Fairbrother and Swann⁽⁵³⁾ showed that, with increasing concentration of either acid or alkali, more and more gelatin goes into solution, that acid or alkali disappears from the solution, and that there is a definite minimum point of solubility in very dilute acid.

(i) **Solubility in Alkali.** The precipitated sericin (1 g.) was put in 100 c.c. of alkali solutions having various hydrogen-ion concentrations under the same conditions described above, and the quantity of dissolved nitrogen was determined by Kjeldahl method and *p*H value of the solutions by the electrical method, (Table 91).

Table 91.

Solubility of Sericins in NaOH Solution at 20°C.

Original NaOH solution *p*H	Sericin A		Sericin B	
	*p*H (2 days after)	Dissolved quantity (%)	*p*H (2 days after)	Dissolved quantity (%)
5.7	6.7	7.96	6.7	9.95
7.2	6.8	8.18	6.8	10.17
11.0	7.4	9.75	7.2	11.15
12.0	8.5	12.38	8.4	12.82

As observed by Fairbrother and Swann, the solubility (g./100 c.c.) of sericin A or sericin B in the alkali solution increases with increasing concentration of alkali and at the same time alkali disappears from the solution, that is, the *p*H value diminishes by the addition of sericin.

The precipitated sericins were found slightly more soluble in KOH solution than in NaOH solution.

---

(53) Fairbrother and Swann, *J. Chem. Soc.*, **121** (1922), 1237.

(ii) **Solubility in Acid.** Under the same conditions described above, I determined the solubilities of the precipitated sericins in acid solutions having various hydrogen-ion concentrations, (Table 92).

Table 92. Solubility of Sericins in HCl Solution.

Original HCl solution *p*H	Sericin A			Sericin B		
	*p*H (2 days after)	Dissolved quantity (%)	*V*	*p*H (2 days after)	Dissolved quantity (%)	*V*
3.8	6.4	7.74	2.8	6.3	9.51	4.0
3.4	6.0	7.07	2.6	5.9	8.85	3.4
3.0	5.4	6.25	2.0	5.3	7.60	2.9
2.75	4.7	5.75	1.9	4.7	7.32	2.7
2.4	4.6	5.67	1.7	4.6	7.35	2.7

V: the quantity (c.c.) of 0.1 N HCl combined with sericins.

The existence of a well-marked point of minimum solubility over a narrow range of hydrogen-ion concentration is characteristic of most proteins, and the point of minimum solubility is *p*H 4.5 for sericin A and *p*H 4.7 for sericin B, as shown in Fig. 39.

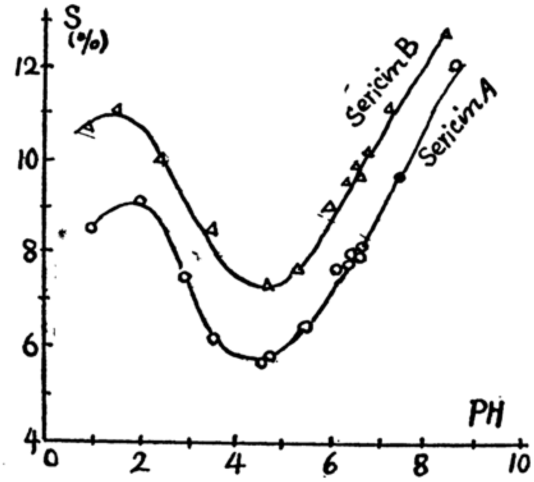

Fig. 39.

On account of slight denaturation on precipitating sericins, their isoelectric points are displaced to the more alkaline side than that of natural sericin.

The point of minimum solubility in sulphuric acid solution was found *p*H 4.4 for sericin A and *p*H 4.6 for sericin B.

The results are summarized as follows:

(*a*) When precipitated sericins are added to the acid or alkali solution, acid or alkali disappears from the solution, sericin combining stoichiometrically with it.(54)

---

(54) J. Errera, *Compt. rend.*, **193** (1931), 1347.

(*b*) The solubility of sericin precipitated with $(NH_4)_2SO_4$ increases with increasing concentration of acid or alkali, reaching a maximum point, and then falling gradually.

(*c*) The precipitated sericins possess a minimum solubility near *p*H 4.5, which corresponds to the isoelectric point of natural sericin.

(*d*) The solubility of sericin A in dilute acid or alkali solution is less than that of sericin B.

(*e*) Both sericin A and sericin B are more soluble in HCl solution than in H_2SO_4 solution.

(b) **Effect of the Temperature on the Solubility.** The solubility of sericins depend on the hydrogen-ion concentration and also on the temperature as it is well known. The effect of the temperature on the solubility of sericins in HCl solution having *p*H 2.4 is shown in Table 93, where constant K was obtained by calculation from the formula $\log S - \log A = K \log t$ or $S = At^K$, S being the solubility of sericins, t the temperature, and A constant.

Table 93.

	Below 30°C.		Above 30°C.	
	K	$\log A$	K	$\log A$
Sericin A	0.027	0.685	0.024	−2.00
Sericin B	1.73	0.740	1.54	−1.55

Near 30°C. the variation in solubility of the precipitated sericins is remarkable, while the solubility of the natural sericin varies rapidly near 60°C.

(c) **Solubility in the Solutions of Sodium Salts of Organic Acids.** The precipitated sericin (1 g.) was introduced into 100 c.c. of 0.01 mol salt solution, and the solubility was determined at 20°C. after 48 hours (Table 94.).

Table 94.

Salt	Sericin A	Sericin B	Salt	Sericin A	Sericin B
HCO_2Na	10.01%	10.65%	$C_3H_7CO_2Na$	10.20%	9.51%
CH_3CO_2Na	9.98	10.01	$C_4H_9CO_2Na$	10.50	9.06
$C_2H_5CO_2Na$	10.01	9.73	$C_{17}H_{33}CO_2Na$	17.91	15.26

With increasing number of carbon atoms in the molecule of the salts, the solubility of sericin A increases slightly, while that of sericin B diminishes as is the case with the natural sericin and gelatin. In the solution of salts of higher fatty acids, such as oleate, sericin dissolves considerably as it is well known.

(2) **Buffer Capacity of Sericin A and Sericin B.** One gram of sericin A or sericin B was dissolved in a solution containing 1 milli-mol NaOH and the resulting solution was made up to 1 liter. Twenty c.c. of this solution were mixed with x c.c. of 0.01 N HCl and $(30-x)$ c.c. of distilled water. pH was determined colorimetrically when there was no interference by colour reactions, and by the quinhydrone electrode, when there was such.

The buffer capacity of precipitated sericins is approximately equal to that of gelatin, and sericin A and sericin B have nearly the same buffer capacity as shown in Fig. 40, where dots are the values for sericin A (sericin B shows nearly the same values), crosses those for gelatin, and v denotes the number of c.c. of 0.01 N HCl added to 20 c.c. of the sericin solution. This fact leads to the conclusion that the isoelectric point of sericin A is near that of sericin B. In fact, the isoelectric points of sericin A and sericin B were found respectively pH 4.1 and pH 4.2 by comparing the degree of turbidity of their solutions.

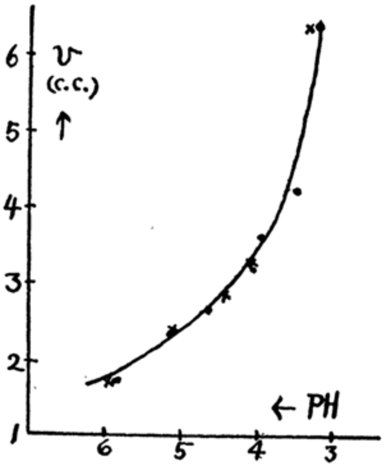

Fig. 40.

Sec. II. The Electric Cataphoresis of Precipitated Sericins.

The isoelectric point of a protein must be the point at which an equal mumber of diffusible anions and cations are present owing to the dissociation of the protein complex, and under this condition the protein presents the amphoteric ion $\mathrm{R}{<}^{\mathrm{NH}_3^{\cdot}}_{\mathrm{COO}'}$. In acid solutions the ionization of the carboxyl-group is suppressed while the ionization of the ammonium-group is increased. The protein becomes then positive complex ion. In alkali solution or in solutions having a higher pH than that of the isoelectric point, the ionization of the ammonium-group is suppressed while the ionization of the carboxyl-group is increased. The protein is, then, electronegative.

One gram of sericin A or sericin B was dissolved in a solution containing 1 milli-mol NaOH and the solution was treated in the same manner as described before. The cataphoresis measurements were made by the microscopic method as shown previously. The results are shown in Tables 95, 96 and Fig. 41.

Table 95.

Cataphoretic Velocity of Sericin A.

Vol. of $\frac{N}{10}$ HCl added (c.c.)	*p*H		U(μ/sec. per 1 volt/cm.)	ζ (m.v.)
1.3	4.7	slightly milky	−0.701	−1.708
1.4	4.5	milky, ppt. later	−0.565	−1.376
1.5	4.35	,, ,,	—	—
1.6	4.2	,, ,,	−0.306	−0.743
1.7	4.05	most ppt.	—	—
1.8	3.95	ppt.	+0.306	+0.748
1.9	3.78	,,	—	—
2.0	3.7	milky, ppt. later	+0.391	+0.952
2.1	3.6	slightly milky	—	—

Table 96.

Cataphoretic Velocity of Sericin B.

Vol. of $\frac{N}{10}$ HCl added (c.c.)	*p*H		U(μ/sec. per 1 volt/cm.)	ζ (m.v.)
1.3	4.7	very slightly milky	−0.896	−2.163
1.4	4.5	milky, ppt. later	−0.767	−1.844
1.5	—	ppt.	—	—
1.6	4.25	most ppt.	0.00	0.00
1.7	—	ppt.	—	—
1.8	4.05	slightly milky	+0.283	+0.683
1.9	3.7	very slightly milky	—	—
2.0	3.6	,, ,, ,,	—	—

The electromotive potential developed on the interface between a protein particle and water may vary with pH of the solution. The charged protein particles can be precipitated from solution under such conditions as reduce the potential to less than 1.5 milli-volts. This reduction can be brought about by dilute solutions of salts, the active precipitating ion being the one carrying the opposite charge to the protein ion, and the effect being proportional to the valency of the ion as already shown. Debye and Hückel's formula indicates that, for a given potential difference E, there is a direct proportionality between the potential of the double layer and the cataphoretic velocity, U (μ/sec.) :

$$\zeta = \frac{6\pi\eta U}{ED} .$$

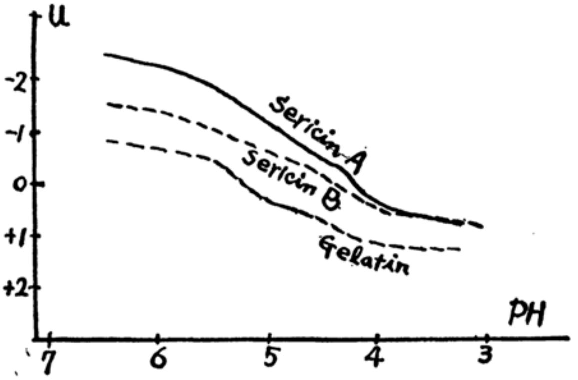

Fig. 41.

From Table 94 and 95 it can be found that the isoelectric points of sericin A and sericin B in very dilute alkaline solutions exist respectively near pH 4.1 and pH 4.25, and that the rate of migration of sericin A is slightly greater than that of sericin B.

The flattened form of the sericin curves in Fig. 41 indicates that sericin remains electrokinetically very sluggish over the whole range. This leads us to the suspicion that sericin, like gelatin, is less reactive both as an acid and as a base than egg albumin. M. Samec(55) showed that amylopectin possesses a greater cataphoretic velocity than any other component of starch though it is the most hydrated.

Effect of Salts. The cataphoretic velocity of sericin is influenced by the presence of salts as well as by pH (Table 97).

The influence of salts on the surface potential of sericin A is shown in Fig. 42. Small quantities of an electrolyte having a monovalent cation raises the boundary charge. Since this increase is due to the negative ion,

---

(55) M. Samec, *Kolloid-Beihefte*, **33** (1931), 269–278.

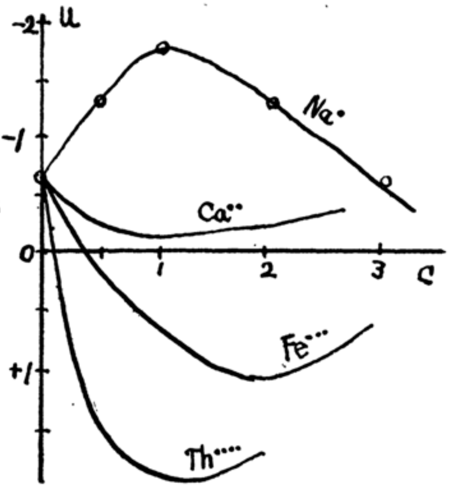

Fig. 42.

Table 97.

Salt	Sericin A		Sericin B	
	U(μ/sec.)	ζ (m.v.)	U(μ/sec.)	ζ (m.v.)
NaCl	−1.797	−4.501	−1.972	−4.873
KCl	−2.01	−5.032	−2.386	−5.895
$CaCl_2$	−0.122	−0.306	−0.487	−1.203
$MgCl_2$	−0.378	−0.946	−0.235	−0.5806
$AlCl_3$	+0.590	+1.470	+0.127	+0.3119
$FeCl_3$	+0.500	+1.245	+0.368	+0.906
$Th(NO_3)_4$	+2.010	+5.032	—	—

The concentration of the salt solutions is 0.001 mol and the volume added is 1.0 c.c.

the influence of the latter in minute concentration seems to exceed the discharging effect of a monovalent cation. In the case of a divalent or trivalent cation, however, the discharging effect of that ion comes immediately into force.

Sec. III. Some Properties of Sericin A and Sericin B.

(1) **The Gold Number.** The gold numbers of sericin A and sericin B in N/3000 NaOH solution are given in Table 98.

Table 98.

Conc. (%)	Sericin A	Sericin B
0.10	0.025	0.035
0.16	0.023	0.0295
0.22	0.0185	0.024
0.30	9.014	0.018

The protective action of sericin A is greater than that of sericin B under the same conditions.

(2) **The Apparent Specific Gravity.** It is known that in most hygroscopic materials a contraction in total volume occurs during adsorption of water, and this is probably true for sorption in general. When such occurs, an *apparent* density will be obtained which is greater than the *true* density.

Sericin A and sericin B swell in various solutions and liquids. The specific gravity of sericin measured by the usual submersion method will, therefore, vary with the liquids used. So the true specific gravity cannot be obtained, but the apparent one depending on the nature of the liquid.

Table 99.

App. density	Liquid used	Author
1.361	Hot water	Müller
1.350	Cold water	J. Kubota(56)
1.33	Benzine	—

Further, from the value of the apparent density we can presume the order of swelling of sericin in liquids. In an active solvent in which sericin swells, the apparent specific gravity is greater than in an inactive solvent. This fact can be seen for the apparent density of raw silk (Table 99).

The apparent density of raw silk depends on the quantity of sericin remaining on the silk fiber, and on the liquid used. The apparent density of sericin A and sericin B is shown in Table 100.

Table 100.

Apparent Specific Gravity of Sericins (15°C.)

Liquid used	Sp. gr. of liquid (15°C)	Sp. gr. of sericin A	Sp. gr. of sericin B	Diff.
Acetone (1)	0.8071	1.412	1.339	0.073
,, (2)	0.7989	1.404	1.322	0.082
C_2H_5OH	0.7955	1.3947	1.3690	0.0257
HCOOH	1.1640	1.374	1.357	0.017
Pyridine	1.0004	1.370	1.353	0.017
CH_3COOH	1.0713	1.370	1.368	0.002
$C_6H_5NO_2$	1.0209	1.3688	1.328	0.0408
CH_3OH	0.8019	1.366	1.353	0.013
C_6H_6	0.8848	1.375	1.344	0.013
Water	1.000	1.356	1.309	0.047
$C_6H_4(CH_3)_2$	0.8642	1.283	1.265	0.018

(1) Merck's acetone.

The active solvents in which precipitated sericin swells are acetone, ethyl alcohol, formic acid, and pyridine. Benzene and xylene which have no or small permanent dipole moment are inactive.

Nitrobenzene, methyl alcohol, and acetic acid are moderately active solvents for sericin. It was impossible to determine the true density of the powdered sericin.

The reciprocal values of densities of proteins are shown in Table 101.

---

(56) J. Kubota, *Bull. Sericul. and Silk Ind. Japan*, **5** (1932), 68–69.

Table 101.

Protein	1/*d*	Protein	1/*d*
Sericin A	0.736 (in benzene)	Gelatin	0.724
Sericin B	0.744 („ „)	Egg-albumin	0.735
Fibroin	0.740 (in water)	Serum albumin	0.735

(3) Colour Reactions of Sericin A and Sericin B.

Table 102.

Solution of Precipited sericins in N/10 NaOH.

Colour reaction	Sericin A	Sericin B
Engel's reaction	none	none
Million's reaction	given	given (deeper red)
Arginine reaction	given (5.95%)*	given (3.50%)*
May and Rose's and Neubauer Rhode's reaction	given faintly	none
Sulphur reaction	given faintly	given faintly
Histidin reaction	given	given
Biuret reaction	violetish red	violetish red
Xanthoprotein reaction	given	given

* By colorimetry.

Recently the existence of tryptophane in sericin has been reported by Abderhalden.(57)

The Biuret Reaction of Sericin A and Sericin B. The red tinge of the biuret colour reaction of sericin sol has qualitatively an intimate relation with the quantity of sericin in it, but the colour is influenced by the presence of other substances in the cocoon layer. So it was impossible to determine quantitatively the degree of "kaijo" of cocoons by the colour of the biuret reaction of sericin sol.

---

(57) Abderhalden, *Z. physiol. Chem.*, **202** (1931), 37, ibid., **207** (1932), 141–146.

(4) Fluorescent Colours of Sericin A and Sericin B Solutions under the Ultraviolet Light. When the sericin A and sericin B solutions in dilute alkali are exposed to the ultraviolet light, both give a slight yellow fluorescence. If a minute amount of glycocoll which is contained in the solution of the natural sericin and gives slight violet fluorescence be added to the sericin solutions, there is no variation in fluorescent colour of the sericin A solution, while the sericin B solution gives immediately a brilliant violet fluorescence. If the sericin A and sericin B solutions contain small amounts of aspartic acid, both give the same violet fluorescence. The fluorescent colours produced by the sericin solutions are influenced by the presence of a minute quantity of salts. From these results it can be seen that the fluorescent colours depend on the impurities adsorped or occluded in the cocoon layers which have close relation with the variety of cocoons, and on the proportion of sericin A and sericin B which is also intimately related to the variety.

(5) Transformation from Sericin A to Sericin B. Sericin A is more sensitive to heat than sericin B. So sericin A is transformed gradually into sericin B by heating it in drying apparatus, by steaming or by heating its solution in a flask.

Sec. IV. The Swelling of the Sericin A and Sericin B.

It will be interesting to note that substances like gelatin, casein, sericin, and starch, which are proved to be amorphous by X-ray analysis of Debye and Scherrer are the very substances that show the phenomenon of swelling. But the stretched sericin A will be shown to be crystalline by X-ray examination as done in the case of fibroin.

Sericin, when dry, is hygroscopic and it contains usually about 10% water. The sericin immersed in water gradually swells, or imbibes water. The swelling of the precipitated sericin in water and in the solutions of acids was investigated by M. Oku,(58) who showed that the swelling of the precipitated sericin in very dilute acid solutions possesses a minimum point in 1/1024 mol HCl or H_2SO_4 solution, and that it swells more in HCl solution than in H_2SO_4 solution.

The swelling of proteins belongs to complex phenomena and it depends on the expansion of colloidal micellæ, and on the adsorption and imbibition of liquid molecules or ions. This phenomenon of swelling has naturally a close relation to the "kaijo" of cocoons. Precipitated sericin (0.2 g.) was

---

(58) M. Oku, *Reports Gunze Phys. Chem. Lab. Japan*, **4** (1929), 15–22.

put in centrifuge tubes with scales, mixed with 10 c.c. of water or a salt solution, and then left to stand for 24 hours at 20°C. After the equilibrium was reached it was treated in a centrifuge for 30 minutes and the increase in volume of the sericin referred to the original was taken as expressing the comparative degree of the swelling.

(1) The Swelling of Sericins in the Solutions of Organic Sodium Salts. **(a) Sericin A.** I used the sodium salts, for their anions are the least surface capillary active (Table 103 and Fig. 43).

Table 103.

	C_m	O
HCO_2Na	0.50	0.73
CH_3CO_2Na	0.33	2.84
$C_2H_5CO_2Na$	0.25	8.93
n-$C_3H_7CO_2Na$	0.21	19.6
n-$C_4H_9CO_2Na$	0.125	68.5

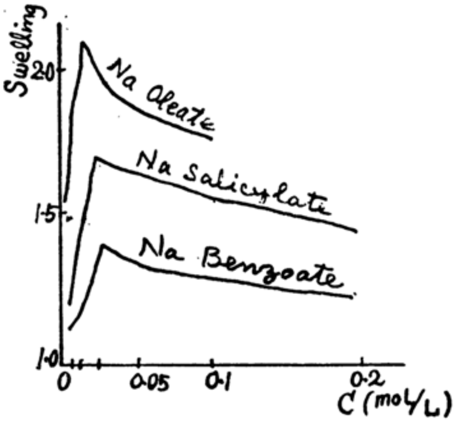

Fig. 43.

In the sodium salt solutions of fatty acids the concentration for the minimum swelling of sericin A, C_m, decreases with increasing number of carbon atoms in their molecules and it has the following relation with capillary activity of the fatty acids, O,

$$\log O = 3.0 - 8.2\, C_m\ .$$

The maximum swelling in the solution of oxalate or salt of an oxy-acid is coincident with the minimum point in the solutions of sodium salts of fatty acids, and at the concentration of 2 mol and in concentrations less than 0.125 mol the arrangement of the anions according to the type and the intensity of their influence on swelling leads to the Hofmeister series: citrate $<$ tartrate $<$ acetate. As the precipitated sericin adsorbs strongly the anion of sodium oleate, it has a maximum point of swelling in very low concentration of the salt (0.012 mol).

The relation between the concentration of the minimum swelling of sericin B and the number of carbon atoms of the salts of fatty acids was contrary to the case of sericin A. The results were similar to those of their solubilities in the solutions of the same salts.

Taking the swelling of the precipitated sericin in distilled water at 20°C. as 100, the effects of salts on swelling are shown in Table 104.

Table 104. (Sericin A).

Mol	2	1	0.5	0.25	0.125	0.0625	0.0312
HCO_2Na	122.5	98.4	77.4	85.5	87.1	93.5	
CH_3CO_2Na	114.5	98.4	83.8	80.6	101.6	109.6	—
$C_2H_5CO_2Na$	150.0	132.2	104.8	98.6	109.6	130.6	—
n-$C_3H_7CO_2Na$	117.7	116.1	112.9	108.0	111.2	114.5	—
n-$C_4H_9CO_2Na$	—	145.1	119.3	112.9	101.6	106.4	109.6

The numbers less than 100 mean contraction in the solutions. In general sericin B swells more in the solutions of these salts than sericin A.

(2) **Swelling in the Solutions of the Sodium Salts of Inorganic Acids.** In the solutions of capillarily inactive sodium salts of inorganic acids sericin A and sericin B swell as well as in the solutions of capillarily active organic salts. But the concentration of the maximum swelling is greater than in the case of organic salts.

(3) **Effect of Acids and Bases.** The swelling of both sericins depends remarkably on the *p*H of the solutions as it is well known, and the minimum swelling of sericin A in HCl solutions corresponds to *p*H 3.75. Different acids causes different degrees of swelling.

Loeb (1922) considered that swelling was affected not only by the hydrogen-ion concentration, but also, in acid solutions, by the valency of the anion. Sericin A and sericin B swell considerably in a very dilute alkali solution and in concentrations higher than 1/40 mol NaOH the former disperses partly into solution.

The swelling of sericins in solutions of HCl and of NaOH is shown in Fig. 44. The acid curve rises sharply to the maximum at an external *p*H of 3.0; the alkaline curve rises slowly at first. The isoelectric point of sericin A exists on the more acid side than that of sericin B as frequently described.

(4) **Effect of Temperature.** The variation in swelling of sericins with rising temperature is shown in Fig. 45, where $\log S$ is given by the equa-

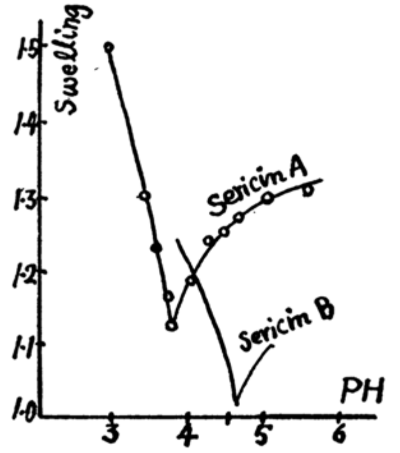

Fig. 44.

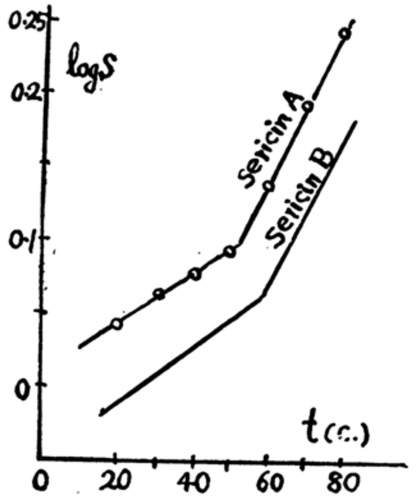

Fig. 45

tion : $\log S = A+Bt$ or $S = A'e^{Bt}$, where A and B are constants, the former depending on pH and on the state of sericin, and the latter on the state only. For example, $\log S = 1.33+0.005\,t$ (below 50°C.), and $\log S = 1.54+0.14\,(t-50)$ (above 50°C.) for sericin A at pH 4.6. The abrupt change at 50°C. caused by the rapid transformation of gel into sol can also be seen in other properties.

Lloyd(59) showed that the quantity of absorbed water in gelatin is proportional to the square of temperature at low temperatures. If the quantity of adsorbed water be proportional to the increase in volume of sericin, the swelling may be proportional to the square of temperature. Such relation can be found in the results of this experiment.

(*To be continued.*)

---

(59) Lloyd, *Biochem. J.*, **21** (1929), 21, 1352; ibid., **22** (1928), 1007; ibid., **24** (1930), 1460; ibid., **25** (1931), 1580.