Action of Alkaline Copper-solution on Silk Fibroin. III.

By Yūjirō Takamatsu.

In his first paper[1] the author ascertained that in an ammoniacal solution of copper oxide i.e. Schweizer's solution, not only is the solubility of fibroin increased by the presence of copper hydroxide as solid phase, but also the copper content of the solution is enhanced because of the dissolution of fibroin, thus bringing about an increased solubility of fibroin in copper-amine solution through the mutual dissolution of fibroin and copper. Also, with regard to the chemical reactions, he determined the chemically combining equivalent of fibroin to copper in a solution of copper oxide-ethylendiamine; and further ascertained the existence of a complex fibroin-copper-ethylendiamine compound, isolating it from solutions in which given amounts of fibroin were dissolved in a great excess of copper hydroxide. This compound was found to have the following composition:

$$\text{Fibroin} : \text{Cu} : en = 1 : 2 : 2$$

$$en = \text{ethylendiamine } H_2N\,CH_2\,CH_2\,NH_2$$

And in his second paper,[2] he investigated the phenomena connected with the dissolution of fibroin in copper-amine solutions, especially the case in which a large quantity of fibroin is placed in a copper-amine solution with a given copper content, and defined the correlation between the maximum amount of fibroin dissolved and the copper and also the amine content of the solution; and further ascertained the existence of a another complex fibroin-copper-amine compound different from the one mentioned above, isolating it from solutions in which the maximum degree of solubility of fibroin was estimated in copper oxide-ethylendiamine solution. This compound be expressed as:

$$\text{Fibroin} : \text{Cu} : en = 1 : 1 : 1$$

Continuing this line of research, the author, in this present work, has studied further on the phenomena connected with the dissolution of silk fibroin in copper-amine solution and on the mechanism of the chemical reactions in this system.

In the first chapter of this paper, the author deals with (1) the chemical natures of the two fibroin-copper-amine compounds mentioned above; (2) the chemical formulae by which the combining state of the compositions of the compounds may be expressed; (3) the mutual relations between these compounds; and (4) the peculiar phenomena shown in the solutions of these compounds which may be caused by the presence of excessive amounts of ethylendiamine.

In the second chapter, he (1) deals with the mechanism of the dissolution of fibroin in copper-amine solution; and (2) gives a detailed explanation of the modes of that dissolution; suggesting, in conclusion (3), the chemical reactions in the system of fibroin-copper-amine.

Chapter I. On the fibroin-copper-amine compounds.

(1)

Given the two kinds of fibroin-copper-amine compounds,

$$\text{Fibroin} : \text{Cu} : en = 1 : 1 : 1 \qquad [I]$$

$$\text{Fibroin} : \text{Cu} : en = 1 : 2 : 2 \qquad [II]$$

the problem is to find a formula by which the manner of their combining state may be expressed.

First of all, in connection with the formula of the compound [II], we can here quote the results gained from the study of the system of cellulose-copper-amine.

According to Traube[3], (i) the chemical equivalent of cellulose to copper in the solution of copper oxide-ethylendiamine is shown by the ratio $C_6 : Cu = 2 : 1$ or $(C_6)_2 : Cu = 1 : 1$, where C_6: $C_6H_{10}O_5$ rest, (ii) a complex cellulose-copper-amine compound such as

$$(C_6)_2 : Cu : en = 1 : 2 : 2 \text{ i.e. } [(C_6)_2Cu][Cu\,en_2]$$

is prepared from the copper-*en* solution in which a given amount of cellulose is dissolved with excessive amounts of copper-amine.

Comparing these facts with the case of fibroin, the first term is seen to correspond to the ratio of fibroin to copper, fibroin (227) : Cu (63.6) = 1 : 1, which had been previously studied by the author; and the second term, that is, the condition of preparation of the complex compounds, is common to both cellulose and fibroin:

$$(C_6)_2 : Cu : en = 1 : 2 : 2,$$

$$\text{Fibroin} : Cu : en = 1 : 2 : 2.$$

From these analogies we may assume that the combining state of the components of the compound [II] is expressed by such form as [Fibroin Cu] [Cu en_2]. Similarly, the compound [I] will also be expressed either by the form [Fibroin_2 Cu] [Cu en_2] or [Fibroin Cu] *en*.

In an effort to establish these proposals more exactly, the author prepared the fibroin-copper-

1) This memoir.; 1932, **7** 1. 2) This memoir.; **7** 306. 3) Traube; Ber. 1927, **60** 43.

alkali compound derived from those compounds [I] and [II] respectively by treating them with alkali, and examined the change of their chemical compositions, especially those of the two components Cu and *en*, that occur when the compounds of fibroin-copper-amine are transformed into those of fibroin-copper-alkali.

Here again the analogy with cellulose[1] is significant. If the complex cellulose-copper-amine compound $[(C_6)_2Cu][Cu\,en_2]$ mentioned above is treated with caustic alkali, it is transformed into the cellulose-copper-alkali compound $[(C_6)_2Cu]Na_2$ (Normann's compound) with its $[Cu\,en_2]$ radical replaced by Na radical, thus being deprived of half of its Cu content and all of its *en* content, thus,

$$[(C_6)_2Cu][Cu\,en_2] \xrightarrow{NaOH} [(C_6)_2Cu]Na_2$$

$$(C_6)_2 : Cu : en = 1:2:2 \quad (C_6)_2 : Cu : Na = 1:1:2.$$

So, in the present case of fibroin, the author prepared the fibroin-copper-alkali compound by treating the solutions of fibroin in copper-amine, from which the compounds [I] and [II] should be isolated, with alcoholic potash of definite concentration.

(2)

Omitting a minute description of the experimental conditions and the analytical method[2], the author will here show the results of his studies.

(a) Chemical formula for the compound [II].

As examples of the composition of the fibroin-copper-alkali compound which should be derived from the compound [II], the following data are given.

Table 1.

Percentages of components				Combining ratios of components			
Fibroin %	Cu %	K %	*en* %	Fibroin	Cu	K	*en*
48.00	13.81	12.87	0.26	1	1.03	1.55	0.02
46.90	14.02	12.05	0.40	1	1.07	1.50	0.03
46.50	13.70	11.67	0.35	1	1.05	1.46	0.03

(Fibroin : Cu : K : *en* = 227 : 63.6 : 39 : 60)

Hence the *en* content being practically negligible in each case, it may be assumed that there exists a fibroin-copper-alkali compound with such combining ratios as

$$\text{Fibroin} : Cu : K \fallingdotseq 1:1:1.5.$$

Therefore, comparing these ratios with those of fibroin-copper-amine compound,

$$\underset{1\quad\ \ 2\quad\ 2}{\text{Fibroin} : Cu : en} \xrightarrow{K} \underset{1\quad\ \ 1\quad\ 1.5}{\text{Fibroin} : Cu : K} \ (en=0).$$

That is to say, by such a treatment the fibroin-copper-amine compound [II] is transformed into the fibroin-copper-alkali compound, being deprived of half of its Cu content and all of its *en* content. This is very similar to the case of cellulose just mentioned, especially in the points of the change in Cu and *en* contents. The discordance in alkali content may be assumed to be chiefly due to an instability of the fibroin-copper-alkali compound (1 : 1 : 2) which should be taken into account because it is apt to be transformed into the more stable compound of the lower composition such as 1 : 1 : 1.[3]

From these results we may well give the expression [Fibroin Cu] $[Cu\,en_2]$ for the formula of the compound [II] as quite comparable with the cellulose-copper-amine compound $[(C_6)_2Cu][Cu\,en_2]$.

(b) Chemical formula for the compund [I].

In the same way, the composition of the fibroin-copper-alkali compound derived from the compound [I] is expressed by the following ratios:

$$\text{Fibroin} : Cu : K = 1:1:1.5$$

and

$$\underset{1\quad\ \ 1\quad\ 1}{\text{Fibroin} : Cu : en} \xrightarrow{K} \underset{1\quad\ \ 1\quad\ 1.5}{\text{Fibroin} : Cu : K} \ (en=0).$$

If we assume that the formula of the compound [I] is either $[\text{Fibroin}_2\,Cu][Cu\,en_2]$ or [Fibroin Cu] *en* as mentioned above, then the change of the combining ratios due to the transformation from the compound of fibroin-copper-amine to that of fibroin-copper-alkali may be expressed respectively as follows:

$$\underset{2\qquad\ 2\qquad\ 2}{[\text{Fibroin}_2\,Cu][Cu\,en_2]} \xrightarrow{K} \underset{2\qquad\ 1\quad\ 2}{[\text{Fibroin}_2\,Cu]K_2} \ (en=0)$$

$$\underset{1\qquad\ 1\quad\ 1}{[\text{Fibroin}\,Cu]\,en} \xrightarrow{K} \underset{1\qquad\ 1\quad\ 2}{[\text{Fibroin}\,Cu]K_2} \ (en=0)$$

Comparing these two cases with the experimental results, especially in the points of the changes of Cu and *en* contents, it will be clearly seen that the form of [Fibroin Cu] *en* is much more adaptable for the compound [I].

Summarising these results, the author proposes the following chemical formulae for the compounds [I] and [II]:

[I] Fibroin : Cu : *en* = 1 : 1 : 1 i.e. [Fibroin Cu] *en*

[II] Fibroin : Cu : *en* = 1 : 2 : 2 i.e. [Fibroin Cu] $[Cu\,en_2]$

1) Traube; Ber. 1923, **56** 268, Normann; Ch. Z., 1906, **30** 584, etc.

2), 3) Reference should be made to J. Soc. Chem. Ind. Japan 1933, **36** 1445.

(3)

Mutual relations between the compounds [I] and [II]

[I] [Fibroin Cu] *en*, [II] [Fibroin Cu] [Cu en_2]

Referring to the conditions for the preparation of these two compounds, we know from the beginning of this paper that the compound [I] is isolated from the copper-*en* solution in which the maximum degree of solubility of fibroin is dissolved, and the compound [II], on the other hand, is prepared from the solution in which a given amount of fibroin is dissolved with excessive amounts of copper hydroxide. To be more exact, if they are expressed with the chemically combining equivalent between fibroin and copper, it can be said that the compound [I] or [II] is prepared from the system of fibroin copper-*en* in which the ratio of fibroin to copper is equal to 1 : 1 or 1 : $\geq$ 2 respectively.

Now, if the ratio is lying between the above two cases, then what kind of compounds may be prepared? The author examined this problem after the case of the compounds [I] and [II], isolating the reaction products from the solutions where the ratio of fibroin to copper is lying between 1 : 1 and 1 : $\geq$ 2, and analysing quantitatively the content of the three components, fibroin, copper and *en*.

The results are shown in Table 2.

Table 2.

Composition of solution			Percentages of 3 components			Combining ratios of 3 components		
Fibroin mgE.	Cu mgMol	*en* mgMol	Fibroin %	Cu %	*en* %	Fibroin	: Cu	: *en*
4.00	6.00	22.0	44.32	18.00	15.63	1	1.44	1.37
7.50	9.50	18.0	48.52	16.15	16.12	1	1.19	1.26

These data show clearly that (1) the compositions of these compounds correspond to those lying between the two compounds [I] and [II]; (2) their copper and *en* contents are almost equal to each other; and (3) their compositions approach to that of the compound [I] or [II] as the ratio of fibroin to copper in solution changes from 1 : 1 to 1 : 2 or conversely. From these facts the author assumes that the compounds thus prepared are not new compounds, but rather the mixture of the compounds [I] and [II].

In addition to this, from the fact, as already examined, that the combining ratio of the compound [I] represents the relation between the maximum degree of solubility of fibroin and the smallest amounts of copper and *en*, and that a compound with higher content of copper and *en* than that of the compound [II] has never been found, the author believes that the compounds [I] and [II] correspond respectively to the compound of the lowest and highest combining ratio shown in the system of fibroin-copper-amine, and that there should be the following mutual relations between them:

$$\text{[Fibroin Cu]}\,en \underset{+\text{Fibroin}}{\overset{+\text{Cu},\ en}{\rightleftarrows}} \text{[Fibroin Cu] [Cu}\,en_2\text{]}.$$

Summarising the foregoing together with his previous studies on the phenomena connected with the dissolution of fibroin, the author proposes the following scheme for the chemical equations of these fibroin-copper-amine compounds representing the mode of dissolving fibroin in copper-amine:

Copper-*en* base:

$$\text{Cu(OH)}_2 + 2\,en \longrightarrow [\text{Cu}\,en_2](\text{OH})_2$$

Compound [I]:

$$\text{Fibroin} + [\text{Cu}\,en_2](\text{OH})_2 \rightarrow [\text{Fibroin Cu}]\,en + en + x\text{H}_2\text{O}$$

Compound [II]:

$$\begin{cases} \text{Fibroin} + [\text{Cu}\,en_2](\text{OH})_2 \rightarrow [\text{Fibroin Cu}]\,en + en + x\text{H}_2\text{O} \\ [\text{Fibroin Cu}]\,en + [\text{Cu}\,en_2](\text{OH})_2 \rightarrow [\text{Fibroin Cu}][\text{Cu}\,en_2] + en + x\text{H}_2\text{O} \end{cases}$$

or

$$\text{Fibroin} + 2\,[\text{Cu}\,en_2](\text{OH})_2 \rightarrow [\text{Fibroin Cu}][\text{Cu}en_2] + 2\,en + y\text{H}_2\text{O}$$

A more detailed explanation of these formulae will be reserved for the next chapter.

(4)

On the peculiar phenomena due to the presence of excessive amounts of ethylendiamine in the system of fibroin-copper-amine.

If excessive amounts of ethylendiamine are present in the aqueous solutions of the fibroin-copper-amine compounds i.e. [Fibroin Cu] [Cuen_2] and [Fibroin Cu] *en*, or in the solutions from which these compounds are prepared, there take place some abnormal changes in the properties of the solutions, such as changes in the colouration, in the electrical conductivity, and in the optical rotating power. These changes can be similarly observed in the system of fibroin-copper-ammonia, which has an intimate relation with the system of fibroin-copper-ethylendiamine mentioned above, but they can hardly be noticed when only ammonia, even in excess, is added instead of ethylendiamine. Therefore we can assume that these changes are caused by the presence of such a special amine as ethylendiamine viz. a amine which takes a particular relation with copper. These property changes will be discussed in the order mentioned.

(a) *The change in colouration of the solution.*

When excessive amounts of *en* are added to the solution of fibroin-copper-amine compound, the colour of the solution changes gradually as follows:

reddish violet→purplish blue→
pure blue→greenish blue.

That is to say, colour of reddish violet, the characteristic of the fibroin solution due to the biuret colour reaction, loses its feature according to the amounts of *en* added, and at last turns to greenish blue, showing the same colour as the copper-amine solution in which fibroin is not dissolved.

To express these colour changes more exactly, the extinction coefficients for these solutions were compared. The estimation of coefficients was done by Nutting's spectrophotometer under the definite light such as yellow line, wave length $=574\,\mu\mu$, selected especially for observing these bluish coloured solutions.

Preceeding the comparison of the extinction coefficients it was necessary to determine whether or not the fibroin-copper-amine compound suffered any changes in its constitution when the concentration of the solution was very diluted. The results of this investigation are as follows:

Table 3.

1) [Fibroin Cu] *en* solution:

Mol/L	1/250	1/500	1/1000	1/2000	mean
Molar extinction coeff. (yellow line)	76.5	78.5	77.0	78 5	78.6

2) [Fibroin Cu] [Cu en_2] solution:

Mol/L	1/250	1/500	1/1000	1/2000	mean
Molar extinction coeff. (yellow line)	72.5	72.5	73.0	74.0	73.0

Namely, within the limits of the concentration as shown above the relation between the concentration of the solution and the extinction coefficient thus examined is wholly conformable to Beer-Lambert's law, i.e.

E=K.c.d. E: Molar extinctinction coeff.
c: concentration of solution.
d: depth of solution.

Therefore it is clear that there takes place no change in the constitution of the compound when its concentration is diluted.

Then, the extinction coefficient for each of the solutions of the compounds with an increasing amount of *en* was estimated and compared with that of $[Cu\,en_2](OH)_2$ compound. The results are tabulated in.

Table 4.

(1) [Fibroin Cu] *en* solution 1/500 Mol/L.

Amounts of *en* added Mol/L	0	1/200	1/100	1/80	1/40
Molar extinction coeff.	77.8	83.5	94.5	101.0	101.5

(2) [Fibroin Cu] [Cu en_2] solution 1/1000 Mol/L.

Amounts of *en* added Mol/L	0	1/200	1/100	1/80	1/40
Molar extinction coeff.	75.5	—	92.0	100.2	99.5

(3) $[Cu\,en_2](OH)_2$ solution 1/500 Mol+1/40 Mol *en*/L

Molar extinction coeff. 101.0

As can be seen from the table the extinction coefficient examined especially under a yellow line increases as excessive amounts of *en* are added until it reaches the same value as that of copper-*en*. solution. Therefore the change of coefficient accounts for the change in the colour of the solution.

From these results together with the well known facts that the coincident of extinction coefficients of any two substances means the presence of the same or a similar chemical constitution between them, it may be also assumed that the constitution of the fibroin-copper-amine compound will change either to an identical or to a similar form as that of the copper-*en* compound, as it is influenced by the presence of *en* in large excess.

(*b*) *The change in electrical conductivity of the solution.*

When excessive amounts of *en* are added to the solution of fibroin-copper-amine compound, the electrical conductivity of the solution is extraordinarily increased in spite of the fact that *en* is a very weak base with a small conductivity.

This change of conductivity, estimated by means of Kohlrausch's method, was compared with that of copper-*en* solution, i.e. $[Cu\,en_2](OH)_2$ or *en* solution itself. In these experiments the concentration of copper in each solution was kept stable. The results are shown in Table 5 and Fig. 1.

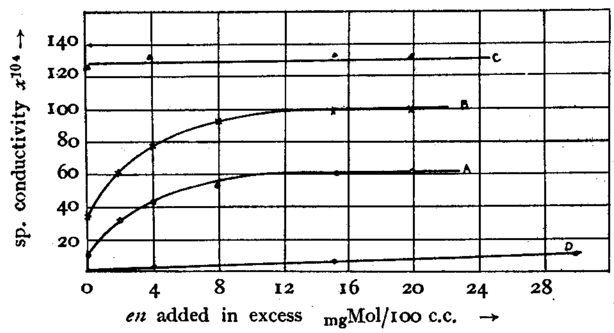
Table 5.

A: 4 mg Mol [Fibroin Cu] *en* /100 c.c.
B: 2 mg Mol [Fibroin Cu] [Cu en_2] /100 c.c.
C: 4 mg Mol $[Cu\,en_2](OH)_2$ /100 c.c.
D: 4—30 mg Mol *en* /100 c.c.

Excessive amounts of *en* added mgMol/100 c.c.		0	2.00	4.00	8.00	15.26	19.68
Specific conductivity $\varkappa^{10^4}$ (at 20°C)	A	12.50	35.62	44.48	51.45	60.19	60.30
	B	32.90	60.95	78.83	93.40	96.40	99.83
	C	125.06	—	133.61	—	136.91	130.05

D. *en* mg Mol/100 c.c.	4.00	15.00	30.00
$\varkappa^{10^4}$ (at 20°C)	3.88	6.48	9.72

Fig. 1.



In these results we find that there is a remarkable difference between the degrees of conductivity of the solutions of $[Cu\,en_2](OH)_2$ and *en*. For example, the degree of basicity of $[Cu\,en_2](OH)_2$ compound is much greater than that of *en*, and the degree of conductivity of the solution of [u en_2] $(OH)_2$, a strong base, is practically unaffected by the coexistence of a very weak base such as *en* in large excess. But contrary to this, it will be found that there takes place a peculiar change in the case of the solution A or B, i.e. [Fibroin Cu] *en* or [Fibroin Cu] [Cu en_2] respectively, a change that is due to the coexistence of *en* in large excess.

From these results, it is safe to assume that *en* thus added in excess exists not as a free amine itself, but rather reacts with the fibroin-copper-amine compound to produce a substance of higher conductivity.

(*c*) *The change in the optical rotating property of the solution.*

The fact that the fibroin-copper-amine solution has a strong optical rotating power and that this power depends on the concentration of both fibroin and copper in the solution remains to be demonstrated. According to Hess and Messmer's well known method[1] the measurement of the optical rotating power was made under a monochromatic light, wave length=436.9 $\mu\mu$, by using a half shadow polarimeter (Haensch and Schmidt). In order to aviod the influence of racemisation commonly observed in an alkaline solution of protein, measurements were taken within the lapse of 4 or 5 hours after the preparation of the fibroin-copper-amine solution. The experimental errors were $\pm 0.03°$. The results are shown in Table 6 and Fig. 2.

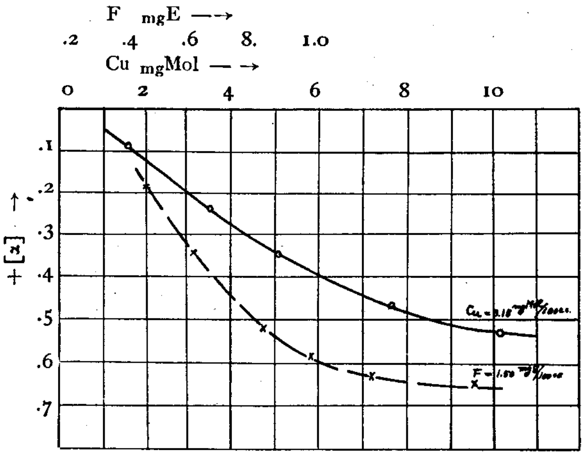
Table 6.

Conc. of fibroin mg E/100 c.c.	Conc. of Cu mg Mol/100 c.c.	Conc. of NH_3 mg Mol/100 c.c.	Angle of ratation $+\alpha$
1.50	2.00	ca 916.0	0.18°
1.50	3.25	916.0	0.40
1.50	4.50	916.0	0.53
1.50	5.75	916.0	0.62
1.50	7.00	916.0	0.61
0.33	3.15	916.0	0.09
0.66	3.15	916.0	9.23
1.00	3.15	916.0	0.34
1.50	3.15	916.0	0.46
2.00	3.15	916.0	0.52

(length of tube. 5 cm, room temp. 9~11°C)

Thus the optical rotating power depends on the concentration of both fibroin and copper in the solution, and their relations will be expressed by a continuous curve without any break such as a maximum or minimum point. Therefore, from these facts together with analogies in the actions of copper-amine solution toward cellulose and fibroin, which had been already appointed by the author, it may be safe to assume that the optical activity in this case is chiefly due to the formation of a complex fibroin-copper-amine compound as

Fig. 2.



in the case of cellulose.

Now, omitting further details of the investigation into the proper nature of optical activity, we will examine what change may be brought about in the optical nature of the solution of fibroin-copper-amine compound to which has been added excessive amounts of *en*.

The results are shown in Table 7 & Fig. 3.

Table 7.

1) Fibroin-Cu-*en* solution.

A { Fibroin 1.50 mg E; Cu 4.00 mg Mol; *en* 10.00 mg Mol

No. of solution	*en* added in excess mg Mol	angle of ratation $+\alpha$
A	0	0.29°
B	5.00	0.19
C	10.00	0.12
D	20.00	0.10

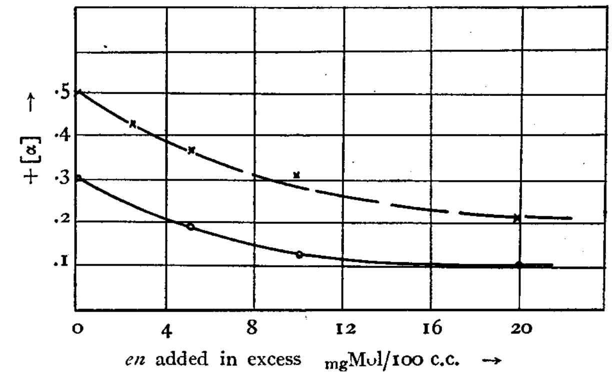
2) Fibroin-Cu-NH_3 solution.

B { Fibroin 1.50 mg E; Cu 4.00 mg Mol; NH_3 ca. 916 mg Mol

No. of solution	*en* added in excess mg Mol	angle of rotation $+\alpha$
A	0	0.50°
B	2.50	0.42
C	5.00	0.35
D	10.00	0.32
E	20.00	0.18

(length of tube. 5 cm., room temp. 9~10°C)

Fig. 3.



× Fibroin-Cu-NH_3
o Fibroin-Cu-*en*

1) Hess & Messmer; Ann., 1923, **435** 7.

That is, as the amounts of *en* is increased, the rotating power of the solution is diminished, in spite of the fact that *en* itself has no optical activity, this diminution of power being shown by a continuous curve.

From these facts together with the knowledge that the rotating nature is chiefly due to the formation of a complex fibroin-copper-amine compound as mentioned above, it can safely be stated that *en* thus added in excess has a destructive effect upon the fibroin-copper-amine compound.

* * *

The peculiar phenomena mentioned above are caused only by the presence of a special amine such as *en*, because they have never been observed in the case of another amine such as NH_3, even when NH_3 is present in greater excess. Therefore, to make clear how such phenomena should arise, we must first take into consideration the singularity of the reaction between copper and *en*. Referring to the actions between copper and *en*, Traube[1] reported that in an aqueous solution of copper-*en* which is prepared by saturating an *en* solution with copper hydroxide, (1 molecule of copper hydroxide reacts with 2 moleenles of *en*) a complex copper-*en* compound with the following composition is formed;

$$Cu\,(OH)_2 + 2\,en \rightarrow [Cu\,en_2]\,(OH)_2.$$

Comparing this with the case of system Cu-NH_3, the case in which at least 50-60 molecules of NH_3 are required to dissolve 1 molecule of copper hydroxide and there coexist few compounds such as $[Cu\,(NH_3)_4]\,(OH)_2$, $[Cu\,(NH_3)_2]\,(OH)_2$ and so on, according to the concentration of NH_3, as shown by Bonsdorff[2] and Hantsch[3], we may assume that $[Cu\,en_2]$ complex is much more stable than $[Cu\,(NH_3)_4]$ complex, and also that the chemical affinity of *en* to Cu is much greater than that of NH_3 to Cu.

Considering these singularities together with the peculiar phenomena mentioned above, it must be concluded that *en* added in large excess to the solution of fibroin-copper-amine does not exist as a free amine along with the fibroin-copper-amine compound, but rather it reacts immediately with the compound because of its strong affinity to copper, depriving the latter of some of the combined copper and thus forming a stable $[Cu\,en_2]$ complex with a strong basic nature. Thus the solulion comes to show much higher conductivity. In a word, under such conditions *en* will have a destructive effect upon the fibroin-copper-amine compound. Similar phenomena can be seen in the case in which fibroin is dissolved into a copper-*en* solution. If there are excessive amounts of *en* in the solution of copper-*en*, not only is the rate of dissolution of fibroin retarded, but also the solubility of fibroin is diminished till it becomes almost zero.

At this point the equatious mentioned in the preceding section as showing the formation of the fibroin-copper-amine compounds should be explained more fully.

$$[I]\quad \text{Fibroin} + [Cu\,en_2]\,(OH)_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + xH_2O$$

$$[II]\begin{cases} \text{Fibroin} + [Cu\,en_2]\,(OH)_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + xH_2O \\ [\text{Fibroin Cu}]\,en + [Cu\,en_2]\,(OH)_2 \rightleftarrows [\text{Fibroin Cu}]\,[Cu\,en_2] + en + xH_2O \end{cases}$$

The arrow pointing to the right means the formation of the compounds, whereas the arrow pointing to the left shows the decomposition of the compounds in return.

Now, if fibroin is put into the solution of copper-*en* with excessive amounts of *en*, it will be reasonably understood from the law of mass action that it is difficult to turn the equilibrium from left to right. This indicates the retardation in dissolution of fibroin—diminution in degree of solubility of fibroin.

On the other hand, if excessive amounts of *en* are added to the solution of the fibroin-copper-amine compound, the equilibrium will be steadily shifted from right to left according to the amounts of *en* added. This brings about the decomposition of the fibroin-copper-amine compound, thus accounting for the colour change, the extraordinary increase of conductivity, and the peculiar diminution of optical rotating power.

Thus, the author concludes that these peculiar phenomena are chiefly attributable to the presence of *en* in excess and the singularity shown in the reaction of copper and *en* thus added.*

Chapter II. On the mechanism of the dissolution of fibroin in copper-amine solution.

(1) Kinetic equation for the dissolution of fibroin.

In this section, the author deals with the equation by which the rate of dissolution of fibroin may be expressed kinetically and also with the manner in which the dissolution is carried out.

1) Traube; Ber. 1911, **31** 19.
2) Bonsdorff; Z. f. angew. Chem. 1900, **47** 147.
3) Hantzsch; Ber. 1908, **41** 4328.
*) Appendix:
These peculiar phenomena are found not only in the system of fibroin-copper-amine, but also in the systems of cellulose biuret-, glycerin-, and sugar-copper-amine, which have relations similar to the case of fibroin. Especially, the author has carried out investigations in the case of cellulose, the conclusions of which will be published in the near future.

In his experiments he adopted as the condition of dissolution of fibroin the case in which fibroin is completely dissolved in a solution of $Cu(OH)_2-NH_3$ or $Cu(OH)_2-en$ with a definite copper content, where the relation of fibroin to copper is that of 1 to 1 (chemical equivalent: Fibroin : Cu = 227 : 63.6), without shaking lest the fibroin be dissolved too quickly. He determined the rate of dissolution of fibroin and also the composition of the solution by the same method as shown in the previous paper. Some of the results are shown in Table 8 and Fig. 4.

Table 8.

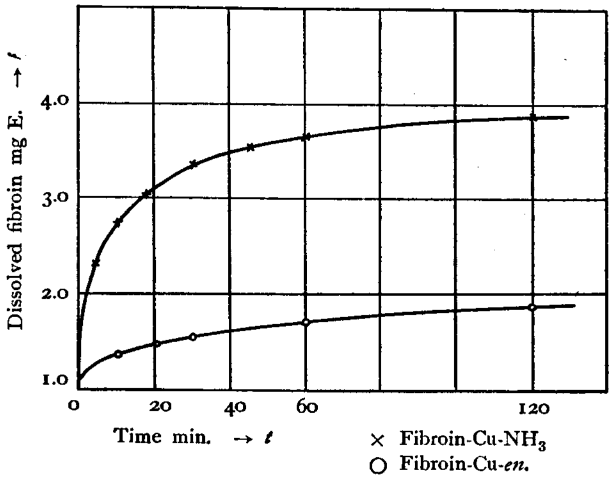
1) System of fibroin-copper-ammonia. (Fig. 4)
Fibroin 5 mg E, $Cu(OH)_2$ 5 mg Mol and NH_3 ca. 500 mg Mol per 150 cc. Temp. 25°C

Time	Composition of solution		Combining ratio of copper to fibroin in solid phase	Solubility of fibroin
min.	Fibroin mg E.	Cu mg Mol	Cu/F	%
5	2.29	4.53	0.17	45.8
10	2.72	4.11	0.39	54.4
18	3.02	3.93	0.47	60.4
30	3.38	4.16	0.52	66.2
45	3.51	4.19	0.54	70.2
60	3.66	4.30	0.53	73.2
120	3.90	4.35	0.59	78.0

2) System of fibroin-copper-*en*.
Fibroin 5 mg E., $Cu(OH)_2$ 5 mg Mol and *en* 8.54 mg Mol per 100 cc. Temp. 25°C.

Time	Composition of solution		Combining ratio of copper to fibroin in solid phase	Solubility of fibroin
min.	Fibroin mg E.	Cu mg mol	Cu/F	%
10	1.39	2.87	0.43	34.8
20	1.44	2.78	0.48	36.0
30	1.55	2.80	0.49	38.8
60	1.72	2.97	0.45	43.0
120	1.89	—	—	47.3
360	2.15	3.10	0.50	53.8
960	2.30	3.20	0.48	57.5

Fig. 4.



As can be clearly seen from the figure, as soon as fibroin is put into the solution, the reagent penetrates into fibroin with the result that a rapid decrease in the concentration of copper in the solution takes place owing to the mutual reaction between fibroin and copper-amine. The combining ratio of copper to fibroin in the solid phase reaches a constant value in a short while; whereas the rate of the dissolution of fibroin is swift at the start but becomes gradually slower, describing a parabolic curve. The concentration of copper in the solution increases again with the dissolution of fibroin-copper-compound, showing a minimum point in the figure. From these facts the manner in which the dissolution of fibroin is carried out may be fairly conjectured.

In order to express more exactly the rate of dissolution, the author tried to apply the following equation[1]:

$$x = k\,t^n \quad \ldots\ldots\ldots\ldots \quad (1)$$

where, x is dissolved amounts or solubility of fibroin in time t, and k and n are constant.

With the logarithms, this equation (1) becomes

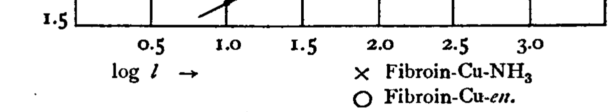
$$\log x = \log k + n \log t \quad \ldots\ldots\ldots\ldots \quad (2)$$

If the rate of the dissolution of fibroin satisfies the equation (2), then the plot of $\log x - \log t$ must be a straight line. As shown in Fig. 5, the observed values of x (solubility of fibroin in %) and t (time in min.) satisfy these relations.

The constant n can be determined from the figure, and the mean value of the constant k is calculated from x, t observed and n thus determined.

Thus the solubility of fibroin calculated conversely from these results, is recorded in Table 9.

Fig. 5.



As can be seen from the table, the calculated values of solubility of fibroin agree well with the observed values. Therefore, we can ascertain that the rate of dissolution of fibroin may be kinetically expressed by the equation:

$$x = k\,t^n,$$

where x is the solubility of fibroin at time t, and

1) c.f. Robertson; J. Physik. Chem. 1910, 14 377.

k and *n* are constant.

Table 9.

1) System of fibroin-Cu-NH_3.

n=0.140

t min.	*x* (obs.) %	*k*	*x* (calc.) %
5	45.8	—	—
10	54.4	39.0	55.5
18	60.4	39.7	60 5
30	66.2	40.4	65.2
45	70.2	40.4	69.1
60	73.2	40.4	72.1
120	78.0	39.0	79.7
	mean	39.8	

2) System of fibroin-Cu-*en*.

n=0.125

t min.	*x* (obs.) %	*k*	*x* (calc.) %
10	34.8	26.1	34.4
20	36.0	24.8	37.5
30	38.8	25.3	39.4
60	43.0	25.8	43.0
120	47.3	26.0	47.0
360	53.8	25.8	53.8
960	57.5	—	—
	mean	25.8	

Now, it is of interest to compare this equation with equations already found for related phenomena:

(1) From his study of diffusion of liquids into a filter paper, Ostwald[1] found that the rate of diffusion is kinetically expressed by the following equation:

$$l=k\,t^n$$

where, l is the distance of diffusion at time t, and k and n are constant.

(2) As the equation for the chemical reaction which follow from the diffusion of reagents, a similar equation has been proposed. For example, Sakurada[2] arrived at the following equation in his studies on acetylation and nitration of cellulose:

$$x=k\,t^n$$

where, x is the degree of acetylation or nitration at time t, and k and n are constant.

(3) For the kinetical equation of extraction of reaction product, Robertson[3] found that the rate of extraction of protamin salmon by hydrochloric acid is also expressed by the equation:

$$x=k\,t^n$$

where, x is the amounts of salmin hydochloride thus extracted at time t, and k and n are constant.

In each of these above equations the constant n has a numerical value of the same order, i.e. 0.15~0.50.

Examining the expression in the general equation of $x=k\,t^n$ and considering it with reference to the dissolution phenomena of fibroin, we may conclude that the mechanism of dissolution of fibroin is as follows:

The dissolution of fibroin can be said to be a continual sequence of reactions in four stages, which occur stepwise one after another, namely: (1) penetration and diffusion of the copper-amine solution into the structure of silk fibroin; (2) formation of the fibroin-copper-amine compound caused by their mutual reactions; (3) swelling of fibre structure followed by solvation of the complex metallic compound; and (4) the dispersion of the solvated compound into solution, the fibre structure being destroyed by increasing swelling. Thus the dissolution of fibroin may be completed by a series of chain reactions such as diffusion, chemical reaction, swelling, and dispersion, where the fibroin-copper-amine compounds have definite compositions such as [Fibroin Cu] [Cn en_2] and [Fibroin Cu] *en*. Moreover, the reaction velocity at these stages can without exception be commonly expressed by the general equation:

$$x=k\,t^n$$

From what has been mentioned, it will be clearly seen that the kinetic equation of the dissolution of fibroin $x=k\,t^n$ should cover not only the rate of dissolution of fibroin, but also the process of chain reactions. It is clear also how important a rôle these modes of diffusion and chemical reaction play during the progress of this dissolution, so that the mechanism of this complex phenomenon of the dissolution of fibroin is elucidated only by the close investigation of these intermediate reactions. This elucidation is well in accord with the case of the system of cellulose-copper-amine[4] in that both contain complex metallic compounds with definite compositions.

To prove that a definite compound may be chemically produced by the mutual reactions at the dissolution of a substance, we can quote here many studies: For example, at the dissolution of cellulose in an organic solvent or a solution of neutral salt, the presence of a compound has been well recognised by study of X-ray analysis together with chemical means.[5] Also as for the dissolution of protein, Sörensen[6] reported that casein is dissolved into an alkaline or acidic solution in the form of a compound formed by mutual reactions between casein and alkali or acid.

As for silk fibroin, the object of this present study, Weimarn[7] proposed that at the dissolution of fibroin in a solution of neutral salt there may exist a certain compound with the following composition, though its composition has never yet been ascertained:

1) Ostwald; Koll. Z. 1908, **20** 2.
2) Sakurada; J. Soc. Chem Ind. Japan 1932, **35** 377, 746.
3) Robertson; J. Biol. Chem. 1916, **25** 351.
4) Hess, Trogus etc.; Z. f. phys. chem. 1929, **145** 401 etc.
5) Hess; Ber. 1931, **351** 374, Katz; Rec. Trav. Chim. 1931, **50** 149.
6) Sörensen; Kollz. 1929, **46** 16.
4) Weimarn; Kollz. 1923, **29** 198, 1928, **46** 40.

$$[D.\ a\,HOH]_c\ [F.\ b\,H\,OH]_d,$$

where the hydrated molecule of salt-dispergator is designated as [D. a H OH], and the hydrated melecule of fibroin as [F. b H OH].

Supplementing these studies, the results of the author's studies mentioned above enable one to ascertain more exactly the presence of chemical reactions at the dissolution of fibroin in copper-amine solution. That is to say, we can here well recognise that the dissolution of fibroin is accompanied by the formation of a reaction product with a definite composition and also by the definite chemical ratios of their reacting components in connection with the high hydrophilic nature of protein.

(2) The modes of dissolution and chemical reaction in the system of fibroin-copper-amine.

The mode of dissolution and chemical reaction in the system of fibroin-copper-amine, where the amine is ethylendiamine, will now be discussed.

(a) chemical equations for the complex fibroin-copper-amine compounds.

$$Cu(OH)_2 + 2\,en \rightleftarrows [Cu\,en_2](OH)_2 \quad \cdots\cdots\cdots (1)$$

[Fibroin Cu] *en* compound:

$$\text{Fibroin} + [Cu\,en_2](OH)_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + xH_2O \quad \cdots\cdots (2)$$

[Fibroin Cu] [Cu *en*$_2$] compound:

$$\left\{\begin{array}{l} \text{Fibroin} + [Cu\,en_2](OH)_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + xH_2O \cdots\cdots \\ [\text{Fibroin Cu}]\,en + [Cu\,en_2](OH)_2 \rightleftarrows [\text{Fibroin Cu}][Cu\,en_2] + en + xH_2O \end{array}\right. \quad (3)$$

or

$$\text{Fibroin} + 2\,[Cu\,en_2](OH)_2 \rightleftarrows [\text{Fibroin Cu}][Cu\,en_2] + 2\,en + yH_2O$$

and,

$$[\text{Fibroin Cu}]\,en \underset{+\text{Fibroin}}{\overset{+\text{Cu},\,en}{\rightleftarrows}} [\text{Fibroin Cu}][Cu\,en_2].$$

In the equations the fact that ethylendiamine is set free in solution with the formation of the complex fibroin-copper-amine compound may be testified by the fact that the amount of copper hydroxide corresponding to that of this *en* is newly brought into the solution according to the equation (1), And also these schemes make it clear that the solubility of fibroin in copper-amine solution is increased by the mutual dissolution of fibroin and copper.

That is to say, if fibroin is put into a solution of copper-amine solution in the presence of large excessive amounts of copper hydroxide in the solid phase, fibroin begins to dissolve according to the equations (2), (3) at the same time that amine is set free in the solution. This amine reacts further with copper hydroxide in the solid phase, forming a copper-amine compound as shown above. Thus, more fibroin is newly brought into solution and consequent'y the solubility of fibroin is much increased as was suggested by the author in his first report.

Thus we are ready to propose that the mode of dissolution of fibroin accords with these chemical equations of the complex fibroin-copper-amine compounds.

(b) Modes of dissolution and chemical reaction in the system of fibroin-copper-amine.

The modes of dissolution and chemical reaction in the system of fibroin-copper-amine can be denoted by the chemical equivalent of fibroin to copper in the following formulae:

In the solution of [Cu *en*$_2$] $(OH)_2$ base;

(i) Fibroin : Cu = 1 : > 2,

This is the case where a given amount of fibroin is put into solution of high concentration. The fibroin is dissolved in the form of the compound [Fibroin Cu] [Cu *en*$_2$], the compound with the highest contents of copper and amine, and there exists an equilibrium between the compound and the Cu-*en* base in excess.

(ii) Fibroin : Cu = 1 : 2,

where the Fibroin is dissolved in the form of the compound [Fibroin Cu] [Cu *en*$_2$].

(iii) Fibroin : Cu = 1 : 1~2,

where the Fibroin is dissolved in the form of the compound either [Fibroin Cu] [Cu *en*$_2$] or [Fibroin Cu] *en* according to the amounts of copper used, and in the following relation:

$$[\text{Fibroin Cu}]\,en \underset{+\text{Fibroin}}{\overset{+\text{Cu},\,en}{\rightleftarrows}} [\text{Fibroin Cu}][Cu\,en_2].$$

(iv) Fibroin : Cu = 1 : 1,

where the Fibroin is dissolved in the form of the compound [Fibroin Cu] *en*, the case where the maximum degree of solubility may be required.

In these four cases the fibroin is completely dissolved in the solution.

(v) Fibroin : Cu = 1 : < 1,

This is the case where a large amount of fibroin is put into a solution of a given concentration. The dissolution phenomena in this case are very abnormal, and are comparable to those usually observed in the dissolution of colloidal substanees in which the solubilities are greatly affected by the amounts of the substances used. As for the chemical reactions in these cases, the followings are given under detinite conditions:

In solution Fibroin : Cu = 1 : 1

In solid phase Fibroin : Cu = 2 : 1

Summary.

(1) The chemical nature of the fibroin copper-amine compounds with the following two kinds of compositions were studied:

Fibroin : Cu : *en* = 1 : 1 : 1 [I]

Fibroin : Cu : *en* = 1 : 2 : 2 [II]

en = ethylendiamine

(2) As for the chemical formulae of these two compounds, the following forms were proposed as being adaptable for expressing their combining states;

[I] [Fibroin Cu] *en*,

[II] [Fibroin Cu] [Cu en_2]

(3) In the formation and the mutual reaction of these two compounds, the following reactions were recognised:

$$[\text{Fibroin Cu}]\,en \underset{+\text{Fibroin}}{\overset{+\text{Cu},\ en}{\rightleftarrows}} [\text{Fibroin Cu}][\text{Cu}\,en_2]$$

(4) Abnormal phenomena was found to result from the presence of large excessive amounts of special amine, such as ethylendiamine, i.e.:

i) a retardation of the rate of dissolution of fibroin, and a decrease of solubility of fibroin in a copper-amine solution;

ii) peculiar changes, such as a change of coloration, a decrease of electrical conductivity, a decrease of optical activity of the solution, etc, when excessive amounts of *en* are present in the solution of fibroin-copper-amine. These abnormal phenomena were found to be chiefly attributable to the amount of *en* excess of that required to form the fibroin-copper-amine compounds, and also to the singularity in the mutual reactions between copper and *en*.

(5) The rate of dissolution of fibroin in copper-amine solution was kinetically expressed by the following equation:

$$x = k\,t^n$$

where, x is the dissolved amounts of fibroin at time t, and k and n are constant; and the mechanism of dissolution of fibroin in copper-amine solution was discussed.

(6) As for the chemical reaction in the dissolution of fibroin in the system of fibroin-copper-amine, the following equations were proposed:

$$\text{Cu(OH)}_2 + 2\,en \rightleftarrows [\text{Cu}\,en_2](\text{OH})_2$$

[Fibroin Cu] *en* compound:

$$\text{Fibroin} + [\text{Cu}\,en_2](\text{OH})_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + x\text{H}_2\text{O}$$

[Fibroin Cu] [Cu en_2] compound:

$$\begin{cases} \text{Fibroin} + [\text{Cu}\,en_2](\text{OH})_2 \rightleftarrows [\text{Fibroin Cu}]\,en + en + x\text{H}_2\text{O} \\ [\text{Fibroin Cu}]\,en + [\text{Cu}\,en_2](\text{OH})_2 \rightleftarrows [\text{Fibroin Cu}][\text{Cu}\,en_2] + en + x\text{H}_2\text{O} \end{cases}$$

or

$$\text{Fibroin} + 2\,[\text{Cu}\,en_2](\text{OH})_2 \rightleftarrows [\text{Fibroin Cu}][\text{Cu}\,en_2] + 2\,en + y\text{H}_2\text{O}$$

(7) The modes of the chemical reaction accompanying the dissolution in the system of fibroin-copper-amine were systematically explained.

Acknowledgment.

The author wishes to express his thanks to former Prof. Ikuzo Fukushima, College of Engineering of Kyoto Imperial University, for his valuable advice and helpful criticism.