The Action of alkaline Copper-solution on Silk-fibroin.

By

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Among the various actions of alkaline copper solutions on fibrous materials, the chemical behaviour of an ammoniacal solution of copper oxide towards cellulose has been recently studied by K. Hess[1] and W. Traube,[2] and their work has rendered great service to the study of the chemistry of cellulose.

According to them, cellulose reacts with this reagent, forming a complex cellulose-copper-amine compound and in this form it is presumed to be dissolved.

Animal fibers, especially silk fibers, are known to be soluble in this solution and also to be characterised by the biurett colour reaction, but the scientific researches in this field are much more scanty than those on cellulose.

We have studied this subject and discussed the phenomena connected with the resolution of silk fibroin in alkaline copper-solution and the mechanism of the chemical reactions in this system.

In the present paper, we deal with the chemical behaviour of alkaline copper-solutions towards silk fibroin.

(A) PHENOMENA CONNECTED WITH THE DISSOLVING OF SILK FIBROIN IN SCHWEIZER'S SOLUTION.

Copper hydroxide dissolved in an aqueous solution of ammonia gives a blue coloured solution, called Schweizer's solution.

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1) Hess; Ber. 1921, **54** 834, Ann. 1923, **435** 7,
" 1922, **55** 2432, Z. f. Phys. Chem. 1927, **126** 369.

2) Traube; Ber. 1922, **55** 1899, Ber. 1927, **60** 43,
" 1922, **56** 2444, " 1930, **63** 2083. etc.

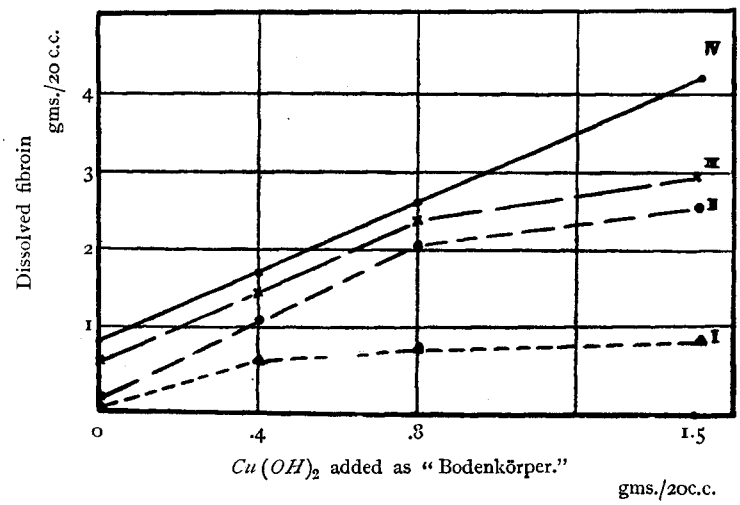
According to Bonsdorf[1] the solubility of the copper hydroxide in Schweizer's solution is not constant, but rather depends on the method of the preparation of the copper hydroxide. Referring to its amount, Traube[2] reported that 50–60 molecular weights of ammonia were required to dissolve only one molecular weight of copper hydroxide.

When silk fibroin is put into such Schweizer's solution, its solubility is only small and hitherto it has been difficult to get a concentrated solution of fibroin. But we have found that not only can the solubility of fibroin be increased by adding copper hydroxide as "Bodenkörper" to this solution, but also the copper content of this solution can be much enlarged.

In our experiments, scoured and defatted silk wadding was used for silk fibroin and Schweizer's solution was prepared by saturating an aqueous solution of ammonia in certain concentrations with copper hydroxide.

Fig. 1. shows the relations between the degree of solubility of fibroin and the amount of copper hydroxide which was added as "Bodenkörper" to such Schweizer's solutions of the following composition:

Fig. 1.



1) Bonsdorf; Z. f. angew. Chem. 1904, 41 184.
2) Traube; Ber. 1911, 3319.

(1) 20. gms. NH_3 / 2.28 gms. Cu / L. (2) 50. gms. NH_3 / 4.07 gms. Cu / L. (3) 100. gms. NH_3 / 8.31 gms. Cu / L.

(4) 210. gms. NH_3 / 13.61 gms. Cu / L.

From this figure it is seen that the solubility of fibroin depends on the concentration of Schweizer's solution and in the case of the same concentration, its solubility is much increased by the presence of copper hydroxide as "Bodenkörper" and there are definite relations among them.

For example, the solubility of fibroin in concentrated Schweizer's solution, Composition. (4), is only 0.799gms./20c.c., but by adding 0.4, 0.8, and 1.5gms. of copper hydroxide in order per 20c.c. of this solution, the solubility of the fibroin is increased to 1.741, 2.548, and 4.054gms./20cc. respectively.

Thus the increase in the solubility of the fibroin goes parallel with the amount of copper hydroxide added as "Bodenkörper", and the latter is also brought into solution.

In the case of other Schweizer's solutions, a similar phenomenon can be seen, but if the quantity of ammonia is small and copper hydroxide is added in large excess, there is no such parallel increase in the solubility of fibroin, and a large amount of copper hydroxide remains undissolved in the solution. Thus, it will be seen that the phenomenon of the increase in the solubility of fibroin due to the addition of copper hydroxide is caused by the mutual resolution of fibroin and copper conditioned by the moderate concentration of ammonia in the solution.

Table 2. shows an example of the increase in the solubility of copper hydroxide (in 20c.c. of 21% ammoniacal solution) brought about by such mutual resolution of fibroin and copper.

Table 2.

Fibroin, dissolved	gms./20 c.c. 0	0.5105	1.0176	2.0040
$Cu(OH)_2$ content	gms./20 c.c. 0.4144	0.8504	1.1116	1.7252

From the results given above we have found that the solubility of fibroin in Schweizer's solution depends on the copper content in the system

and the latter also increases owing to the dissolving of fibroin. Thus, in the system of fibroin and ammoniacal solution of copper oxide the phenomenon of the mutual resolution of fibroin and copper is progressive.

Traube,[1] Linkmeyer[2] and Langhans[3] found such a phenomenon in the system of cellulose and ammoniacal solution of copper oxide and this has now been applied in the preparation of "copper silk".

According to Traube, these phenomena are represented as follows:

$$Cu(OH)_2 + 4NH_3 \rightleftarrows [Cu(NH_3)_4](OH)_2 \quad (1)$$

$$2(C_6H_{10}O_5) + 2[Cu(NH_3)_4](OH)_2 \rightleftarrows [(C_6H_8O_5)_2Cu][Cu(NH_3)_4] + 4NH_3 + 4H_2O \quad (2)$$

That is to say, when cellulose is subjected to the same treatment, cellulose is dissolved in the form of a complex cellulose-copper-amine compound as in Eq. (2) and at the same time ammonia is set free in solution. This ammonia reacts with copper hydroxide, if it exists in excess in the system as "Bodenkörper," forming again a copper-amine base according to Eq. (1). Thus, more cellulose is newly brought into solution and consequently the solubility of cellulose is much increased.

(B) CHEMICAL REACTIONS BETWEEN FIBROIN AND ALKALINE COPPER-SOLUTIONS.

With regard to the chemical reactions of alkaline copper-solutions toward silk fibroin we know the following facts in connection with their resolution phenomena.

In the previous chapter, it was seen that the mode of resolution of fibroin in ammoniacal solution of copper oxide resembled to the case of cellulose, in which cellulose formed a complex compound; (Cellulose Cu) $[Cu(NH_3)_4]$.

More over the fibroin in an alkaline copper-solution, like other proteins of this kind, is characterised by the biurett colour reaction, which has been studied by many chemists. More particularly, Traube[4] has found

1) Traube; Ber. 1922 **55**, 1899.
2) Linkmeyer; Franz. P. Nr. 346722.
3) Langhans; D. R. P. Nr. 140247.
4) Traube; Ber. 1927, **60** 43.

recently that it is attributable to the chemical combination between copper, alkali and biurett, and has also acertained the existence of a complex compound with the experimental formula [Biurett Cu][$Cu(NH_3)_4$], which is similar to the case of cellulose.

Thus, we may consider that so far as the reactions are concerned there is something in common between cellulose, biurett and silk fibroin. But we have no accurate knowledge with regard to silk, such as its chemical constitution and molecular weight, and so, as the first step in the study of this subject, we tried to estimate the chemical equivalent of fibroin to copper and examine the composition of the reaction product which was isolated from the solution.

1) CHEMICAL EQUIVALENT OF FIBROIN TO COPPER.

For the estimation of the chemical equivalent of fibroin to copper, we adopted Traube's method[1] and examined it in a copper oxide-ethylendiamine solution.

According to Traube, in an aqueous solution of copper oxide-ethylendiamine, which is prepared by saturating an ethylendiamine solution with copper hydroxide, I molecule of copper hydroxide reacts with exactly 2 molecules of ethylendiamine, leaving no free ethylendiamine in solution.

The reaction is;

$$Cu(OH)_2 + 2(H_2NCH_2CH_2NH_2) = [Cu(H_2NCH_2CH_2NH_2)_2](OH)_2.$$

We applied this property in our cases of fibroin. So, if the copper content in this solution is increased by fibroin, being dissolved we cannot but interpret it as due to the fact that this increased amount of copper has been consumed in the chemical combination with fibroin. Therefore, by estimating this increase of copper, we can conversely calculate the chemical equivalent of fibroin to copper.

In our experiments, we estimated, on the one hand, the copper content of a copper oxide-ethylendiamine solution which was prepared by saturating ethylendiamine solutions of different concentrations with cop-

1) Traube; Ber. 1930, **63** 2083.

per hydroxide and on the other hand, we estimated it in the case of the resolution of given amounts of fibroin. And, from the difference in the copper contents in these two cases, we calculated the chemical equivalent of fibroin to copper.

The results are given in Fig. 2, and Table 3.

Fig. 2.

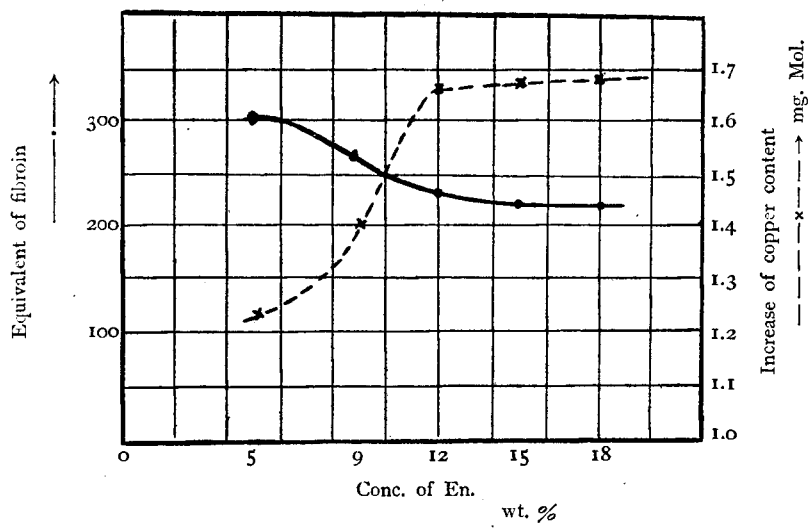


Table 3.

a) Fibroin: Silk wadding.

(1) Conc. of ethylendiamine %	(2) Fibroin, dissolved. gm.	(3) Increase of copper content mg. Mol. *Cu.*	(4) Equivalent of fibroin corresp. to 1 Mol. *Cu*	
5	0.1845	0.57	326.5	
5	0.3690	1.16	318.1	
5	0.3690	1.19	310.1	
5	0.5535	1.77	312.7	
9	0.3690	1.36	271.4	
12	0.1845	0.82	226.4	
12	0.3690	1.61	229.2	
12	9.3690	1.58	233.6	Mean 227.
15	0.3690	1.66	222.3	
15	0.3690	1.60	230.6	
18	0.3690	1.62	227.8	
18	0.3690	1.67	221.0	
b) Fibroin: Silk yarn. (from different source)				
15	0.3690	1.57	235.0	
18	0.3690	1.64	225.6	

As can be clearly seen from the above results, the increase of the copper content, viz. the amounts of copper which react with fibroin, are increased with the concentration of ethylendiamine in solution and they reach the maximum constant value at concentrations of from 12% up to 18%.

Calculating from these results, we can confirm that in these conditions 1 Mol. *Cu* combines always with 227 gms of fibroin or in other words, the chemical equivalent of fibroin to copper corresponds to 227 on the average.

It will also be seen that the values of the equivalent of fibroin to copper are almost invariable in the same conditions, irrespective of the kind of the silk fibroin and also of the amounts of fibroin used in these experiments.

2) FIBROIN-COPPER-AMINE COMPOUND.

When the solution of fibroin in copper oxide-ethylendiamine is poured into a large quantity of alcohol, a sticky purple mass is immediately precipitated.

Prior to an analysis of this reaction product, we tried a few preliminary experiments on the chemical nature of the fibroin regenerated from the solution.

We attempted elementary analysis on the original and regenerated fibroins, but we could not find any noticeable differences in their chemical compositions :—

	C	H	N	O
Original fibroin	48.26%	6.08%	18.27%	27.38%
Regenerated fibroin	48.22	6.08	18.32	27.40

Next, we examined the degree of hydrolysis undergone upon the regenerated fibroin as a result of such treatment of the alkaline copper solution. For this purpose, we dissolved fibroins, original and regenerated, in a neutral solution of calcium nitrate (1:4) at 80°C. for 30 mins., and compared their content of free amino-acid by means of Sörensen's method.[1]

1) Sörensen; Opperheimer, Die Methodik der Ferment **4**, 967.

As is shown below, the content of free amino-acid in regenerated fibroin differs very little from that in the original.

	Amino-acid-nitrogen	$\frac{\text{amino-acid-nitrogen}}{\text{total nitrogen}}$
Original fibroin	0.22%	1.20%
Regenerated fibroin	0.33	1.81

Thus we found that the fibroin regenerated from the reaction product was almost the same, at least in the point of chemical composition, as the original one, though undergoing a small degree of hydrolysis on account of the alkaline copper-solution.

Now, we examined the composition of these reaction products prepared with copper oxide-ethylendiamine solutions of different concentrations. As these precipitates are sticky and are inconvenient for analysis, we dried them in a desiccator of calcium chloride till they could be powdered. (After this drying they became deep purplish black.)

In the case of these samples, we analysed quantitatively the content of 3 components, viz. fibroin, copper and ethylendiamine, of which these products should be composed, and knowing from the preceding section that the chemical equivalent of fibroin corresponds to 227, we calculated their combining ratios in such molecular proportions as;

Fibroin : $Cu : En. = 227 : 63.6 : 60$

where, $En.$ = ethylendiamine.

The results are shown in the Table 4.

Table 4.

Sample Nr.	Conc. of En., used	Percentages of 3 components			Combining ratios of 3 components		
		Fibroin	*Cu*	*En.*	Fibroin	*Cu*	*En.*
		%	%	%			
1	5	40.40	22.45	18.12	1	2.00	1.70
2	12	40.33	21.88	18.58	1	1.96	1.76
3	15	39.50	21.06	17.85	1	1.92	1.72

Thus the combining ratios of three components are almost constant in each case, irrespective of the concentration of ethylendiamine in solution. From these results of analysis, though the value of the ethylendiamine

content is somewhat small, we may assume that there is a compound with such combining ratios as ;

$$\text{Fibroin}\,;\quad Cu\ :\ En.\ \fallingdotseq\ 1\ :\ 2\ :\ 2$$

And with regard to the way in which these three are combined, we can further conjecture from the above results and also from analogy with the cases of cellulose and biurret, that this compound may be expressed in the form of a complex fibroin-copper-amine compound such as [Fibroin Cu][$Cu(En.)_2$]; that is to say, of the two coppers, one combines directly with the fibroin and the other reacts with the ethylendiamine.

(C) SUMMARY.

From the results of our researches on the chemical reaction of alkaline copper-solutions towards silk fibroin, the following conclusions were obtained :—

(1) The solubility of fibroin in Schweizer's solution is increased by the mutual resolution of fibroin and copper.

(2) By estimating the increase of the copper content in copper oxide -ethylendiamine solution due to the resolution of fibroin, the chemical combining equivalent of fibroin to copper and vice versa was determined.

(3) From the solution of fibroin in copper oxide-ethylendiamine a complex fibroin-copper-amine compound was isolated with the approximate composition ;

$$\text{Fibroin}\ :\ Cu\ :\ En.\ \fallingdotseq\ 1\ :\ 2\ :\ 2\ \textit{i.e.}\ [\text{Fibroin}\ Cu][Cu(En.)_2]$$

where, $En.$ = ethylendiamine.

(D) EXPERIMENTAL PARTS.

Raw materials :

In our experiments the following were taken as raw materials.

1) Silk fibroin :

Silk wadding of rather high grade was purified by the following operations.

20 gms. of silk were put into a cage of nickel wire and scoured

for 30 mins. with 2 L. of water containing 20 gms. of neutral soap, the cage being shaken from time to time, and then the soap solution was displaced with boiling water 3 times. The silk was subjected to a second scouring just as before with soap solution of the same concentration and washed throughly with boiling water. After further washing with distilled water, the silk was treated with alcohol, ether and was dried at room temperature.

2) Copper hydroxide :

Copper hydroxide was prepared by Gipson's method[1] from copper sulphate. It was dried at 40°C.

3) Ethylendiamine :

Ethylendiamine hydrate, a chemical reagent prepared by Kaühlbaum was taken.

EXP. 1. SOLUBILITY OF FIBROIN IN SCHWEIZER'S SOLUTION.

(cf. Table 1. & Fig. 1.)

Preparation of Schweizer's solution :

Schweizer's solution was prepared by saturating aqueous ammoniacal solutions of different concentrations with copper hydroxide, these being shaken for days at room temperature, and after they had settled fully, the supernatant solutions were separated.

The copper and ammonia content of these solutions were estimated as usual by means of electrolysis and acidimetry respectively.

Estimation of solubility of fibroin :

Excessive amounts of fibroin were put into 20 c.c. of these Schweizer's solutions of the compositions shown in the Table (1, 2, 3 & 4), given amounts of copper hydroxide being added as "Bodenkörper".

After they had been shaken for 4 hours at room temperature, undissolved fibroin was filtered with a Jena glass filter G. No. 1, and washed with distilled water acidulated with dilute acetic acid and dried

1) Gipson; J. Chem. Soc. 1920, **117** 492.

at 100°C. to constant weight. This weight being subtracted from the weight of fibroin originally used the amounts of dissolved fibroin were calculated.

The results are given in Table 1.

Table 1.

	Composition of Schweiz. sol. NH_3 gms./L	Cu. gms./L	$Cu(OH)_2$ added as "Bodenkörper gms./20 c.c.	Dissolved fibroin gms./20 c.c.	Fibroin-content Wt. %	Remarks
1	20	2.28	0	0.016	0.1	
	"	"	0.40	0.629	3.0	Solid $Cu(OH)_2$ remains.
	"	"	0.80	0.819	3.8	Dito.
	"	"	1.50	0.850	3.9	Dito.
2	50	4.07	0	0.127	0.7	
	"	"	0.40	1.145	5.4	Solid $Cu(OH)_2$ is almost soluble.
	"	"	0.80	2.066	9.2	A little solid $Cu(OH)_2$ remains.
	"	"	1.50	2.578	10.9	Solid $Cu(OH)_2$ remains.
3	100	8.31	0	0.594	2.8	
	"	"	0.40	1.417	6.7	Solid $Cu(OH)_2$ is almost soluble.
	"	"	0.80	2.354	9.3	A little solid $Cu(OH)_2$ remains.
	"	"	1.50	2.827	11.3	Solid $Cu(OH)_2$ remains.
4	210	13.61	0	0.799	4.1	
	"	"	0.40	1.741	8.3	Solid $Cu(OH)_2$ is almost soluble.
	"	"	0.80	2.548	11.7	Solid $Cu(OH)_2$ is almost soluble.
	"	"	1.50	4.054	16.5	A little solid $Cu(OH)_2$ remains.

EXP. 2. COPPER CONTENT IN SCHWEIZER'S SOLUTION.

(cf. Table 2.)

Estimation of copper content:

Given amounts of fibroin were dissolved in 20 c.c. of 21% aqueous ammoniacal solution in the presence of a large excess of solid copper

hydroxide, and the whole shaken for 4 hours at room temperature. Undissolved copper hydroxide was centrifuged and the supernatant liquor was filtered with a glass filter G. No. 3, and the copper content in the filtrate was electrolytically analysed.

EXP. 3. CHEMICAL EQUIVALENT OF FIBROIN TO COPPER.

(cf. Table 3. & Fig. 2.)

With regard to this estimation, refer to Traube's method.[1)]

In our experiments, the moisture contents in fibroin and copper hydroxide were 3.10% and 2.30 % respectively.

The results are summarized in Table 3. & Fig. 2.

EXP. 4. FIBROIN-COPPER-AMINE COMPOUND.

(cf. Table 4.)

Preparation :

1) From 5% ethylendiamine solution ;

1.80 gms. of fibroin and 3.50 gms. of copper hydroxide were dissolved in 37.8 gms. of 5% ethylendiamine solution, and shaked for 2 hours at room temperature. Undissolved copper hydroxide was centrifuged and filtered as in the preceding experiments. When 23.8 gms. of this filtrate were poured, with stirring, into 200 c.c. of absolute alcohol, a sticky purple mass was immediately precipitated. To get rid of remaining copper hydroxide and ethylendiamine, the precipitate was washed with 50 c.c. of absolute alcohol 3 times and pressed with filter papers and dried in a desiccator of calcium chloride for 2 days. The yield was ca. 2.01 gms.

2) From 12% ethylendiamine solution ;

1.00 gm. of fibroin and 2.60 gms. of copper hydroxide were dissolved in 18.5 gms. of 12% ethylendiamine solution as in the preceding case and 17.7 gms. of this filtrate were poured into 200 c.c. of alcohol.

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1) Traube; Ber. 1930, **63** 2083.

The subsequent operations were the same as in the former experiment. The yield was ca. 1.00 gms.

3) From 15% ethylendiamine solution:

1.00 gms. of fibroin and 3.20 gms. of copper hydroxide were dissolved in 20.3 gms. of 15% ethylendiamine solution and 15.5 gms. of the filtrate were poured into 400 c.c. of alcohol. The subsequent operations were the same as before. The yield was ca. 0.60 gms.

Method of analysis of fibroin-copper-amine precipitate;

In the case of these samples of fibroin-copper-amine precipitates we estimated the content of 3 components, viz. fibroin, copper and ethylendiamine, of which these precipitates should be composed, by the following methods.

1) Fibroin content:

The sample, ca. 0.30 gms., was treated for 8 hours (or overnight) at room temperature with a mixture of 100 c.c. of 95% alcohol and 20 c.c. of 10% sulphuric acid, shaking from time to time. By this treatment the copper-amine base was decomposed, the remaining fibroin being almost pale white. The precipitate was filtered with a glass filter G. No. 3, washed with distilled water completely and dried at 100°C. to constant weight.

2) Copper content:

The sample, ca. 0.20 gm., was dissolved in water acidulated with dilute sulphuric acid, and fully decomposed by boiling for about 20 mins. The copper content was electrolytically analysed.

3) Ethylendiamine content:

The ethylendiamine content was calculated by the following estimation of the nitrogen contents.

a) Total nitrogen content:

The sample, ca. 0.3 gms., was taken and its nitrogen content was estimated as usual by Kjeldahl's method. By this estimation, we can find the sum of the nitrogen contents due to fibroin and ethylendiamine in the sample.

b) Nitrogen content due to fibroin:

The nitrogen content of the regenerated fibroin was similarly estimated as the previous case.

Then, from the difference between these 2 nitrogen content, we can calculate the ethylendiamine content in the sample.

The results are given in Table 4.

The values of these 3 contents have already been given, but they may be given here again, and it will be seen that their sum reaches about 80% in each case and there remains about 20% unknown.

Conc. of *En.* Wt. %	Fibroin %	*Cu* %	*En.* %	Sum %
5	40.40	22.45	18.12	80.17
12	40.33	21.88	18.58	80.76
15	39.50	21.06	17.85	78.41

These unknown remainders may be attributed to the following causes :—

1) In this analysis, fibroin, copper and ethylendiamine were estimated as the components which should compose these precipitates.
2) For convenience in analysis, the sample was dried only to such an extent that it could be powdered. Therefore, in the sample, there still remained a fair amount of moisture and a small amount of the alcohol which was used to purify the precipitate.
3) The sample changed in colour to dark purplish black owing to this drying. From this fact it may be considered that the sample suffered oxidation, which was also recognized in the case of cellulose. So, some of the copper content in the sample should be expressed as *CuO* in stead of *Cu*.
4) In this analysis :
   a) Fibroin was regenerated from the sample, treating with a mixture of alcohol and acid.
   b) The ethylendiamine content was calculated indirectly.
   c) There was an experimental loss of about 1% in each case.

Thus, we may consider that the remainders are to be attributed

chiefly to the existence of moisture in the sample, though somewhat to experimental loss and the effect of oxidation, so we next tried to examine the results of the elimination of this moisture as follows.

1) Drying in vacuo;

The sample, prepared from 5% ethylendiamine solution, was dried in a vacuum desiccator for several days at room temperature and was analysed.

As can be seen from the following table, the contents of the 3 components rose to 87% of the sample, but their combining ratios were the same as before without any change due to this drying.

	Percentages of 3 components				Combining ratios of 3 components		
	Fibroin	*Cu*	*En.*	Sum	Fibroin	*Cu*	*En.*
	%	%	%	%			
Before drying	40.40	22.45	18.12	80.97	1	2.00	1.70
After drying	43.22	24.00	19.32	86.54	1	2.00	1.70

2) Drying at 100°C.

When the sample, dried in vacuo, was further dried at 100°C. till to constant weight (for about 17 hours), the weight was decreased by 8.54%. If it is assumed that this loss was caused by the elimination of the remaining moisture, the sum of the 3 components can be reckoned to be about 95% of the sample.

We analysed this sample by the above analytical methods and compared the results with those obtained by calculation from the composition of the sample before drying, the loss of weight being taken into consideration :—

		Fibroin content	Copper content	Total nitrogen content
		%	%	%
Before drying (at 100°C)		43.22	24.00	16.93
After drying	Obs.	32.39	26.01	17.84
	Calc.	47.23	26.23	18.53

From the above table, we can observe;

1) The copper content is almost unchanged.
2) The total nitrogen content is very slightly decreased.
3) The fibroin content is much decreased.

But, from the fact that the nitrogen content of the regenerated fibroin remains unchanged in comparison with the original silk, we can assume that there takes place no decomposition in the fibroin during this drying. The decrease of fibroin content, above observed, may, therefore, be caused by the resolution of a part of the fibroin in the alcohol mixture owing to superheating, the regeneration of the fibroin becoming incomplete.

It may be added that the colour of the sample changes to a very dark greenish black.

From the above results, it is clear that though the moisture may be considered to be almost entirely eliminated, the sample thus treated suffers chemical or physical changes in its composition and in consequence we can not apply our analytical methods in this case.

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