Action of alkaline copper-solution on silk fibroin. II.

By

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In his previous studies[1] the author ascertained that in an ammoniacal solution of copper oxide, 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 owing to the dissolution of fibroin, and thus an increased solubility of fibroin in Schweizer's solution through the mutual dissolution of fibroin and copper is presented. Also, with regard to the chemical reactions, he determined the chemically combining equivalent of fibroin and copper in a solution of copper oxide-ethylendiamine, and further ascertained the existence of a complex fibroin-copper-ethylendiamine compound of the following approximate composition, isolating it from solutions in which given amounts of fibroin were dissolved in a great excess of copper hydroxide:

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

en=ethylendiamine $H_2NCH_2CH_2NH_2$

Continuing this line of research, he has 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 definite copper content, and has found that the dissolution of fibroin in this case is very abnormal and is comparable to what is usually observed in the solution of colloidal substances, in which their solubility is greatly affected by the amounts of the substance used.

In the present work, accordingly, he started out by studying the conditions required the dissolution of fibroin and the method of determining its solubility in order to adopt a method of procedure by means of which he could suitably carry on his investigations. He then went on to elucidate

1) This memoir, 1932, **7** 1.

the correllation between the maximum amounts of fibroin dissolved and the copper and also the amine content of a copper-amine solution of a certain concentration ; and further ascertained the existence of another complex fibroin-copper-amine compound, different from the one mentioned above.

He thus tried to study closely the abnormal phenomena observed in the dissolving of fibroin as governed by the chemical reaction.

(A) CONDITIONS WHICH AFFECT THE DISSOLUTION OF FIBROIN AND THE METHOD OF ESTIMATING ITS SOLUBILITY.

To estimate the degree of solubility of fibroin the following method was used :

Excessive amounts of fibroin were put into a solution of amine with given amounts of copper hydroxide. After they had been shaken for several hours at room temperature, undissolved fibroin was filtered with a Jena glass filter (G. No. I) and washed with distilled water acidulated with dilute acetic acid and dried at 100°C to constant weight. This weight being substracted from the weight of the fibroin originally taken, the amounts of fibroin dissolved were calculated.

In the experiments, scoured and defatted wadding was used for silk fibroin and the purity of the copper hydroxide was determined as usual by the electrolytical method.

To ascertain the conditions under which the solubility of fibroin can best be estimated, the following experiments were made.

(1) Time required to accomplish the dissolution of fibroin.

The followings are the data with regard to the amount of shaking required :

fibroin	1.500	gms.	Temp. 18—20°C
$Cu(OH)_2$	6.00	mg Mol.	
NH_3	17%	50 c.c.	

Duration of shaking	hrs.	1/2	1	2	4	18
Fibroin dissolved	gms.	1.102	1.252	1.303	1.329	1.354

From this table it will be seen that the dissolution of fibroin in Schweizer's solution is so rapid and smooth that in half an hour the amount of fibroin dissolved reaches about 90% of the whole and it is almost completely dissolved in 4 hours. With longer treatment the solubility of fibroin may be increased, but in that case the effect of oxidation due to air and that of the basicity of concentrated ammonia towards a protein such as fibroin must be taken into consideration.

(2) The effect of oxidation due to air during the treatment.

Since air affects the solubility of fibroin through oxidation, the estimation of the solubility of fibroin was carried out in an atmosphere of inert gasses such as nitrogen or hydrogen gas instead, and the results were compared. During the operation of displacing air with an inert gas, care was taken to minimize the experimental loss due to an escape of ammonia by using an inert gas which had been previously saturated with ammonia.

The results were as follows:

fibroin	2.500	gms.	Shaking	4 hrs.
$Cu(OH)_2$	10.00	mg Mol.	Temp.	18—20°C
NH_3	17%	50 c.c.		

Kind of atmosphere		Air	Nitrogen	Hydrogen
Fibroin dissolved	gms.	2.072	2.070	2.058

Thus the effects of oxidation due to air are almost negligible in those conditions, especially in such speedy dissolution.

(3) The effect of the amount of fibroin used.

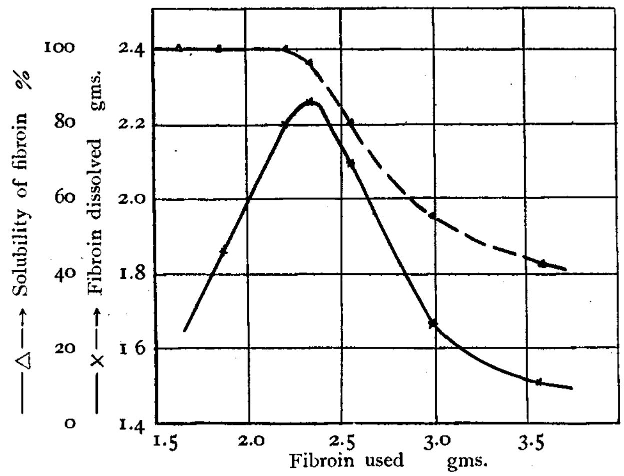
The relation between the amount of fibroin used and the solubility of the fibroin in the definite conditions is shown in Table 1 and Fig. 1.

Table 1.

NH_3	17%		Shaking	4 hrs.
$Cu(OH)_2$	10.00	mg Mol / 100 c.c.	Temp.	18 - 20°C

Fibroin used gms.	Fibroin dissolved gms.	Solubility of fibroin %
1.620	1.620	100.0
1.820	1.820	100 0
2.202	2.202	100.0
2.338	2.261	96 6
2.545	2.071	81.2
2.969	1.639	55.2
3 582	1.516	42.3

Fig. 1.



As can be clearly seen from the above results, the solubility of fibroin in Schweizer's solution is increased as the amount of fibroin used increases, and in those conditions all the fibroin is dissolved without leaving a solid phase till it reaches its maximum degree of solubility.

If the amount of fibroin is increased beyond this point, however, the degree of its solubility is decreased and some of it remains in an undissolved state in the solution. Thus, the solubility of fibroin becomes inconstant, in relation to the amount of the solid phase.

Now inspecting these dissolution phenomena, it may be observed that the first of them are quite comparable to those seen in the dissolution of crystalloid, for example, cane sugar in an aqueous solution or metallic powder in an acidic solution, in which the solubility is increased with the amount of these solutes until it reaches the maximum degree of solubility i.e. the definite degree of solubility of these substances as conditioned by the given temperature, pressure and so on.

The later phenomena, however, are very different from those seen in crystalloids and have rather something in common with those of colloidal substances. For examples of this may be taken such cases as the dissolution of cellulose in Schweizer's solution, reported by Sakurada[1], or the case of

1) Sakurada; Kollz. 1931, **54** 43.

the dissolution of casein in an alkaline solution, examined by Sörensen[1]. Ostwald[2] and his collaborators have proposed to explain these phenomena by their own theory of " Bodenkörperregel ".

Thus the phenomena seen in the dissolution of fibroin are abnormal, like those of colloidal substances, so in this study the author estimated the maximum degree of solubility of fibroin in a copper-amine solution with a given copper content under the conditions and by the method of estimation stated below, on the other hand, examined the relations between the maximum degree of solubility of fibroin and the copper content of the solution and then discussed the abnormal phenomena found in the dissolution of fibroin.

Method of estimation of the maximum degree of solubility of fibroin :

Given excessive amounts of fibroin were put into the amine solutions with given amounts of copper hydroxide, successively little by little, and shaking each time. After the whole of the fibroin had been placed in the solutions, thay were shaken for over 4 hours at room temperature in an air atmosphere in a closed vessel. The subsequent operations and the method of finding the solubility of fibroin were the same in the previous experiments.

(B) RELATIONS BETWEEN THE MAXIMUM DEGREE OF SOLUBILITY OF FIBROIN AND THE COPPER CONTENT OF THE SOLUTION.

The maximum degree of solublity of fibroin in the amine solution containing given amounts of copper hydroxide was determined by the method of estimating the solubility of fibroin just mentioned above.

The results are shown in Tables 2 & 3 and figs. 2 & 3.

Table 2.

Amine : Ammonia

(1) 17% ammoniacal solution

$Cu(OH)_2$ added	mg Mol	2	4	6	11	16
Max. amount of fibroin dissolved	gms.	0.417	0.920	1.330	2.468	3.563
	mg *E.*[3]	1.8	4.0	5.9	10.9	15.7
Fibroin / *Cu*	mg *E.* / mg Mol	0.91	1.00	0.98	0.99	0.99

1) Sörensen; Kollz. 1929, **49** 61.
2) Ostwald; Kollz. 1929, **49** 188. 414, 414; 1930, **50** 65.
3) mg *E.*; mg Equivalent.

(2) 8.5% ammoniacal solution

$Cu(OH)_2$ added	mg Mol	3	4	7	9	11
Max. amount of fibroin dissolved	gms.	0.659	0.855	1.488	2.072	2.521
	mg *E*.	2.9	3.8	6.6	9.1	11.1
Fibroin / *Cu*	mg *E*. / mg Mol	0.97	0.95	0.94	1.01	1.01

Table 3.

Amine: Ethylendiamine

(1) 2% ethylendimine solution

$Cu(OH)_2$ added	mg Mol	2	3	4
Max. amount of fibroin dissolved	gms.	0.450	0.683	0.899
	mg *E*.	1.9	3.0	3.9
Fibroin / *Cu*	mg *E*. / mg Mol	0 99	1.00	0.99

(2) 5% ethylendiamine solution

$Cu(OH)_2$ added	mg Mol	4	5	8
Max. amount of fibroin dissolved	gms.	0.915	1.121	1.810
	mg *E*.	4.0	4.9	8.0
Fibroin / *Cu*	mg *E*. / mg Mol	1.01	0.99	1.00

Fig. 2.

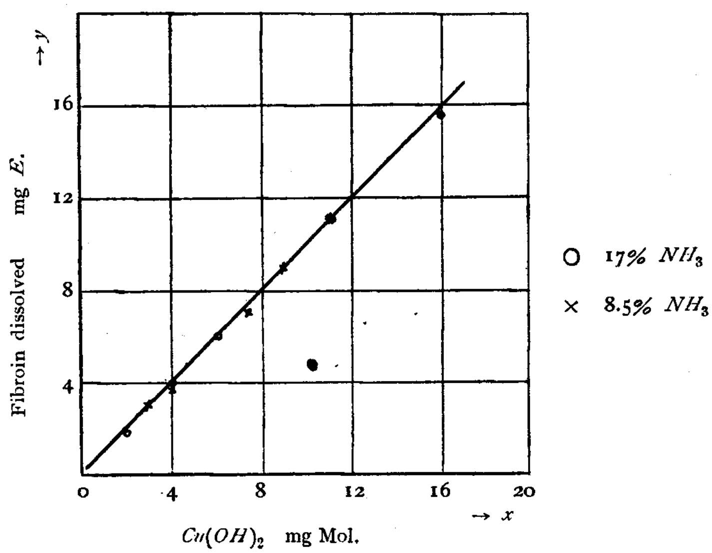
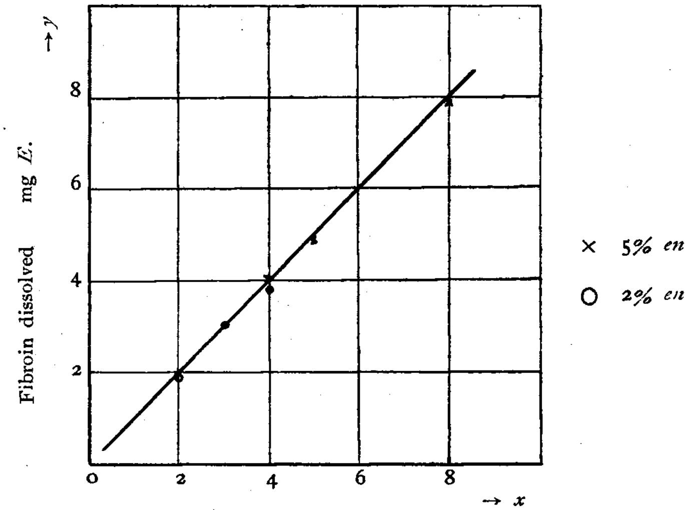


Fig. 3.



The fact that the solubility of fibroin is increased as the amount of copper hydroxide increases is the same as mentioned in the previous report, but on inspecting the table we can see that the maximum degree of solubility of fibroin shows almost the same value irrespective of the kind of amine employed, and that the relations between the maximum degree of solubility of fibroin and the copper content of the solution are expressed by the following equation in terms of their combining ratios, Fibroin : $Cu=227$: 63.6, which were determined in the previous study :—

$$\frac{\text{Max. degree of solubility of fibroin}}{\text{Copper content of the solution}} = 1$$

And also from the figures we can see that their relations are denoted by a straight line :

$$y=x$$

where, $y=$max. amount of fibroin dissolved

and $x=$copper content of the solution.

Thus the relation between the maximum degree of solubility of fibroin and the copper content of the solution can be expressed by the simple ratio of the chemical equivalent of fibroin to that of copper.

Now, if these results are compared with those found in the case of cellulose, the following interesting facts may be derived.

According to Hess[1], Trogus, Uhl and Sakurada, the solubility of cellulose not only depends on the conditions under which it may be determined, but is also greatly affected by the uniformity and purity of the fibre used, and the mode of dissolution is rather complicated.

Hess and his collaborators systematically investigated these complicated phenomena and explained them by their idea of what they called the "Fremd Hautsystem". To be more exact, for example, according to them, in the case of the dissolution of ramie fibre in Schweizer's solution, the relations between the degree of solubility of cellulose and the copper content of the solution are expressed by a curve of S-shape as is usual in the case of substances which are not uniform in their properties, but if the fibre is scoured and purified or if its impure membrane ("Fremd Haut") is broken and removed, then the curve of solubility approaches a straight line from the S-shaped curve. Also in the case of artificial fibres such as viscose silk, which are regenerated from colloidal solutions of cellulosic fibres, the curve above mentioned is almost always a straight line and moreover the relations between the maximum degree of solubility of cellulose and the copper content of the solution may be denoted by simple ratios such as those of their chemical equivalents i.e. cellulose (162): *Cu* (63.6) = 1 : 1, which were determined by Hess' well known work on the polarimetric investigation of cellulose in alkaline copper-amine solutions[2].

Thus, examining these phenomena connected with the dissolution of fibroin and cellulose in copper-amine solutions, we can see that the mode of dissolution of fibroin is quite comparable with the case of cellulose, especially with that of artificial silk, and that fibroin is dissolved as if it were a substance with uniform properties, without such subtle and complicated phenomena as are found in the case of cellulosic fibre with the so-called "Fremd Haut".

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1) Hess, etc.: Z.f. Physik. Chem. 1929, **145** 401 : Ber. 1930, **63** 2030.

2) Hess; Ann. 1924, **435** 7 etc.

From this, together with the fact that the chemical behaviour of copper-amine solutions towards fibroin and cellulose is almost the same, as was shown in the previous report, we can now conclude that fibroin is a very suitable substance to use for the purpose of investigating the phenomena connected with the dissolution of these fibres in copper-amine solutions and the mechanism of the chemical reactions in these systems.

Turning to the experimental results again, we may decide that the fact that the relations between the maximum degree of solubility of fibroin and the copper content of the solution are expressed by a straight line signifies that the dissolution of fibroin is probably caused by the chemical reactions between fibroin, copper and amine, which will be dealt with in the next chapter.

(C) A FIBROIN-COPPER-AMINE COMPOUND.

In the previous paper it was shown that the mode of the dissolution of fibroin was quite similar to that of cellulose and the existence of a complex fibroin-copper-amine compound with the composition, given below was also reported, this compound being isolated from a solution of fibroin in copper oxide-ethylendiamine with excess of copper oxide, analogous to that shown in the case of cellulose, $[(C_6)_2\ Cu]\ [Cu\ en_2]$:

$$\text{Fibroin} : Cu : en = 1 : 2 : 2 \qquad \text{i.e. } [\text{Fibroin } Cu]\ [Cu\ en_2] \qquad (1)$$

en = ethylendiamine

From this, together with the results mentioned in the previous chapter, results that indicate clearly the existence of the relation, fibroin : $Cu = 1 : 1$ between the maximum degree of solubility of fibroin and the copper content of the solution, we may infer that so far as the chemical reactions are concerned, there are some differences in the chemical behaviours of fibroin, copper and amine according to the amounts of these substances and also the dissolving conditions.

In order to throw some light on the chemical reaction occurring in the case where the ratio of fibroin to copper is 1 : 1, the author examined the composition of the reaction product on the analogy of the case of compound (1).

In his experiments he prepared the reaction product, isolating it from a solution of fibroin in copper oxide-ethylendiamine where fibroin : $Cu=1:1$, by adding a large quantity of alcohol, and analysed quantitatively the content of the three components, fibroin, copper and ethylendiamine, of which the product should be composed, and calculated from the molecular proportions,

$$\text{Fibroin} : Cu : en = 227 : 63.6 : 60$$

The results are shown in Table 4.

(For the method of preparation and analysis, reference should be made to the previous report.)

Table 4.

Sample	Composition of solution			Percentages of 3 components			Combining ratio of 3 components		
No.	Fibroin mg *E*.	*Cu* mg Mol	*en* mg Mol	Fibroin %	*Cu* %	*en* %	Fibroin	: *Cu*	: *en*
1	4.00	4.00	10.27	54.82	14.67	15.43	1	0.96	1.06
2	8.00	8.00	24.10	53.80	14.90	14.69	1	0.99	1 03

We can thus assume that there is a compound with the combining ratios,

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

That is to say, both *Cu* and *en* each combine in the proportion of one equivalent to one equivalent of fibroin. From these results we can also ascertain that there is a new fibroin-copper-amine compound different in composition from compound (1).

Now, examining the combining ratios of this newly found compound (2), we find that the ratio of fibroin to copper corresponds to that of the maximum degree of solubility of fibroin with regard to the copper content of the solution and on the other hand, as may be seen from Table 5, the amounts of amine combining with fibroin and copper are the smallest amounts necessary to bring these amounts of fibroin and copper into solution.

Table 5.

Fibroin	5 mg *E*.	Shaking	4 hrs.
$Cu(OH)_2$	5 mg Mol	Temp.	18—22°C

Amount of *en* used	Fibroin dissolved	Solubility of fibroin	Remarks
mg Mol / 30 c.c.	mg *E*.	%	
10.20	5.00	100.0	The solution is transparent and $Cu(OH)_2$ is wholly dissolved.
8.05	5.00	100.0	Do.
6.50	5.00	100.0	Do.
5.00	5.00	100.0	Do.
4.75	4.52	90.4	$Cu(OH)_2$ is almost entirely dissolved.
4.20	3.39	67.9	A little $Cu(OH)_2$ remains.
2.85	1.61	32.2	$Cu(OH)_2$ remains.

It may be said therefore, that both the composition of the fibroin-copper-amine compound which is thus determined and also the relations of the minimum amounts of copper and amine necessary to dissolve the given amount of fibroin can be expressed by the same equation (Equn. 2). The way in which these two compounds (1) & (2) are composed and also their mutual relations, will be reported on in the next paper.

(D) ON THE ABNORMAL PHENOMENA CONNECTED WITH THE DISSOLUTION OF FIBROIN.

As can be clearly seen from the previous chapter (A), although the maximum degree of solubility of fibroin in copper-amine solutions may be determined under definite conditions, the solubility of fibroin, generally speaking, is greatly affected by the amount of fibroin used. For example, if a large excess of fibroin is dissolved in the solution with the given copper content, not only does part of them remain undissolved, but also the amount of fibroin dissolved decreases in relation to the increase in the amount of solid fibroin present and thus the phenomena connected with the dissolution of fibroin in copper-amine solutions are assumed to be abnormal, just like those seen in the case of colloidal substances.

In addition to these facts, the following reactions as regards the chemical reactions between fibroin, copper and amine can not be over looked :

According to the relative amounts of these three components several different cases can be observed and two kinds of compounds each with a definite composition can be found among their reaction products. The smallest amounts of copper and amine required to dissolve the given amounts of fibroin is expressed by the next equation ;

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

Therefore, to investigate such abnormal phenomena connected with the dissolution of fibroin, it is neccssary to elucidate the way in which these three components react or combine both in the solution and in the solid phase.

Table 6.

(1)

NH_3 17% ; $Cu(OH)_2$ 3.97 mg Mol / 100 c.c. Shaking 4 hrs. Temp· 18—20°C

Fibroin used mg *E.*	In the solution			In the solid phase			Cu/F in solution / Cu/F in solid phase
	Fibroin mg *E.*	*Cu* mg Mol	Cu/F	Fibroin mg *E.*	*Cu* mg Mol	Cu/F	
3.97	3.94	3.97	1.01	—	—	—	—
4.45	3.27	3.34	1.02	1.18	0.63	0.53	1.93
4.96	2.72	2.77	1.01	2.24	1.20	0.52	1.91
6.45	2.54	2.42	0.95	3.94	1.57	0.40	2.37
8.78	1.71	1.65	0.96	7.07	2.32	0.33	2.91

(2)

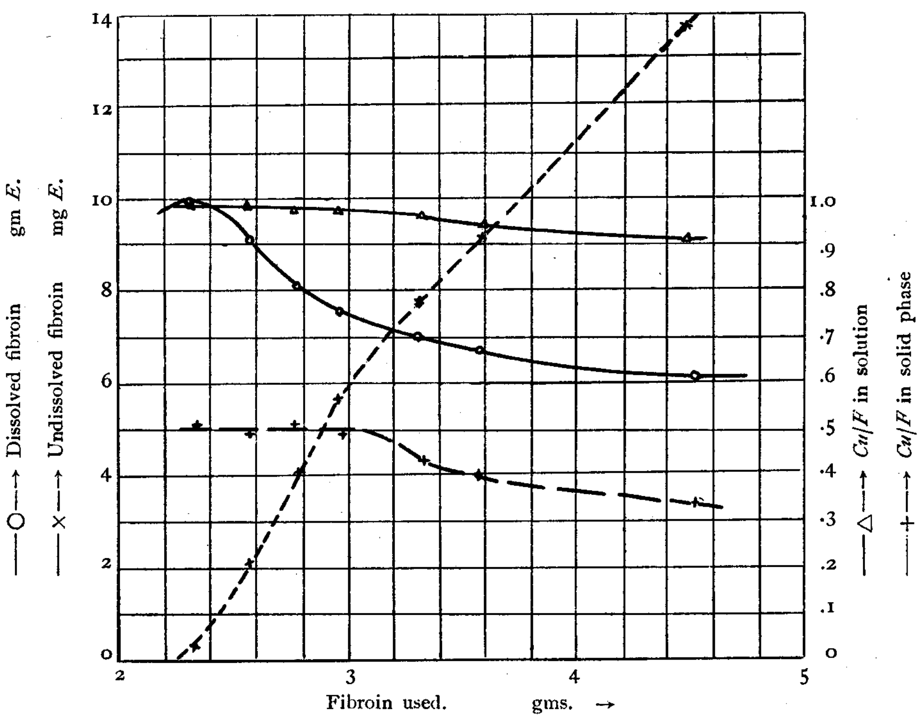
NH_3 17% ; $Cu(OH)_2$ 10.00 mg Mol / 100 c.c. (c. f. Fig. 4)

Fibroin used mg *E.*	In the solution			In the solid pfase			Cu/F in solution / Cu/F in solid phase
	Fibroin mg *E.*	*Cu* mg Mol	Cu/F	Fibroin mg *E.*	*Cu* mg Mol	Cu/F	
6.00	soluble			—	—	—	—
9.70	dito.			—	—	—	—
10.29	9.96	9.83	0.93	0.33	0.17	0.51	1.93
11.21	9.12	8.98	0.98	2.09	1.02	0.49	2.00
12.23	8.18	7.90	0.97	4.05	2.10	0.51	1.91
13.08	7.45	7.22	0.97	5.63	2.78	0.48	2.01
14.61	7.04	6.75	0.96	7.60	3.25	0.43	2.24
15.78	6.68	6.30	0.94	9.10	3.70	0.40	2.35
19.75	6.12	5.57	0.91	13.63	4.43	0.33	2.76

In the expriments, given amounts of fibroin were put at one time into a solution of copper tetramine ($Cu(OH)_2$-NH_3 solution) with a given copper content and shaken for several hours at room temperature. After the dissolution of the fibroin had been completed, the amounts of fibroin dissolved and the copper content of the solution before and after the dissolution were determined, and the ratio of copper to fibroin and the combining ratios of fibroin and copper both in the solution and in the solid phase were calculated.

The results are shown in Table 6 and Fig. 4.

Fig. 4.



These results show that so long as the amounts of fibroin used are less than or equal to the amounts of copper, both calculated from their chemical equivalents, i.e. Fibroin : $Cu = 227 : 63.6$, the fibroin is wholly dissolved, forming a complex fibroin-copper-amine compound such as (1) or

(2) and it is necessary at least for one equivalent of copper to dissolve one equivalent of fibroin, so, if the amount of fibroin is excessively increased, there must be insufficient copper to form a complex compound, or in other words, to bring the fibroin into solution. Hence arise a solid phase.

Now examining the state in which fibroin and copper combine both in the solution and in the solid phase, we can see from the above table that there are two different cases according to the amount of fibroin used.

(1) In this case, those relations are expressed by the following equations:

In the solution:	Dissolved fibroin : copper $\fallingdotseq 1:1$
In the solid phase:	Undissolved fibroin : copper $\fallingdotseq 2:1$

That is to say, in the solution, the combining ratio of dissolved fibroin to copper can be denoted by the same equation which shows the relations between the maximum degree of solubility of fibroin and the copper content of the solution. And in the solid phase there is also a definite ratio of undissolved fibroin to the copper content. Therefore, the ratio of copper to fibroin both in the solution and in the solid phase is constant, irrespective of the amount of fibroin used.

These facts lead to the conclusion that the mutual behaviour of fibroin and copper in the solid phase is probably caused rather by the chemical reaction than by simple adsorption and also there should be present a definite system of reaction between fibroin, copper and amine.

(2) In this case, though the relations between fibroin and copper in the solution may be observed to be the same as in the former case, the relation in the solid phase are changed in relation with the excessive increase of fibroin and there exsist no definite constant ratio between them.

Also, the transition from the former to the latter case, as may be seen from the figure, exists near the point where the amounts of dissolved fibroin are almost equal to those of undissolved fibroin and so each of the curves representing these phenomena has a niche in it.

To clarify the relations of amine to fibroin and copper, they were further examined in the fibroin-copper-ethylendiamine system, the experimental method being quite the same as in the previous case.

The results are shown in Table 7.

Table 7.

$\begin{cases} Cu(OH)_2 \\ en \end{cases}$ 10.00 mg Mol / 13.02 mg Mol / 100 c.c. Shaking 6 hours Temp. 18–20°C.

No. of exp.	Fibroin used mg *E.*	In the solution: Fibroin mg *E.*	*Cu* mg Mol	*en* mg Mol	In the solid phase: Fibroin mg *E.*	*Cu* mg Mol	*en* mg Mol	Combining ratios: Fibroin	*Cu*	*en*
1	10.00	10.00	10 00	13.02	—	—	—	—	—	—
2	12.00	9.50	9.01	11.78	2.50	1.16	1.24	1	0.47	0.50
3	13.00	7.70	7.45	10.57	5.30	2.55	2.45	1	0.48	0.46
4	14.00	6.92	6.68	9 68	7.08	3.32	3.34	1	0.47	0.47
5	16.00	5.80	5.45	8.46	10.20	4.55	4.56	1	0.45	0.45
6	18.00	5.35	5.35	8.05	12.65	4.96	5.07	1	0.39	0.40

In this table it can be seen that the amounts of *en* in the solution in each of the experiments are greater than those of copper and these excessive amounts of *en* are reckoned to be just about 3.02 mg Mol which had been originally used in excess in these experiments i.e. 13.02 mg Mol *en*: 10.00 mg Mol *Cu.* So, assuming that these amounts of *en* may be present freely apart from the chemical reactions between fibroin, copper and amine, then the amounts of copper and amine which would be really combined with fibroin will be denoted by the ratios Fibroin : *Cu* : *en* ≒ 1 : 1 : 1 as is shown in the following table.

In the solution

No. of exp.	Combining amounts: Figroin mg *E.*	*Cu* mg Mol	*en* mg Mol	Free *en* mg Mol	Combining ratios: Fibroin	*Cu*	*en*
1	10.00	10.00	10.00	3.02	1	1.00	1.00
2	9.50	9.01	8.76	3.02	1	0.93	0.92
3	7.70	7.45	7.55	3.02	1	0.96	0.98
4	6.92	6 68	6.68	3.02	1	0.96	0.96
5	5.80	5.45	5.44	3.02	1	0.96	0.96
6	5.35	5.35	5.03	3.02	1	1.00	0.95

In these results, the same phenomena as were shown in the previous case viz. the fibroin-copper-ammonia system, can be seen, so that the relations given below exist, and also the noteworthy fact is seen that the combining ratios of copper and ethylendiamine to fibroin are almost the same in the solid phase.

In the solution : Dissolved fibroin : $Cu : en \doteqdot 1 : 1 : 1$

In the solid phase : Undissolved fibroin : $Cu : en \doteqdot 2 : 1 : 1$

or generally expressed $\doteqdot 1 : x : x$

Summarizing the foregoing, we can assert that there exist definite chemical reactions between fibroin, copper and amine not only in the solution, but also in the solid phase, and from the study of these chemical reactions we can further conclude that the abnormal phenomena connected with the dissolution of fibroin in copper-amine solutions are chiefly attributable to the nature of the distribution of copper and amine to fibroin and also to the combining ratios among these three components that occur when fibroin is added in great excess.

Ostwald[1] and his collabolators, however, have explained such abnormal phenomena by their own theory of "Bodenkörperregel", and in fact these phenomena may be clearly explained as examples of cases observed in the so-called colloidal peptisation of colloidal substances.

But when we inspect the present case of fibroin, we find, as mentioned above, that in the first step of the dissolution of fibroin the complex fibroin-copper-amine compound is being formed by their mutual reactions, the dissolution phenomena proving to be governed by the chemical reactions, and that the abnormal decrease in the solubility of fibroin may be chiefly attributable to the combining ratios of copper and amine to fibroin that occur when fibroin is added in great excess. In view of all this, it is easier to explain such abnormal phenomena by holding that what takes place is a chemical resolution than to deem it wholly a colloidal peptisation. This explanation is rather analogous to that given by Sörensen[2], for example, who examined chemically the similar abnormal case of casein in alkaline solutions and explained it by quoting the example of the dissolution of tartaric

1) loc cit.
2) loc cit.

acid in caustic alkaline solutions. But we have studied more closely the mode of this chemical resolution and the system of reactions than the latter author did.

(D) SUMMARY.

From the results of our researches on the phenomena seen in the dissolution of fibroin in copper-amine solutions and also on their chemical reactions, especially in the case of dissolving a large quantity of fibroin in a solution of a given copper content, the following conclusions were obtained.

(1) The dissolution phenomena of fibroin in this case are very abnormal and are comparable to a similar case usually observed in the dissolution of colloidal substances, in which the solubility is greatly affected by the amounts of the substances used.

(2) The maximum degree of solubility of fibroin in copper-amine solutions with a given copper content was determined under definite dissolving conditions.

Expressing these relations in terms of their chemical equivalents i.e. Fibroin : $Cu = 227 : 63.6$, which had been ascertained by the author in his previous study, the following equation can be derived:

$$\frac{\text{Max. degree of solubility of fibroin}}{\text{Copper content of the solution}} = 1$$

(3) From the dissolution of fibroin in a copper oxide-ethylendiamine solution in which the ratio of fibroin to copper is equal to that shown in (2), a complex fibroin-copper-ethylendiamine compound was isolated with the following composition:

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

where, en = ethylendiamine.

It was ascertained that the relations of the smallest amounts of copper and amine necessary to dissolve the given amounts of fibroin can be also expressed by these ratios.

(4) The abnormal phenomena connected with the dissolution of fibroin in copper-amine solutions, are chiefly attributable to the nature of the

distribution of copper and amine to fibroin and also to the combining ratios between these three components that occur when fibroin is added in large excess and there are definite chemical reactions between fibroin, copper and amine not only in the solution, but also in the solid phase.