CXLI. INVESTIGATIONS ON GELATIN. PART VI. THE INFLUENCE OF THE TREATMENT OF THE PRECURSOR ON THE CHARACTER OF THE GELATIN.

By JOHN KNAGGS AND SAMUEL BARNETT SCHRYVER.

From the Imperial College of Science and Technology.

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THE experiments described in this communication had the following objects:
A. The determination of the character of the products separated from gelatin during flocculation in an electrical field. B. To determine whether gelatins after repeated purifications by flocculation possessed the same properties when obtained from different precursors, or different treatments of the same precursor, in other words, to ascertain whether gelatin could be obtained as a definite chemical entity.

A. CHEMICAL CHARACTERS OF THE PRODUCTS SEPARATED DURING THE FLOCCULATION OF GELATIN IN AN ELECTRIC FIELD.

General Method of Experiment.

The gelatin prepared from purified ossein was freed from electrolytes by washing with frequent changes of distilled water for 4 days, and finally the last traces of soluble salts were removed by electrolysis of a 20 % gel. The Hausmann numbers of the fractions obtained were determined.

5 litres of a 2 % solution of this purified gelatin were prepared, and flocculated in the apparatus described. When the number of milligrams of nitrogen per 100 cc. of the supernatant liquid reached a constant value, which was after the current had passed through the solution for 5 days, the clear supernatant liquid was syphoned off and filtered to remove any particles of undissolved gelatin. This liquid was concentrated under reduced pressure at 40°, and then hydrolysed with 20 % hydrochloric acid.

The flocculated gelatin was rendered free from the supernatant liquid by repeatedly washing by decantation with distilled water. A portion of the flocculated and washed gel was hydrolysed with 20 % hydrochloric acid, the remainder was made up to a 2 % solution by adding the calculated quantity of water and warming to 37°. This solution was reflocculated by the electric current.

The liquid in the cathode compartment which contained the part which diffuses through the membrane, was reduced to small bulk by evaporating under reduced pressure at 40°, and hydrolysed with 20 % hydrochloric acid.

The Hausmann numbers of the three fractions were determined.

The process of flocculation was repeated several times, and each time the Hausmann numbers of the three fractions were found.

The above experiment was carried out using a gelatin prepared from ossein which had been treated for 60 days with 0.2 % NaOH and for 1 day with 0.5 % HCl. The ossein was thoroughly washed in running water to remove all traces of free acid before extraction of the gelatin. The experiment was repeated using gelatin extracted from ossein which had had a prolonged treatment with 0.2 % NaOH only, and in these experiments the supernatant liquid and liquid in the cathode compartment were acidified with dilute hydrochloric acid before evaporation to small bulk to retain in solution any ammonia or volatile nitrogen compounds. The following are the results of the first experiment:

Table I.

<i>Exp. 1. Gelatin from ossein treated for 60 days with 0.2 % NaOH and for 1 day with 0.5 % HCl</i>					
	Amide N	Humin N	Diamino N	Mono-amino N	Non-amino N
A. Original gelatin	2.0	1.1	22.86	—	—
B*. Electrolyte-free gelatin	1.97	1.86	22.59	64.12	9.46
<i>First flocculation of 2 % solution of B</i>					
Supernatant liquid	1.90	2.12	20.94	—	—
Flocculated gelatin	2.01	2.01	21.38	60.49	11.94
Liquid which diffused to cathode	1.6†	1.57	15.95	—	—
<i>Second flocculation</i>					
Supernatant liquid	1.97	2.16	20.61	—	—
Gelatin flocculated twice	2.06	1.93	21.59	61.37	13.05
Liquid which diffused to cathode	1.54†	1.30	12.73	—	—
<i>Third flocculation</i>					
Supernatant liquid	2.63	2.83	19.71	—	—
Gelatin flocculated three times	1.96	1.99	21.68	57.49	16.88
Liquid which diffused to cathode	2.41†	1.73	13.72	—	—
<i>Fourth flocculation</i>					
Flocculated gelatin	2.06	2.16	21.61	56.03	18.14
<i>Seventh flocculation</i>					
Flocculated gelatin	1.86	2.42	21.68	55.99	18.05
<i>Eighth flocculation</i>					
Flocculated gelatin	1.91	2.33	21.76	55.30	18.7
<i>Exp. 2. Gelatin from ossein treated with 0.2 % NaOH only for 60 days.</i>					
C. Original gelatin	1.54	1.26	24.09	—	—
D†. Electrolyte-free gelatin	1.78	1.31	26.27	60.40	10.24
<i>First flocculation of 2 % solution of D</i>					
Supernatant liquid	2.55	3.02	23.03	58.21	13.19
Flocculated gelatin	1.91	3.77	24.12	55.21	14.99
Liquid which diffused to cathode	3.89	3.28	21.02	38.8	33.01
<i>Second flocculation</i>					
Supernatant liquid	2.57	1.73	20.69	61.21	13.8
Flocculated gelatin	1.74	1.18	24.89	53.04	19.15
Liquid which diffused to cathode	10.08	2.60	12.76	43.28	31.28
<i>Third flocculation</i>					
Supernatant liquid	6.31	3.06	17.95	58.11	14.577
Flocculated gelatin	1.76	1.49	24.68	52.17	19.91
Liquid which diffused to cathode	9.35	7.79	8.83	31.66	42.37

* Electrolytes removed from A by electrolysis of 20 % gel.

† The percentage of amide nitrogen is low because the liquid which diffused to the cathode compartment was not acidified with hydrochloric acid before concentration under reduced pressure.

‡ C was washed with water and a 20 % gel electrolysed.

The deductions from Exps. 1 and 2 may be summarised as follows:

(a) *Supernatant liquid.* This is the more acidic fraction as it collects round the anode, and this is borne out by the percentage of diamino nitrogen being lower than that of the flocculated gelatin. The non-amino nitrogen number is also lower than that for the precipitated gelatin and remains fairly constant throughout the experiment.

The amide nitrogen values in Exp. 2 are much higher than the corresponding values in Exp. 1, as precautions were taken in the second experiment to retain any volatile nitrogen compounds before concentration under reduced pressure. These high values may be due to a slight oxidising effect of the anode.

(b) *Flocculated gelatin.* The diamino nitrogen number becomes constant after the first flocculation, but the percentage of non-amino nitrogen increases at each flocculation to a constant value. This observation is treated in greater detail in the next communication.

(c) *The liquid which diffuses through the membrane to the cathode.* This fraction has abnormally low values for the percentage of diamino and mono-amino nitrogen. This fraction is basic in character seeing that it is attracted to the negative pole, and it would therefore be expected that the percentage of diamino nitrogen would be greater than that of the other fractions. The results in Exps. 1 and 2 do not show this to be the case, the reason being that no precautions were taken to prevent secondary reactions due to the reducing action of the cathode. The high percentage of amide nitrogen in Exp. 2 would indicate that ammonia or its simple derivatives were among the products formed, and the high percentage of non-amino nitrogen that the diamino and mono-amino nitrogen groups underwent molecular rearrangement forming ring compounds such as pyrroles, pyridines and piperazines, etc.

(d) *The differences in the gelatins used in the above experiments.* The most striking result indicated by Exps. 1 and 2 is the difference in the Hausmann numbers of the gelatins which have been extracted from ossein treated with acid and alkali respectively. When the last treatment has been with acid a gelatin is obtained which yields a lower diamino nitrogen number than a gelatin extracted from an ossein the last treatment of which has been with an alkali. These results were obtained in spite of the fact that the ossein had been subjected to very thorough washing with water, whether the last treatment had been with an acid or an alkali.

B. INFLUENCE OF THE TREATMENT OF THE PRECURSOR ON THE CHARACTER OF THE GELATIN.

The above experiments indicate that gelatins differ according to whether the precursors from which they have been extracted received a final treatment with acid or alkali. These experiments have been extended to other precursors and similar results have been obtained which are shown in Table II.

The horn-pith and Achilles tendon of the ox were used. They received a preliminary treatment similar to that described for ossein, in that they were extracted with 0.2 % NaOH for 60 days to remove organic nitrogen impurities, and then thoroughly washed in running water. The precursors were then treated as follows:

(a) with 0.2 % NaOH,

(b) with 0.5 % HCl

for 5 days. The excess of free acid or alkali was removed by washing and the gelatin, extracting at 80°, concentrated, dried in a current of air, washed with water and twice flocculated from a 1 % solution in an electric field, taking the precautions described on p. 1095.

Table II.

Precursor	Final treatment of precursor before washing and extraction	Amide N	Diamino N	Difference in Diamino N
Horn-pith	0.2 % NaOH	1.30	24.20	1.26
Horn-pith	0.5 % HCl	1.45	22.94	
Tendon*	0.2 % NaOH	3.02	29.50	3.50
Tendon*	0.5 % HCl	3.45	26.00	
Bone†	0.2 % NaOH	3.2	21.39	1.61
Bone†	0.5 % HCl	3.1	19.78	
Ossein	0.2 % NaOH	1.74	24.89	3.30
Ossein	0.5 % HCl	2.06	21.59	

* The tendons were cut into small pieces and treated with 0.2 % NaOH for 14 days. They were washed free from alkali and preserved in alcohol for several months when they were dried and treated for several weeks with 0.2 % NaOH. The Hausmann numbers of the tendon gelatin differ markedly from those of horn-pith or ossein gelatin. Whether this difference is due to the fact that the gelatins are not the same, or whether tendon gelatin is contaminated with other products which do not exist in horn-pith or ossein cannot be as yet definitely stated.

† The bone was treated with the calculated quantity of 0.5 % HCl to remove the carbonate, washed free from acid and the gelatin extracted at 95°. The acid-treated bone was treated with 0.2 % NaOH for 2 days, well washed, and the gelatin prepared from it. These samples of gelatin contain impurities such as chondroitinsulphuric acid, and they were not purified either by washing or flocculation, yet they show a difference in the percentage of diamino nitrogen.

In all the above samples of gelatin, there is a difference in the percentage of diamino nitrogen depending on the previous treatment of the precursor. A gelatin with the higher percentage of diamino nitrogen was always obtained from a precursor which had had a final treatment with alkali before being washed and extracted, the one with the lower value for diamino nitrogen being obtained from a precursor which had had acid treatment.

A probable explanation of this fact may be found in assuming that gelatin can exist in two tautomeric forms, keto and enol [compare Dakin, 1910, 1912, 1913; Dakin and Dudley, 1913; Robertson, 1920; Jordan Lloyd, 1920], and that ordinary gelatin is an equilibrium mixture of the two. The same modifications will exist in the precursors of gelatin, and the keto form will be changed into the enol form and *vice versa*, according to conditions, i.e. the reaction is reversible and will probably depend on the p_H .

Precursor of gelatin (keto form) $\rightleftharpoons$ Precursor of gelatin (enol form).

The gelatins obtained from horn-pith, tendon or ossein, are markedly different in their physical properties. The above numbers indicate that they are different chemically.

A similar result was obtained from the hydrolysis products of the precursors. If the latter are rapidly hydrolysed after treatment with acid they yield a smaller amount of diamino acids than if hydrolysed after treatment with alkali. The difference in the diamino acids amounts to nearly the same as was obtained from the corresponding gelatins. The hydrolysis products of the precursors will be dealt with in a later communication.

RATE OF EXTRACTION.

In view of the above results it was of interest to ascertain whether differences could be observed in the rate of extraction of gelatin according to whether the final treatment of the precursor had been with acid or alkali. The following experiments were carried out and the method employed was that described by Manning and Schryver [1921].

Rate of Extraction of Gelatin from Horn-pith, at 100°.

The horn-pith was purified in the same way as the ossein by washing for several weeks with 0.2 % NaOH and was then treated as follows, and the rate of extraction followed for samples A, B, C and D.

A. Horn-pith treated with 0.5 % HCl for 2 days.

B. A was electrolysed for 10 days in the apparatus described for flocculating gelatin.

C. D was electrolysed for 10 days.

D. Horn-pith treated with 0.2 % NaOH.

The results are shown graphically in fig. 1.

In these experiments all the free acid or alkali was removed from the tissues A and D by a prolonged washing in running water and finally with distilled water.

Rate of Extraction of Gelatin from Ossein at 92°.

The ossein used was freed from all organic nitrogen impurities by prolonged treatment with 0.2 % NaOH. The following results were obtained (Table III):

Table III.

Ossein treated with caustic soda previous to extraction		Ossein treated with hydrochloric acid previous to extraction	
Time in hours	% extracted	Time in hours	% extracted
0.5	21.8	0.5	20.8
1.25	35.4	1.5	57.3
2.0	46.0	2.5	76.1
3.0	61.5	3.5	92.0
4.0	71.9	4.16	95.9
5.0	78.1	5.16	99.7
6.0	81.9	6.16	99.0
—	—	7.33	100*

* It was assumed in these calculations that all the gelatin was extracted after 7 hours. The percentages in both columns are based on this assumption.

It is seen that the gelatin is extracted more rapidly from the acid-treated precursor than from either the alkali-treated precursor or a precursor which had been submitted to electrodialysis. A similar result was obtained by following the rate of extraction of gelatin from finely powdered bone. If the bone is partially decalcified with 0.5 % HCl the rate of extraction is much quicker than if the same sample is treated with 0.2 % NaOH for 2 days after the acid treatment, for example, after 6 hours at 95°

43% of gelatin is extracted from acid-treated bone,
37% " " " " alkali-treated "

Another point worthy of notice is that a precursor which had been treated with acid, washed and dried, was more deeply coloured than the same precursor which had had alkali treatment.

Fig. 1.

The gelatin extracted from an acid-treated precursor has always a deeper shade of colour than the gelatin from the alkali-treated precursor. Gelatin from alkali-treated horn-pith is almost colourless. It seems, therefore, probable that the acid-treated precursor and the alkali-treated precursor are not chemically identical.

CONCLUSIONS.

(1) A highly purified gelatin on flocculation from 2 % solution in an electric field separates into three fractions. The supernatant liquid round the anode contains a fraction with a low diamino nitrogen value; the portion which diffuses through the membrane to the cathode is reduced by the pole into simple products. The amount of these two fractions is relatively small compared with the amount of gelatin which is flocculated.

(2) Repeated flocculation of the gelatin from 1 % solution in an electric field does not alter the percentage of diamino nitrogen, but the non-amino nitrogen increases at each flocculation to a maximum of 20 (approx.).

(3) Gelatins differ in their Hausmann numbers and this depends on the previous treatment of the precursors before extracting the gelatin. Gelatin extracted from a precursor which has had acid treatment before extraction has a lower value for the diamino nitrogen than the gelatin extracted from the precursor which has had alkali treatment. These differences amount to between 1.5–3.5 %. A possible explanation of these facts is that the precursor undergoes the keto-enol change.

(4) The rate of extraction of gelatin from an acid-treated precursor is much greater than from the same precursor which has had alkali treatment, or has been submitted to electrodialysis.

(5) There is no reason for regarding "gelatin," even after submission to elaborate methods of purification as a definite chemical entity.

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