LXV. STUDIES ON GELATIN. PART I. THE DYNAMICS OF THE FORMATION OF GELATIN FROM OSSEIN.

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THESE researches were undertaken with the object of throwing some light on the processes involved in the extraction of gelatin from its precursors in the animal body. In the present communication the results are confined to the investigation of the dynamics of the formation of gelatin from decalcified bones.

PREPARATION OF THE MATERIALS.

For the purposes of this research, the material used consisted entirely of the middle portions of the femurs of young oxen (about two years old). These were collected from the slaughter-house as soon as possible after the animals were killed, and their preparation for experiment was commenced immediately after they were brought into the laboratory. They were first washed in running water until the washings were clear, and then sawn into two portions along their length, and the marrow and adhering fatty matter was removed. They were then dehydrated and de-fatted, first by treatment with alcohol and then by ether, and afterwards air-dried. They were again cleaned by hand to remove the last portions of adhering tissues. They were then crushed in rock-crushers, for the use of which the authors are indebted to Prof. Truscott. The crushed particles were then sieved, the largest size used passing through $\frac{1}{4}$ -inch mesh, but not through a $\frac{3}{16}$ -inch mesh; the smallest sized bones used passed a $\frac{1}{32}$ -inch mesh. Altogether five grades of bone were decalcified separately, 3 % cold hydrochloric acid (not above 5°) being used for the purpose. The course of the decalcification was followed by the titration of the acid from day to day. This process required from three to seven days, the rate depending on the size of the bone particles. After decalcification, the bones were washed with water till acid-free, then with 1 % ammonia solution, again with water, and finally with alcohol and ether; after which they were dried by a fan.

METHOD OF EXPERIMENT.

The rate of extraction of gelatin from decalcified bones of various graded sizes has been measured at 100° and at 90°.

For the extraction at 100° a weighed quantity of the dried decalcified bones was added to a known volume of boiling water in a flask fitted with a reflux condenser. The time was taken, the mixture was kept gently boiling and at convenient intervals a small sample of the liquid was withdrawn and filtered through cotton wool. The nitrogen content of the filtered sample was determined by Kjeldahl's method, and the boiling continued until no further increase in nitrogen content occurred. To measure the rate of extraction at 90° the same method was used except that the flask was immersed in a thermostat at the required temperature and its contents kept in motion by a glass stirrer.

RESULTS.

Fig. 1 shows the results for the extraction of gelatin at 100°. In each case 20 g. of decalcified bones and 300 cc. water were taken. The results are plotted as percentages of the total amount of nitrogen extracted. The total nitrogen extracted varied slightly with different samples of bones, due to differences in degree of dryness. It lay between 0.85 and 0.95 g. per 100 g. solution.

Fig. 2 shows the results of extraction at 90°.

Fig. 1. Extraction of gelatin at 100°.

The extraction curves for the larger sized particles have a point of inflection, and are similar to the curves for autocatalytic reactions. The process of extraction, however, is not autocatalytic. The rate of extraction of a fresh quantity of decalcified bones is unaffected by the presence in solution of some previously extracted gelatin.

The rate of extraction increases with the finer division of the bones, except for the very finest ($\frac{1}{32}$ -inch and under). The abnormal curve obtained for the latter size appears to be due to aggregation of the particles. Thus in the presence of sodium salicylate ($N/4$), a salt which markedly lowers the surface tension of water and which would therefore tend to prevent aggregation, the

Fig. 2. Extraction of gelatin at 90°.

curve fits normally into the series. A control experiment with $\frac{3}{16}$ -inch bones in presence of $N/4$ sodium salicylate showed that the salt had no direct effect on the rate of extraction. The reaction rate was slightly greater than in water, but this could be ascribed to the raising of the boiling point of the water. A similar curve was obtained when the reaction with $\frac{3}{16}$ -inch bones was carried out in $N/4$ sodium chloride solution.

In the hope that some light might thereby be thrown on reactions in heterogeneous systems in general, it was decided to investigate further the relation between these curves. Other investigations have shown that ossein is the chief substance remaining after the decalcification of bones, and there

is reason to suppose that this is an anhydride of gelatin. Hoffmeister [1879] showed that when gelatin has been heated to 130° it is not readily soluble in water, but is apparently converted into an anhydride, from which gelatin can be regenerated completely only after boiling for several hours with water. The authors have been able to confirm this observation of Hoffmeister, and have found that the curves representing this regeneration are very similar to those which represent the formation of gelatin from ossein. The details of these experiments are reserved for a subsequent communication.

The extraction of gelatin from bones consists essentially in the hydrolysis of an anhydride and the solution of the gelatin. Apart from the rate of this process itself there are, however, other controlling factors amongst which the initial surface exposed to the action of water plays a very considerable part.

An estimate of the initial surface area of the decalcified bones was made by determining the average number of particles contained in a gram of substance. Assuming that the density is the same throughout and that the shape of the particles of different sizes is similar, the relative surface can be approximately calculated. It is proportional to $\sqrt[3]{n}$ where n = number of pieces to 1 g. In this way the following numbers were obtained:

Size of particles	Surface area (in arbitrary units)
$\frac{1}{4}$ -inch	3.75
$\frac{1}{8}$ "	5.71
$\frac{1}{16}$ "	9.65
$\frac{1}{32}$ "	13.75

Plotting these values against the estimated initial rate gives the curves shown in Fig. 3. For the $\frac{1}{8}$ -inch and larger sizes the points lie on smooth curves, approximately parabolas. The abnormality of the smaller sizes may probably again be attributed to aggregation.

For the other sizes, $\frac{1}{8}$ -inch and greater, we may write:

$$\text{Initial rate} = kS_0^2.$$

This suggests as a general formula for the group of curves:

$$\frac{dx}{dt} = k \{S_0^2 + f(x)\} (a - x)$$

where x = quantity of gelatin extracted;

a = total quantity of gelatin extractable, and

k = constant.

The simplest assumption to make with regard to the form of $f(x)$, which represents the change in the surface effect as the reaction proceeds, is that

$$f(x) = x.$$

This gives

$$\frac{dx}{dt} = k (S_0^2 + x) (a - x)$$

which on integration gives

$$\frac{1}{a + S_0^2} \log \frac{a(S_0^2 + x)}{S_0^2(a - x)} = kt.$$

This represents a group of curves of the type found experimentally. When the initial surface is small the curve has a point of inflection, but beyond a certain critical value, given by $S_0^2 = a$, the point of inflection disappears. With the smaller sizes the curve approaches that of a unimolecular reaction. This would be expected from the equation when S is large compared with x .

Fig. 3.

Tabulated on p. 528 are corresponding values of x and t taken from the curves and the calculated values for k .

Rate of extraction of gelatin from decalcified bones at 100°.

Size of particles $\frac{1}{4}$ -inch $S_0 = 3.75$			Size of particles $\frac{1}{8}$ -inch $S_0 = 9.65$		
Time in hours	% extracted	k	Time in hours	% extracted	k
1.14	10	.00491	0.23	10	.00470
1.90	20	.00510	0.475	20	.00455
2.6	30	.00504	0.74	30	.00445
3.33	40	.00488	1.02	40	.00440
4.13	50	.00468	1.28	50	.00455
4.95	60	.00456	1.58	60	.00464
7.1	80	.00432	2.32	80	.00496
Mean $k = .00478$			3.28	90	.00470

Mean $k = .00463$

Size of particles $\frac{3}{16}$ -inch $S_0 = 5.71$		
Time in hours	% extracted	k
0.75	10	.00375
1.28	20	.00415
1.73	30	.00440
2.18	40	.00450
2.67	50	.00460
3.50	65	.00460
4.73	80	.00450

Mean $k = .00436$ Average k for different sized particles = .00444.*Rate of extraction of gelatin from decalcified bones at 90°.*

Size of particles $\frac{1}{4}$ -inch $S_0 = 3.75$			Size of particles $\frac{3}{16}$ -inch $S_0 = 5.71$		
Time in hours	% extracted	k	Time in hours	% extracted	k
2.3	10	.00244	1.5	10	.00187
3.9	20	.00248	2.56	20	.00206
5.3	30	.00247	3.63	30	.00210
6.85	40	.00237	4.65	40	.00212
8.4	50	.00230	5.75	50	.00213
10.25	60	.00220	7.0	60	.00211

Mean $k = .00238$ Mean $k = .00206$

Size of particles $\frac{1}{8}$ -inch $S_0 = 9.65$		
Time in hours	% extracted	k
0.53	10	.00202
1.07	20	.00202
1.74	30	.00189
2.45	40	.00183
3.22	50	.00180
4.05	60	.00181
6.35	80	.00182

Mean $k = .00189$ Average k for different sized particles = .00211.

The average temp. coeff. for the interval 90°–100° is 2.1.

In the search for a suitable expression to represent the experimental results an attempt was made to arrive at a more accurate estimate of the initial surface by adsorption experiments. Decalcified bones markedly adsorb iodine and some dyes.

The unexpected result was obtained that the surface was the same independently of the state of division of the bones. The only variation was in

the time required to arrive at equilibrium. This is shown in the curves for iodine adsorption in Fig. 4. In each case 5 g. decalcified bones and 100 cc. $N/50$ iodine were put in a stoppered bottle and shaken. From time to time 10 cc. was withdrawn and titrated with standard thiosulphate. Precisely similar results were obtained with other initial concentrations of iodine and also with diazine-green (a dye of not very great diffusibility and chosen on this account) as adsorbed substance. The conclusion must be drawn therefore

Fig. 4.

that owing to the high porosity of the bone there is the same surface available whatever the size of the particles, and that the different behaviour must be attributed to the time taken for the adsorbed substance to diffuse along the pores and so arrive at the surface.

Similarly in the extraction of gelatin the variation of rate with size of particles is not a true surface effect. The effect is indeed dependent on the apparent surface (original surfaces + fissure surfaces), the dependence being expressed by the formula above. But, except for very small particles, the rate of extraction is controlled largely by the rate at which gelatin diffuses out and water diffuses in through the pores; it is essentially a capillary phenomenon.

REFERENCE.

Hoffmeister (1879). *Zeitsch. physiol. Chem.* 2, 314.