THE SWELLING OF GELATIN AND THE VOLUME OF SURROUNDING SOLUTION.

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The theory of the swelling of gelatin in acid as developed by Procter and Wilson and by Loeb (1) is based on the Donnan equilibrium and assumes that the swelling is due to the osmotic pressure caused by the unequal distribution of the ions of the acid inside and outside of the gelatin. At equilibrium this osmotic pressure for strong monovalent acids is given by the expression

$$2Y + Z - 2X \tag{1}$$

X = concentration acid in supernatant liquid.
Y = " free acid in gelatin.
Z = " non-diffusible ion = concentration Cl^- equivalent to ionized protein.

It follows that with pure gelatin at equilibrium in acid the swelling depends only on the *concentration* of acid outside the gelatin and is independent of the *volume* of the acid. The equilibrium concentration of acid and hence the swelling will depend, however, on the quantity as well as the concentration of the acid originally taken. The quantitative statement of this effect is complicated and has been discussed by Hitchcock (2). Qualitatively, however, the result may be simply predicted. When the swelling of gelatin in HCl is plotted against the pH of the acid there is found a maximum at about pH 3.0. If increasing volume of acid solution having a pH of 3.0 or higher is added to a series of constant amounts of gelatin, therefore, the pH of the solution at equilibrium will approach the initial pH of the acid as more acid is added. The swelling will therefore increase with increasing amount of acid solution and finally reach a maximum constant value corresponding to the pH of the pure acid. This will be true as soon as the amount of acid which combined with the gelatin is negligible compared to the total amount of acid added. If acid of greater strength than pH 3.0 is used the pH will decrease again as the amount of acid solution added is increased and will again become constant when the amount of solution added is so large that there is no change in pH on addition of the gelatin. The swelling will now increase with the volume of solution, reach a maximum (at pH 3.0) and then decrease to a constant value. Since it is necessary for equilibrium that there be some excess solution, *i.e.*, that sufficient solution is used so that some remains after the gelatin is swollen, it would be possible with very strong acid to bring the gelatin to a pH less than 3.0 before there is any excess solution. In this case, after enough solution has been added to furnish an excess of liquid the swelling will decrease as the amount of acid solution added increases. If the swelling obtained in the different series is plotted against the volume of the original acid solution used, therefore, the swelling will increase, decrease or pass through a maximum with increasing volume of solution, depending on the original concentration of the acid solution. Such results have been recorded in the literature (3). If, however, the swelling values from these experiments are plotted against the pH of the solution (either of the gelatin or supernatant solution) at equilibrium, then the swelling should be independent of the volume of solution. According to Küntzel (3), however, this is not the case. Küntzel found that even when the swelling was plotted against the pH of the solution at equilibrium, the swelling increased with increasing volume of the solution. This result is contrary to that expected from the theory for the case of pure acid and gelatin, but is the expected result if the *gelatin contained neutral salts*. The swelling is depressed by neutral salts and hence the less the volume of acid added the greater the depressing effect of the salt. The swelling even at constant pH will therefore increase with the volume of acid added until the salt is so dilute as to have no effect. This is the result obtained by Küntzel (3). Küntzel used gelatin without previous purification and hence salts were present. In order to be sure of this explanation, however, experiments similar to Küntzel's were done with ordinary gelatin and also with isoelectric gelatin. The results with isoelectric gelatin are given in Fig. 1 in which the swelling at equilibrium has been plotted against the volume of acids of different concentration added. As predicted, the swelling with dilute acids increases with the quantity of acid. The swelling in M/50 acid passes through a maximum while stronger acid gives decreasing swelling with increasing volume. When these curves are plotted against pH at equilibrium (either of the gelatin or outside

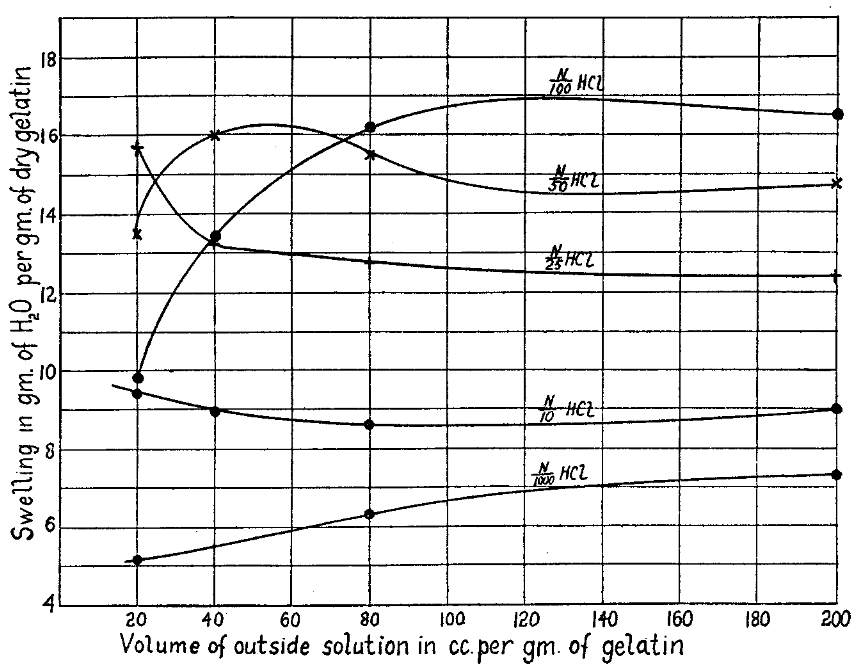

FIG. 1. Swelling of gelatin as determined by the volume of acid of various concentrations.

solution) they coincide as shown in Fig. 2, giving the usual pH swelling curve for gelatin in HCl. When the experiment is performed with unpurified gelatin the results shown in Fig. 3 are obtained which agree with those of Küntzel. As stated above the increase in swelling with increased volume in this case is due to the decreased salt concentration. It may be mentioned that the absolute amount of swelling cannot be compared in experiments done with different samples of gelatin since this property varies markedly in different preparations.

Experimental Procedure.

Cooper's powdered gelatin was used. The isoelectric gelatin was prepared as described previously (4). The experiments were carried out in a water bath at 5°C. 200 cc. of acid was placed in a series of 500 cc. Erlenmeyer flasks and brought to the temperature of the bath. The flasks were equipped with mechanical stirrers which rotated at approximately the same speed. The gelatin was then

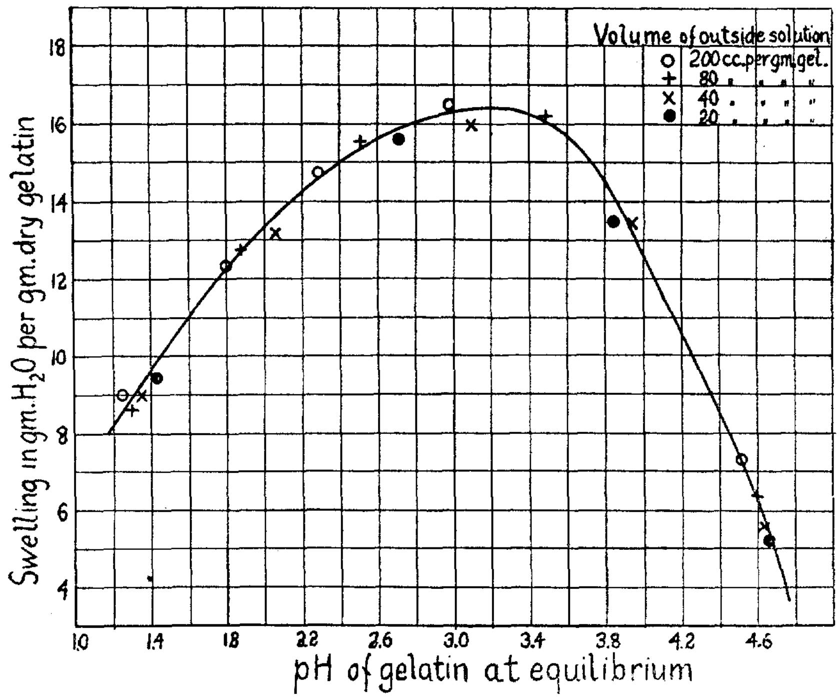

FIG. 2. Swelling of purified gelatin with increasing volume of acid solution as a function of the pH of the gelatin.

added and the suspensions stirred for 5 hours. Preliminary experiments showed that equilibrium was reached in about 2 hours. The gelatin was then filtered off with suction through coarse filter paper and the concentration of the swollen gelatin determined by drying a weighed sample to constant weight at 100°C. Dry weight determinations were also made on the supernatant solution. No appreciable amount of gelatin was found in the supernatant. The pH of the melted gelatin and of the outside solution were determined electrometrically at 30°C.

It may be pointed out that a number of sources of error must be guarded against in swelling experiments of this type. If the temperature is much above 5° there will be some solution of the gelatin and this will increase with the acidity and volume of the solution. The final volume depends to a slight extent on the time required for the swelling to take place, since a true equilibrium is never reached but the mass of gelatin continues slowly to swell indefinitely. If precautions

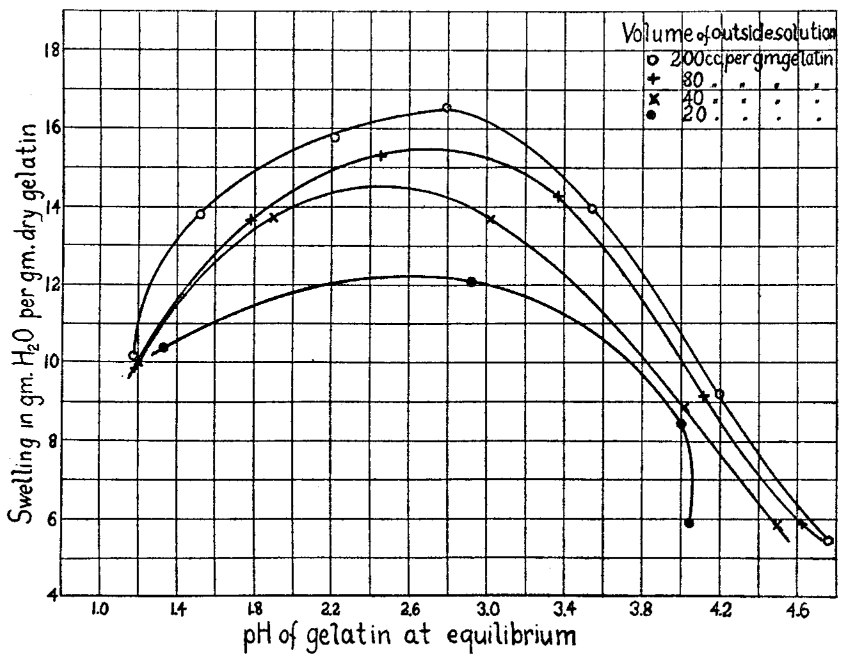

FIG. 3. Swelling of gelatin containing electrolytes with increasing volume of acid solution as a function of the pH of the gelatin.

for rapid stirring are not taken, therefore, the gelatin in a large volume of acid will swell more slowly than that in a small volume and the figure obtained for the equilibrium value will be slightly different.

SUMMARY.

The swelling of isoelectric gelatin added to various volumes of acid of different concentration at 5°C. has been determined. The swelling is determined only by the concentration of the supernatant solution at equilibrium and is independent of the volume of acid.

Similar experiments with *unpurified* gelatin show that in this case, owing to the presence of neutral salts the swelling is a function of the volume as well as the concentration of acid.

Both results are predicted by the Procter-Wilson-Loeb theory of the swelling of gelatin.

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