

STUDIES ON THE REGULATION OF OSMOTIC PRESSURE.

I. THE EFFECT OF INCREASING CONCENTRATIONS OF GELATIN ON THE CONDUCTIVITY OF A SODIUM CHLORIDE SOLUTION.

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INTRODUCTION.

In a study of the factors influencing the regulation of osmotic pressure of the body fluids, the physical properties have been determined in a series of blood sera from normal and pathological individuals. In the case of some patients, there was found a strikingly low percentage of serum protein. The conductivity of the salts in blood with a low percentage of serum protein was high compared with the conductivity of the same salts in normal blood. It occurred to us that possibly the protein molecules offer mechanical interference to the passage of the current through the solution, hence, when the protein content of the solution is diminished the observed conductivity will increase without a corresponding increase in the concentration of ions present. It was to throw light upon this hypothesis that a study of the relation between conductivity and concentration of gelatin was undertaken.

In 1898 Bugarszky and Tangl¹ pointed out that, of the non-conducting substances in the serum, protein is the only one present in amounts sufficient to affect the electrical conductivity. These authors derived a formula by the addition of serum protein in varying quantities to a salt solution of constant conductivity. The serum protein was obtained by dialyzing blood serum for two months and concentrating under reduced pressure. The hydrogen ion concentration was not controlled in their experiments.

¹ Bugarszky, St., and Tangl, F., *Arch. ges. Physiol.*, 1898, lxxii, 531.

EXPERIMENTAL.

Powdered gelatin was used in these experiments and was purified at the isoelectric point according to the method described by Loeb.² The percentage of gelatin in the final solutions was determined by drying samples to constant weight. The hydrogen ion concentration was determined by a gas chain and the conductivity measurements were made with a Kohlrausch bridge at 25°C. The physical methods were standardized by simple inorganic solutions. Duplicate observations were made in each case. The concentrations of gelatin ranged from 0.8 per cent to 6.5 per cent and the experiments were carried out at three different hydrogen ion concentrations. One series of observations was made at pH 5.1, near the isoelectric point of gelatin,

TABLE I.
Conductivity of Pure Gelatin Solutions.

Gelatin. <i>per cent</i>	Conductivity $\times 10^{-4}$ at pH 3.3	Conductivity $\times 10^{-4}$ at pH 5.1	Conductivity $\times 10^{-4}$ at pH 7.4
0.8	6.6	0.3	1.3
1.7	10.7	0.6	2.4
3.3	19.1	1.0	4.2
4.9	25.7	1.3	5.9
6.5	30.9	1.5	7.2

another at 3.3 because of the relatively high ionization of gelatin at that acidity, and finally one at pH 7.4, approximately the reaction of blood. The gelatin was brought to these hydrogen ion concentrations by the addition of HCl or NaOH.

A preliminary experiment, Table I, was carried out on pure gelatin solutions varying in concentration from 0.8 per cent to 6.5 per cent of gelatin. The conductivity of these solutions was determined at the three different hydrogen ion concentrations mentioned above, *viz.* 5.1, 7.4, and 3.3. Fig. 1 shows the concentrations of gelatin as the abscissæ and the specific conductivities $\times 10^{-4}$ as ordinates. The curves at the three reactions are reduced to the same scale and plotted at equal intervals above each other. The conductivity in-

² Loeb, J., *J. Gen. Physiol.*, 1918-19, i, 341.

creases with each increment of gelatin added but the curve is not a straight line in any instance. The actual change in conductivity as the concentration of gelatin increases from 0.8 per cent to 6.5 per

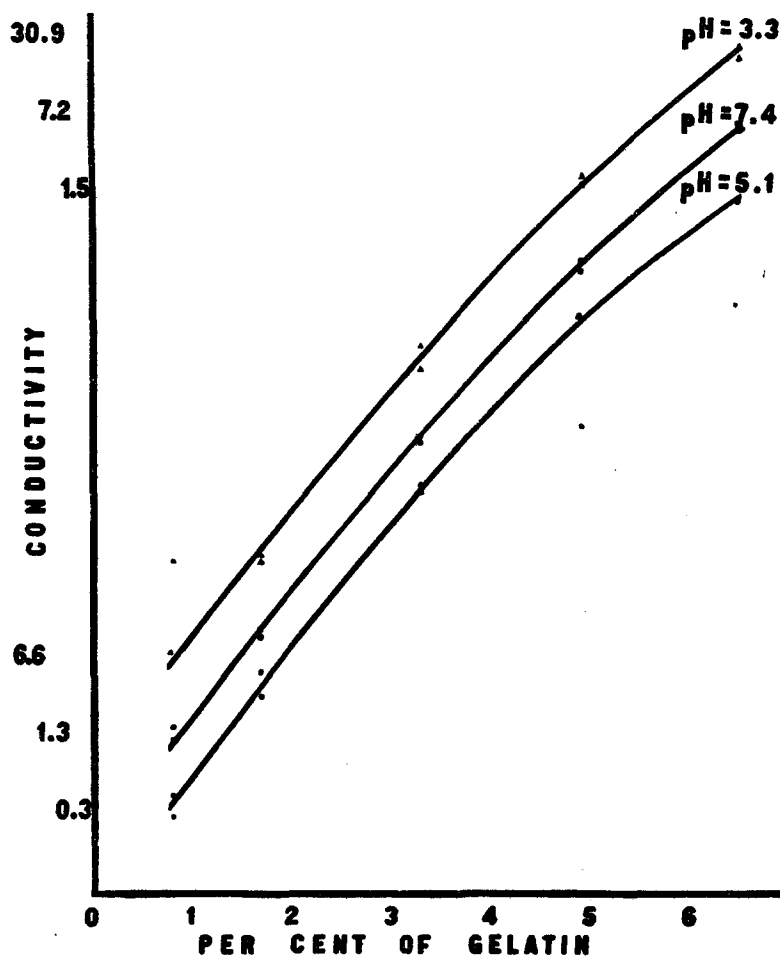


FIG. 1. Conductivities of pure gelatin solutions. The abscissæ represent varying concentrations of gelatin in per cent and the ordinates represent specific conductivities $\times 10^{-4}$. With increase in concentration of gelatin from 0.8 per cent to 6.5 per cent it is seen that the conductivity of gelatin at pH 5.1 increases from 0.3 to 1.5, at pH 7.4 it increases from 1.3 to 7.2 and at pH 3.3 it increases from 6.6 to 30.9.

cent is from 0.3 to 1.5 at pH 5.1 while at 3.3 the range is from 6.6 to 30.9. This difference in ionization with change in hydrogen ion concentration has been thoroughly studied in 1 per cent gelatin solutions by Loeb.³

The next series of experiments, Table II, bears more directly upon the original problem and consists of observations on the effect of gelatin upon the conductivity of a 0.6 per cent sodium chloride solution. The concentrations of gelatin and the reactions of the final solutions were the same as were used in the first experiments. Fig. 2 shows the results plotted on a common scale, conductivities again as ordinates and concentrations of gelatin in per cent as abscissæ. The curves for each pH are appropriately labeled. All three curves

TABLE II.
Conductivity of 0.6 per cent NaCl Solutions with Increasing Gelatin.

Gelatin.	Conductivity $\times 10^{-4}$ at pH 3.3	Conductivity $\times 10^{-4}$ at pH 5.1	Conductivity $\times 10^{-4}$ at pH 7.4
<i>per cent</i>			
0.8	114.5	108.9	109.8
1.7	115.2	106.7	108.2
3.3	117.3	102.6	105.9
4.9	119.8	98.1	104.2
6.5	121.7	94.1	101.0

are apparently straight lines. At pH 3.3 the conductivity increases consistently with the concentration of gelatin; at pH 5.1, however, the conductivity decreases strikingly with increasing amounts of gelatin. At pH 7.4 the conductivity again decreases with each increment of gelatin but the depression of the conductivity is less marked than at pH 5.1. Clearly the addition of gelatin at pH 3.3 to a solution of sodium chloride increases the conductivity of the solution; while the addition of gelatin near the isoelectric point (pH 5.1) decreases the conductivity in proportion to the amount of gelatin added. At the reaction of blood, also, the addition of gelatin to a sodium chloride solution depresses rather than increases the con-

³ Loeb, J., *J. Gen. Physiol.*, 1920-21, iii, 247.

ductivity of the resulting solutions. This latter result is evidence in favor of our original hypothesis that decreasing the percentage of protein in blood serum increases the conductivity of that serum.

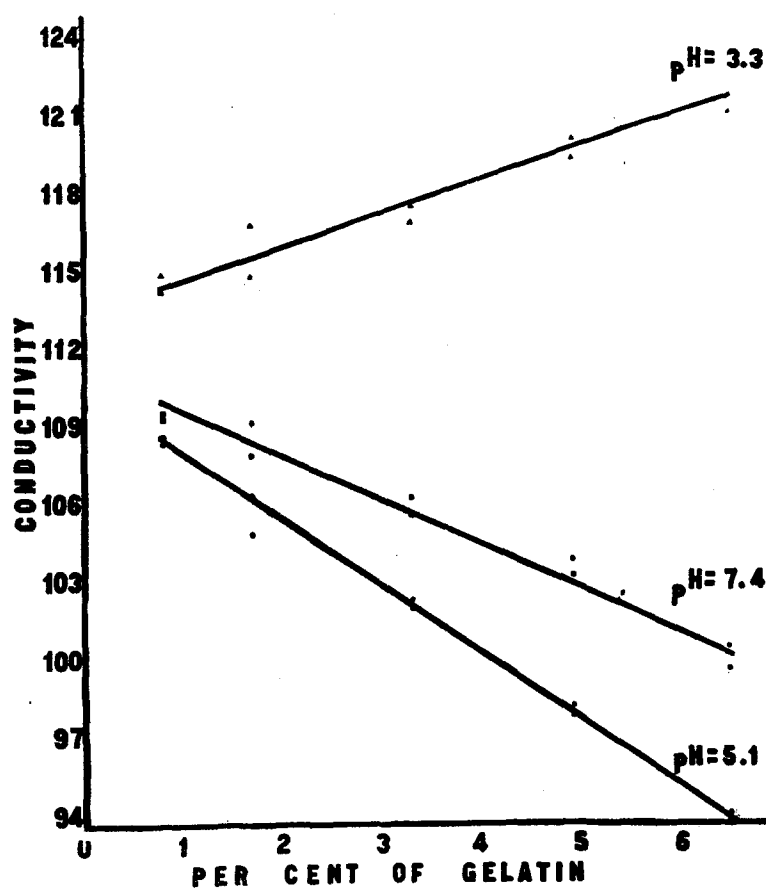


FIG. 2. Changes in conductivity of 0.6 per cent NaCl solutions with increasing concentration of gelatin (from 0.8 per cent to 6.5 per cent) at various hydrogen ion concentrations. At pH 3.3, the specific conductivity $\times 10^{-4}$ increases from 114.5 to 122. At pH 5.1, the conductivity decreases from 108.8 to 94.2. At pH 7.4 the conductivity decreases from 109.7 to 101.

DISCUSSION.

The results charted in Fig. 2 may be explained as follows. The addition of gelatin to a solution of sodium chloride has two possible effects, which tend to oppose each other, (1) to increase the conductivity by the addition of ionized gelatin salts and (2) to decrease the conductivity by the mechanical interference of the large undissociated gelatin molecules. It could be predicted from the newer conceptions of the physical chemistry of the proteins that the first of these effects would be considerably greater at pH 3.3 than at pH 5.1, which is near the isoelectric point of gelatin and furthermore, it would be expected that the results obtained at pH 7.4 would be more nearly like the results observed at pH 5.1 than like those observed at pH 3.3. Whether the addition of gelatin at a given hydrogen ion concentration increases the conductivity or depresses it depends, therefore, upon the degree of ionization of the gelatin at that reaction. It is obvious that at the reaction of blood, the pure protein, gelatin, is so little ionized that the mechanical interference predominates and the conductivity decreases with each increment of gelatin.

CONCLUSIONS.

1. In pure gelatin solutions the conductivity of the solution increases with increasing concentrations, regardless of the hydrogen ion concentration. The actual value of the specific conductivity is greater at that reaction where the degree of ionization is greater.

2. The addition of gelatin in increasing concentrations to a 0.6 per cent sodium chloride solution affects the conductivity of that solution in two ways: (a) At pH 3.3, (where gelatin is highly ionized) the conductivity increases with each added increment of gelatin. (b) At pH 5.1 and 7.4 (where gelatin is less highly ionized) the conductivity decreases with each added increment of gelatin.

A similar study is being made of crystalline egg albumin.