CHEMICAL ANTAGONISM OF IONS.

III. EFFECT OF SALT MIXTURES ON GELATIN ACTIVITY.

By HENRY S. SIMMS.

(From the Department of Animal Pathology of The Rockefeller Institute for Medical Research, Princeton, N. J.)

(Accepted for publication, December 16, 1928.)

I

INTRODUCTION.

Two previous papers¹ showed that the anomalous effect of Mg^{++} ions² on the activity of oxalate diions ($C_2O_4^{--}$) could be counteracted by the addition of salts of Na^+ or K^+ . A similar antagonism was found between SO_4^{--} and Cl^- , as well as between Mg^{++} and SO_4^{--} in their effect on the oxalate diion. No antagonism was found between Na^+ and K^+ .

In this paper we will extend our observations to the effect of single salts and of salt mixtures upon the ionic activity of gelatin.³ It will be shown that, of the three ions Na^+ , K^+ and Mg^{++} , each has its individual effect when present alone with gelatin; and that the effect of a mixture of two or more of these cations is quite different from the effect of one alone.

The experimental data were obtained in the pH range from 6.7 to 7.6 in order to be near the physiological range, the pH of the solutions without salt being 7.367. An accuracy reproducible to 0.001 to 0.003 pH was obtained, although with difficulty.

The ionizable groups of gelatin⁴ are given below together with their degree of ionization at pH 7.367, which was the pH of the gelatin solution without salts in our experiments.

¹ Simms, H. S., *J. Gen. Physiol.*, 1928, xii, 241, 259.

² Simms, H. S., *J. Phys. Chem.*, 1928, xxxii, 1121, 1495.

³ Simms, H. S., *J. Gen. Physiol.*, 1928, xi, 613.

⁴ Simms, H. S., *J. Gen. Physiol.*, 1928, xi, 629.

Acid groups (ionized at pH's higher than pG')	pG' values ⁵	100 α = per cent of ionization	Basic groups (ionized at pH's lower than pG')	pG' values ⁵	100 α = per cent of ionization
Aspartic and glu- tamic acids (R — COOH)	3.5	100	Prearginine ⁴	4.6	0
(Tyrosine R Φ — OH)	9.4	1	Histidine	6.1	5
			Arginine	8.1	84
			Lysine	10.6	100

Arginine is the only group having appreciable amounts of both the ionized and the non-ionized form at this pH (84 and 16 per cent respectively).

To express this in a different way: the change in pH of the gelatin solutions due to addition of salt is an indication of a change in the ionic activity of one or more of the ionic species present. There are only two ionic species of the gelatin present in appreciable quantities;⁶ one in which the arginine group is ionized, the other in which it is non-ionized. The other groups remain essentially the same.

The same statements should apply to any other protein in this range and our results should be typical of proteins in general. At present we have not made observations in other pH ranges.

II.

RESULTS.

A. Solutions Containing Na⁺ and K⁺ Ions.

The effect of Na⁺ (as NaCl) on Na gelatinate may be seen in Figs. 1 and 2A. All the solutions of Na gelatinate contained 1.25 per cent gelatin to which enough NaOH has been added to bring the pH to 7.367 without addition of salt. The pH of this solution is seen to decrease very rapidly with small additions of NaCl; the influence of

⁵ The pG' values (titration indices) represent the mid-points of the buffer ranges due to the respective groups. Simms, H. S., *J. Am. Chem. Soc.*, 1926, **xlvi**, 1239.

⁶ We neglect the histidine group which is only 5 per cent ionized. That this is permissible is shown by the data on K gelatinate with a salt-free pH of 7.203 where the histidine group is 7 per cent ionized and the arginine group is 89 per cent ionized.

further additions becomes less and less as the concentration of salt increases—but the effect up to above 1.0 molar is to *decrease the pH*.

KCl on the other hand *first decreases* the pH until about 0.01 molar K^+ ion is present. The curve then reverses and further additions of KCl *increase* the pH. This is true whether the KCl is added to Na

FIG. 1. Effect of NaCl and KCl on the pH of Na gelatin and K gelatin.

We have plotted the square root of the total ionic strength, including that of the Na (or K) gelatin (0.0032μ). The solid lines represent data with Na gelatin; the broken lines with K gelatin. The two lighter lines are for the effect of one type of chloride on the opposite type of gelatin.

The Z-shaped curves are for solutions containing appreciable amounts of both cations, one being constant and the other varied.

gelatinate or to K gelatinate⁷ (see Figs. 1 or 3A), although in the former case the initial decrease (up to 0.01 M K⁺ ion) is much greater. Above 0.01 M K⁺ ion concentration the two curves are seen to be parallel.

The Na gelatinate contains 0.0032 M Na⁺ ion. In order to observe the effect of more Na⁺ ion we then used Na gelatinate containing 0.0125 NaCl (or a total of 0.0157 M Na⁺). Addition of KCl then produces quite the opposite effect. Up to about 0.09 M K⁺ concentration KCl *increases* the pH; a slight addition of KCl then *decreases* the pH, up to 0.125 M K⁺; and beyond that further additions *increase* the pH.

The effect of NaCl on K gelatinate is seen to be essentially the same as its effect on Na gelatinate (*i.e.*, a consistent decrease in pH).

The effect of NaCl on K gelatinate containing 0.050 M KCl is almost identical with the reverse process described in the second paragraph above (*i.e.*, first an increase, then a decrease and finally an increase in pH). Furthermore the concentrations of Na⁺ ion at the two critical points agree with those of K⁺ ion described above, namely, 0.09 M and 0.125 M. It will be seen below that the solutions containing Mg⁺⁺ have critical points nearly identical with these.

B. Solutions Containing Mg⁺⁺, Na⁺ and K⁺ Ions.

We now pass to solutions containing Mg⁺⁺ ions in addition to Na⁺ or K⁺ or both. The curve for the effect of MgCl₂ on gelatin is given in both Figs. 2A and 3A. We used Na gelatinate for this experiment and hence have 0.0032 μ of Na⁺ ion present. Should we use Mg gelatinate the curve would undoubtedly fall considerably to the left as shown by the extrapolation of the other curves in Figs. 2A or 2B to zero concentration of Na⁺ ion.

If we take solutions of Na gelatinate and add a definite amount of MgCl₂ plus various amounts of NaCl, the effect up to 0.10 molar Na⁺ ion is to *increase* the pH. At 0.10 molar there is a sharp break and a

⁷ The K gelatinate was prepared like the Na gelatinate except that KOH was used instead of NaOH. The pH without salt was 7.203. Hence 0.164 was added to all these values plotted in Figs. 1 and 3 in order that the data might be compared with the Na gelatinate data.

slight further addition of NaCl causes a marked *decrease* in pH. At 0.15 molar Na^+ ion this effect ceases and further additions of NaCl have very slight effect on the pH. This is demonstrated by the lighter curves in Fig. 2A. In Fig. 2B are plotted the differences between these curves and the curve of NaCl alone (*i.e.*, the observed

FIG. 2A. Effect of NaCl and MgCl_2 on Na gelatin.

The square root of the total ionic strength is plotted against pH. The two heavy lines represent the effect of NaCl or MgCl_2 alone on Na gelatin. In the latter case the presence of the 0.0032 M Na^+ ion has doubtless affected the curve. Extrapolation of the curves for the mixtures shows that in the absence of Na^+ , MgCl_2 would cause a much more marked depression of the pH.

The lighter curves show the effect of addition of NaCl to Na gelatin containing a fixed amount of MgCl_2 .

pH with MgCl_2 and NaCl , minus the pH with NaCl alone at the same ionic strength). It is obvious that in the presence of MgCl_2 the addition of NaCl produces breaks in the curves at definite concentrations which are independent of the amount of MgCl_2 present (at least from 0.0042 to 0.17 M MgCl_2). Comparing the curves in the higher concentrations of NaCl , it is evident that in this range, addition of MgCl_2 lowers the pH.

It might be added that the effect on pH is not due to the sequence of addition of the salts. Three solutions were prepared with the same amounts of NaCl , MgCl_2 and Na gelatinate, but the components were

FIG. 2B. Effect of $\text{Na}^+ - \text{Mg}^{++}$ mixtures relative to that of NaCl alone.

The values for ΔpH represent the observed pH values minus the pH with the same ionic strength of NaCl . These are plotted against the square root of the Na^+ concentration. (The Mg^{++} is not included.) It is seen that the "breaks" are independent of the amount of MgCl_2 .

mixed in different order in each case. All three had essentially the same pH.

The effect of KCl on gelatin containing MgCl_2 is almost identical up to 0.12 M K^+ ion with the effect of NaCl (compare Fig. 3A with

FIG. 3A. Effect of KCl and MgCl_2 on the pH of Na gelatin and K gelatin. Also the effect of a mixture of Mg, Na and K.

Curves are given for the effects of MgCl_2 alone on Na gelatin, KCl alone on Na gelatin and KCl alone on K gelatin (indicated "Na-free").

The effects of KCl on Na gelatin containing fixed amounts of MgCl_2 are represented by solid Z-shaped curves. Using K gelatin gives the Z-shaped curve marked "Na-free" (dash line).

The effect of appreciable amounts of all three cations is given by the dotted line.

2A and Fig. 3B with 2B). Above the point of the second break (0.12 M K^+ ion) the curves in the case of KCl go to the right (higher pH) which is not surprising since KCl alone in higher concentrations causes an increase in pH.

In Fig. 3B we have plotted the difference between the curves with $MgCl_2$ plus KCl and the curve with KCl alone. This gives the effect of the mixture, and eliminates the effect of KCl alone. The dash line gives the observations on the addition of KCl to K gelatinate containing

FIG. 3B. Effect of $K^+ - Mg^{++}$ mixtures or a $K^+ - Na^+ - Mg^{++}$ mixture, relative to that of KCl alone.

The values of ΔpH equal the observed pH values minus the pH with the same ionic strength of KCl (on Na gelatinate or K gelatinate as the case may be). These are plotted against the square root of K^+ plus Na^+ concentration. (The Mg^{++} is not included.) It is seen that the "breaks" are independent of the amount of $MgCl_2$. (Cf. Fig. 2B.)

MgCl_2 . Since no Na^+ was present the effect of this ion does not enter in, but it will be seen that this curve is essentially like those in Fig. 2B (with Na gelatinate and NaCl).

The *heavy* lines in Fig. 3B (also 3A) represent data on Na gelatinate and hence contain 0.0032 M Na^+ ion. These are seen to be essentially like that with K gelatinate. The *dotted* line represents similar solutions with an added amount of NaCl, raising the Na^+ ion concentration to 0.0157 M. This curve is essentially like the others except that the critical points come at slightly lower concentration of $[\text{Na}^+ + \text{K}^+]$, namely at 0.07 M $[\text{Na}^+ + \text{K}^+]$ and at 0.08 M $[\text{Na}^+ + \text{K}^+]$. There is a third break at 0.12 M, but not a sharp one.

III.

DISCUSSION.

The above observations, while incomplete, may throw light on some of the physiological anomalies, such as the antagonistic effects of cations; the failure of Na^+ alone or K^+ alone to supplant a mixture of the two in physiological systems; and the unequal distribution of ions between the various physiological fluids, secretions and tissues in a given organism.

The essential qualitative generalizations to be made from the above observations are:

1. Mixtures of NaCl and KCl behave differently from either salt alone, in their effect on gelatin activity.
2. Mixtures of MgCl_2 and NaCl (or KCl) behave differently from these salts alone, the effect being slightly different if KCl is used in place of NaCl.
3. Mixtures containing all three cations, Mg^{++} , Na^+ and K^+ in appreciable quantities, behave similarly to Mg^{++} plus NaCl or KCl, but the curve is complicated by three breaks instead of two.

IV.

THEORY.

Before attempting to explain the above phenomena, we may remind the reader that previous observations^{2,8} have shown a tendency of cations to affect the ionic activity of simple amines and ampholytes, in

⁸ Simms, H. S., *J. Phys. Chem.*, 1929, xxxiii, in press.

a manner not in agreement with the Debye-Hückel theory and corresponding to the complex salts observed by Pfeiffer and collaborators.⁹ These deviations with gelatin also agree with the combinations of ions with proteins found by Northrop and Kunitz¹⁰ in a different manner.

It would seem that the various cations have an affinity for certain portions of the protein molecule but it is at first difficult to understand how two cations tending individually to produce one effect can produce the opposite effect in a mixture.

Let us consider the gelatin in this pH range to consist of two ionic species. The first, which we will call *A*, has its arginine group ionized, and predominates at lower pH values. The second, *B*, has its arginine group non-ionized, and predominates at higher pH.

Let us assume, for example, that in a given concentration, the tendency for Na⁺ ion to combine with *A* is such as to produce negligible effect on its activity, while Na⁺ reduces the activity of *B* to 0.9; then its effect on the arginine dissociation index (8.1) of gelatin would be represented by the logarithm of the ratio of γ_B/γ_A . The change in pH would in this range equal the change in pK; hence:

$$\Delta\text{pH} = \Delta \text{pK}' = \log \gamma_B/\gamma_A$$

or

$$\Delta\text{pH} = \log 0.9/1.0 = -0.05.$$

Let us assume furthermore that a given concentration of Mg⁺⁺ ion reduces the activity of *A* to 0.5 and the activity of *B* to 0.3; then

$$\Delta\text{pH} = \log 0.3/0.5 = -0.22.$$

Thus Mg⁺⁺ produces an effect in the same direction as Na⁺.

Let us assume thirdly that in a mixture of the same amounts of Na⁺ and Mg⁺⁺ ions the species *A* is affected principally by Mg⁺⁺ ion ($\gamma_A = 0.5$) and that *B* is affected principally by Na⁺ ion ($\gamma_B = 0.9$). It is then obvious that the effect on the pH will be in the *opposite direction* from the effect with Na⁺ or Mg⁺⁺ alone, since

⁹ Pfeiffer, P., and Wittha, F., *Ber. chem. Ges.*, 1915, xlviii, 1289. Pfeiffer, P., Würgler, J., and Wittha, F., *Ber. chem. Ges.*, 1915, xlviii, 1938; *Z. physik. Chem.*, 1924, cxxxiii, 22; cxxxiv, 180; cxxxv, 16; cxliii, 265.

¹⁰ Northrop, J. H., and Kunitz, M., *J. Gen. Physiol.*, 1928, xi, 481.

$$\Delta\text{pH} = \log 0.9/0.5 = +0.25.$$

The first two of the above assumptions are legitimate and reasonable. The third assumption is made to agree with the observations. If it is correct it means that in a mixture of Na^+ and Mg^{++} these ions do not combine with the two ionic species of gelatin in the same ratio that they do when present alone, but that Na^+ combines more with one ionic species and Mg^{++} more with the other. (Although this might be accounted for with Na^+ and Mg^{++} at low concentrations on the basis of the charge, such an explanation would fail in the case of Na^+ and K^+ .)

V.

EXPERIMENTAL.

2.50 per cent gelatin solutions were made up in 250 cc. amounts, and containing 16 cc. 0.100 M NaOH, or KOH. These were used as mother solutions in all measurements.

For each observation 5.00 cc. of mother solution was placed in a 10 cc. volumetric flask; the requisite amounts of the various salts were added, and the solution made up to 10.00 cc. (1.25 per cent gelatin). The salts were added volumetrically from stock solution in three concentrations (0.025μ , 0.25μ and 2.50μ).

The pH of each solution was measured at 25.0°C. in a water-jacketed hydrogen electrode of the bubbling type.¹¹

The accuracy of the measurements needs mentioning. It is difficult to get accurate readings on protein solutions in this range, but by frequent and careful plating of the electrodes, and by passing the hydrogen over red hot copper, it was possible to make readings reproducible in general to 0.0001 volt (0.001 pH). As an illustration, fresh mother solution with NaOH was made up six times, and each time the pH without salt was exactly 7.367. (Two solutions with KOH had the same pH of 7.302.)

The readings with salts were discarded if at least two consecutive readings out of three readings on each solution did not agree within 0.0002 volt (0.003 pH). Furthermore all data which from the nature of the curve seemed doubtful were repeated, and were discarded if they were not corroborated. New solutions were then observed with new electrodes.

The data are given in Tables I to XI.

¹¹ Simms, H. S., *J. Am. Chem. Soc.*, 1923, xlv, 2503.

TABLE I.
Effect of NaCl on Na Gelatinate.

C_{NaCl}	pH	C_{NaCl}	pH	C_{NaCl}	pH
0	7.367	0.025	7.120	0.500	6.954
0.0025	7.284	.050	7.073	.750	6.921
.0050	7.243	.075	7.041	1.000	6.907
.0075	7.213	.125	7.018	1.125	6.903
.0125	7.164	.250	6.987	1.250	6.900

TABLE II.
Effect of KCl on Na Gelatinate.

C_{KCl}	pH	C_{KCl}	pH	C_{KCl}	pH
0	7.367	0.025	6.927	0.500	7.147
0.0025	7.283	.050	6.973	.750	7.176
.0050	7.103	.075	7.025	1.000	7.249
.0075	6.968	.125	7.046	1.125	7.267
.0125	6.949	.250	7.086	1.250	7.311

TABLE III.
Effect of KCl on K Gelatinate.
0.164 is added to the following pH values to give those plotted in Figs. 1 and 3A.

C_{KCl}	pH	C_{KCl}	pH	C_{KCl}	pH
0	7.203	0.025	7.178	0.500	7.281
0.0025	7.169	.050	7.209	.750	7.318
.0050	7.152	.075	7.225	1.000	7.374
.0075	7.135	.125	7.256	1.125	7.406
.0125	7.156	.250	7.269	1.250	7.436

TABLE IV.
Effect of NaCl on K Gelatinate.
0.164 is added to these pH values to give those plotted in Fig. 1.

C_{NaCl}	pH	C_{NaCl}	pH	C_{NaCl}	pH
0	7.203	0.025	7.068	0.500	6.921
0.0025	7.137	.050	7.032	.750	6.895
.0050	7.115	.075	7.005	1.000	6.873
.0075	7.099	.125	6.968	1.125	6.863
.0125	7.081	.250	6.941	1.250	6.846

TABLE V.

Effect of KCl on Na Gelatinate Containing 0.0125 M NaCl.

(pH without salt = 7.367)

 μ for each solution equals $C_{KCl} + 0.0157$.

C_{KCl}	pH	C_{KCl}	pH
0	7.164	0.112	7.281
0.0125	7.318	.125	7.203
.025	7.419	.250	7.354
.050	7.499	.500	7.416
.075	7.549	.750	7.453
.087	7.563	1.000	7.548
.100	7.359		

TABLE VI.

Effect of NaCl on K gelatinate Containing 0.050 M KCl.

(pH without salt = 7.203)

0.164 is added to these pH values to give those plotted in Fig. 1.

 μ for each solution equals $C_{NaCl} + 0.0532$.

C_{NaCl}	pH	C_{NaCl}	pH
0	7.213	0.125	7.042
0.0125	7.249	.250	7.117
.0250	7.298	.500	7.164
.0500	7.393	.750	7.198
.0750	7.446		

TABLE VII.

Effect of $MgCl_2$ on Na Gelatinate.

C_{MgCl_2}	pH	C_{MgCl_2}	pH	C_{MgCl_2}	pH
0	7.367	0.100	7.108	0.750	6.942
0.0125	7.293	.125	7.074	1.250	6.910
.050	7.203	.500	6.963		

TABLE VIII.
Effect of $MgCl_2$ Plus $NaCl$ on Na Gelatinate Containing $MgCl_2$.
 (pH without salt = 7.367)

A. 0.00416 M $MgCl_2$ plus various amounts of $NaCl$ ($\mu = C_{NaCl} + 0.0157$)					
C_{NaCl}	pH	ΔpH^*	C_{NaCl}	pH	ΔpH^*
0	7.285	0.117	0.100	7.438	0.411
0.0125	7.305	.177	.125	7.287	.274
.0250	7.306	.216	.250	7.184	.201
.0500	7.331	.287	.500	7.149	.200
.0750	7.414	.373	.750	7.101	.177
.0875	7.497	.463	1.000	7.083	.174
B. 0.0167 M $MgCl_2$ plus various amounts of $NaCl$ ($\mu = C_{NaCl} + 0.0532$)					
C_{NaCl}	pH	ΔpH^*	C_{NaCl}	pH	ΔpH^*
0	7.203	0.131	0.125	7.296	0.294
0.0125	7.516	.461	.250	7.120	.141
.025	7.526	.485	.500	7.054	.110
.050	7.543	.518	.750	7.021	.100
.075	7.563	.545			
C. 0.0416 M $MgCl_2$ plus various amounts of $NaCl$ ($\mu = C_{NaCl} + 0.128$)					
C_{NaCl}	pH	ΔpH^*	C_{NaCl}	pH	ΔpH^*
0	7.074	0.057	0.112	7.167	0.178
0.0125	7.151	.138	.125	6.977	-.010
.025	7.169	.159	.250	6.922	-.045
.050	7.186	.184	.500	6.905	-.048
.075	7.205	.209	.750	6.845	-.072
.100	7.319	.328	1.000	6.827	-.076
D. 0.167 M $MgCl_2$ plus various amounts of $NaCl$ ($\mu = C_{NaCl} + 0.503$)					
C_{NaCl}	pH	ΔpH^*	C_{NaCl}	pH	ΔpH^*
0	6.963	0.013	0.125	6.780	-.156
0.0125	7.384	.436	.250	6.746	-.180
.025	7.413	.467	.500	6.724	-.185
.050	7.465	.521	.750	6.711	-.190
.075	7.523	.583	1.000		

* The values of ΔpH in this table equal the above pH value minus the pH of the same ionic strength of Na gelatinate plus $NaCl$ (Fig. 1 or 2A).

TABLE IX.
Effect of KCl on Na Gelatinate Containing MgCl₂.
 (pH without salt = 7.367)

A. 0.0167 M MgCl ₂ plus various amounts of KCl ($\mu = C_{\text{KCl}} + 0.0532$)					
C_{KCl}	pH	ΔpH^*	C_{KCl}	pH	ΔpH^*
0	7.203	0.131	0.125	7.193	0.130
0.0125	7.563	.560	.250	7.311	.212
.025	7.600	.580	.500	7.452	.300
.050	7.585	.550	.750	7.548	.347
.075	7.416	.370			
B. 0.0416 M MgCl ₂ plus various amounts of KCl ($\mu = C_{\text{KCl}} + 0.128$)					
0	7.074	0.057	0.125	6.856	-0.230
0.025	7.174	.120	.250	7.133	+ .016
.050	7.227	.164	.500	7.250	.083
.075	7.301	.230	.750	7.421	.205
.100	7.371	.292	1.000	7.491	.220
.112	7.258	.176			
C. 0.167 M MgCl ₂ plus various amounts of KCl ($\mu = C_{\text{KCl}} + 0.503$)					
0	6.963	0.013	0.125	6.773	-0.394
0.0125	7.398	.252	.250	6.861	-.330
.025	7.450	.302	.500	6.927	-.316
.050	7.514	.360	.750	6.956	-.344
.075	7.416	.260			

* The values of ΔpH in this table equal the above pH values minus the pH of the same ionic strength of Na gelatinate plus KCl (Fig. 1).

TABLE X.

Effect of KCl on Na Gelatinate Containing 0.0125 M NaCl plus 0.00417 M MgCl₂.
(pH without salt = 7.367)

μ for each solution equals $C_{\text{KCl}} + 0.0282$.

C_{KCl}	pH	ΔpH^*	C_{KCl}	pH	ΔpH^*
0	7.305	0.378	0.100	7.441	0.394
0.0125	7.372	.420	.125	7.457	.400
.0250	7.398	.425	.250	7.484	.387
.0500	7.470	.452	.500	7.504	.354
.0625	7.323	.295	.750	7.514	.320
.0750	7.370	.334	1.000	7.538	.288
.0875	7.406	.363			

* The values of ΔpH in this table equal the above pH values minus the pH of the same ionic strength of Na gelatinate plus KCl (Fig. 1 or 3A).

TABLE XI.

Effect of KCl on K Gelatinate Containing 0.0167 M MgCl₂.

(pH without salt = 7.203)

0.164 is added to these pH values to give those plotted in Figs. 3A and 3B.

μ for each solution equals $C_{\text{KCl}} + 0.0532$.

C_{KCl}	pH	ΔpH^*	C_{KCl}	pH	ΔpH^*
0	7.014	-0.194	0.125	7.154	-0.113
0.0125	7.035	-.183	.250	7.193	-.078
.0250	7.073	-.153	.500	7.298	+.012
.0500	7.171	-.073	.750	7.350	+.020
.0750	7.289	+.030			

* The values of ΔpH in this table equal the above pH values minus the pH of the same ionic strength of K gelatinate plus KCl (Fig. 1 or 3A). It will be noted that these solutions are free from Na.

VI.

SUMMARY.

1.25 per cent gelatin solutions containing enough NaOH to bring them to pH 7.367 (or KOH to pH 7.203) were made up with various concentrations of NaCl, KCl and $MgCl_2$, alone and in mixtures, up to molar ionic strength. The effects of these salts on the pH were observed.

$MgCl_2$ and NaCl alone *lower* the pH of the Na gelatinate or the K gelatinate, in all amounts of these salts.

KCl first *lowers* the pH (up to 0.01 M K^+), then *raises* the pH.

Mixtures of NaCl and KCl (up to 0.09 M of the salt whose concentration is varied) *raise* the pH; then (up to 0.125 M Na^+ or K^+) *lower* the pH; and finally (above 0.125 M) behave like KCl alone.

Mixtures of $MgCl_2$ and NaCl *raise* the pH up to 0.10 M Na^+ , and *lower* it up to 0.15 M Na^+ *regardless of the amount of $MgCl_2$* . Higher concentrations of NaCl have little effect, but the pH in this range of NaCl concentration is lowered with increase of $MgCl_2$.

Mixtures of $MgCl_2$ and KCl behave as above described (for $MgCl_2$ and NaCl) and the addition of NaCl *plus* KCl to gelatin containing $MgCl_2$ produces essentially the same effect as the addition of either alone, except that the first two breaks in this curve come at 0.07 M and 0.08 M $[Na^+ + K^+]$ and there is a third break at 0.12 M.

In this pH range the free groups of the dicarboxylic acids and of lysine are essentially all ionized and the prearginine and histidine groups are essentially all non-ionized. The arginine group is about 84 per cent ionized. Hence we are studying a solution with two ionic species in equilibrium, one with the arginine group ionized, and one with it non-ionized.

It is shown that the effect of each salt alone depends upon the effect of the cation on the activity of these two species due to combination. The anomalous effects of cation mixtures may be qualitatively accounted for if one or both of these species fail to combine with the cations in a mixture in proportion to the relative combination in solutions of each cation alone.

Special precautions were taken to ensure accuracy in the pH measurements. The mother solutions gave identical readings to 0.001 pH and the readings with salts were discarded when not reproducible to 0.003 pH. All doubtful data were discarded.