

CHEMICAL ANTAGONISM OF IONS.

IV. EFFECT OF SALT MIXTURES ON GLYCINE ACTIVITY.

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I.

INTRODUCTION.

The three previous papers of this series¹ demonstrated that Na⁺ ions counteract the anomalous effect of Mg⁺⁺ ions on the activity of oxalate di-ions (C₂O₄⁻); and also that Cl⁻ ions counteract the effect of SO₄⁼ ions on oxalate di-ions. Furthermore, NaCl, KCl, or MgCl₂ each affect gelatin activity in the same manner when only one of these salts is present. Each causes a *lowering* of the pH. However, the addition of a small amount of NaCl or KCl to a gelatin solution already containing another of the above salts causes a very marked *rise* in pH (an effect opposite to that produced by one salt alone). This occurs up to 0.10 molar Na⁺ or K⁺ ion concentration. Further additions cause a sharp lowering of pH up to 0.15 molar Na⁺ or K⁺. Beyond that there is little effect.

The analogy of these observations to physiological antagonisms is obvious. In order to investigate the mechanism it is desirable to see if still simpler substances than gelatin behave in the same way. In the present paper it will be shown that NaCl, KCl, MgCl₂, and CaCl₂, singly and in mixtures, affect the activity of glycine (a simple amino acid) in practically the same manner that they affect gelatin activity.

II.

RESULTS.

The results obtained in these experiments are obvious from the accompanying figures. Fig. 1 shows the influence of salts on the pH

¹ Simms, H. S., *J. Gen. Physiol.*, 1928, xii, 241, 259; 1929, xii, 511.

of a 0.0100 molar solution of glycine containing one-half equivalent of NaOH. NaCl alone or KCl alone *lower* the pH in the manner shown. However, if we start with 0.00416 molar KCl and add increasing amounts of NaCl, we get first a *rise* in pH until 0.007 M NaCl is present. Then there is a sharp break and further additions *lower* the pH until 0.035 M NaCl is present. Further additions of NaCl *increase* the pH as shown.

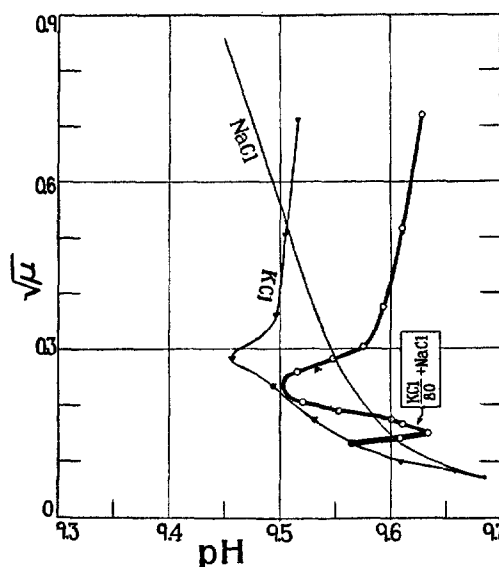


FIG. 1. Antagonistic effects of KCl + NaCl mixtures compared with the effects of KCl or NaCl alone on the pH of glycine solutions (containing 0.5 equivalent of NaOH). Note that both cations are monovalent.

Fig. 2 shows that the same phenomena take place when NaCl is added to glycine solutions containing $MgCl_2$. The breaks come at 0.015 molar and 0.085 molar NaCl concentration *regardless of the amount of $MgCl_2$ present*. This is the same result that was observed with gelatin.

Fig. 3 shows that $CaCl_2 + NaCl$ mixtures behave essentially the same as $MgCl_2 + NaCl$ mixtures and $KCl + NaCl$ mixtures.

Fig. 4 shows the effect of adding $CaCl_2$ to glycine solution containing $MgCl_2$. The same sort of curve is produced as is found with the other

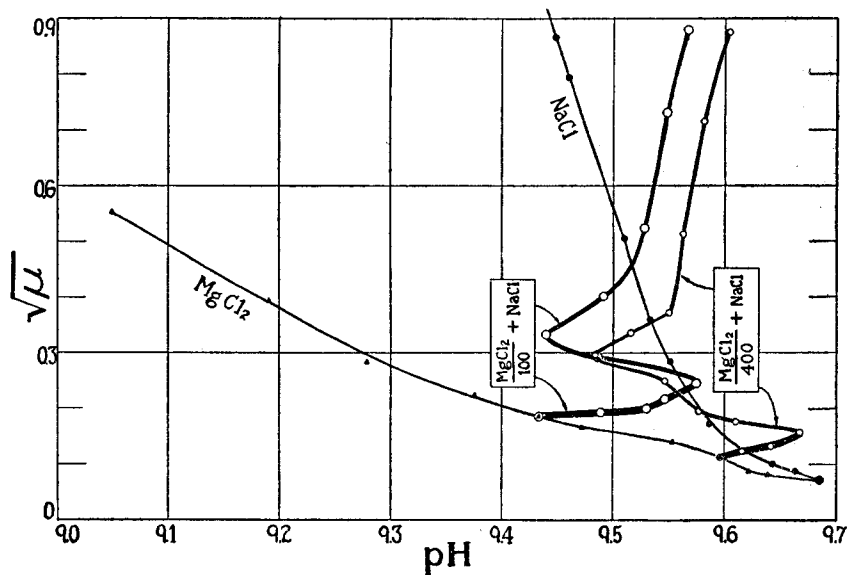


FIG. 2. Effect of $MgCl_2 + NaCl$ mixtures on the pH of glycine solutions, compared with the effect of each salt alone. One divalent and one monovalent cation.

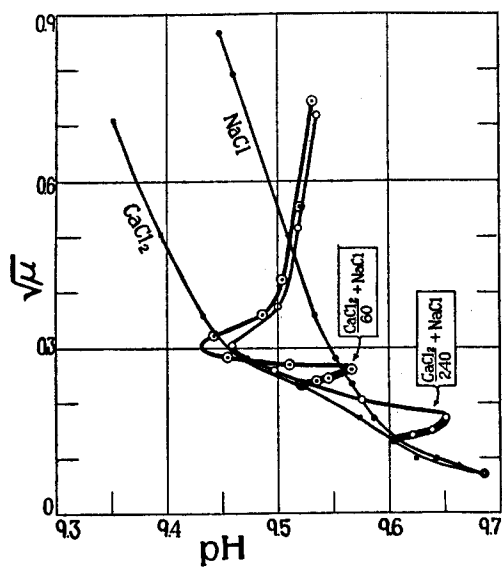


FIG. 3. Effect of $CaCl_2 + NaCl$ mixtures on the pH of glycine solutions, compared with the effect of each salt alone. One divalent and one monovalent cation.

salt mixtures. In Fig. 4 it is noteworthy that after the first break the curve for the mixture of MgCl_2 and CaCl_2 becomes almost identical with that for MgCl_2 alone. After the second break it swings to the right and there is a third break near where it crosses the curve for CaCl_2 alone. There appears to be a tendency toward the same phenomena in Figs. 1, 2, and 3.

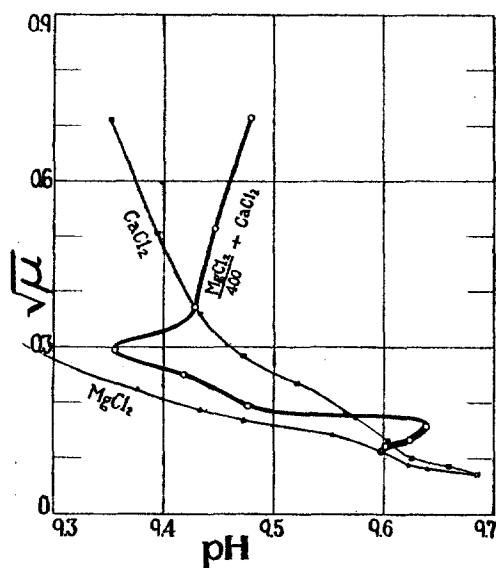


FIG. 4. Effect of $\text{MgCl}_2 + \text{CaCl}_2$ mixtures on the pH of glycine solutions. Both cations are divalent.

In Fig. 5 the curves for the salt mixtures $\text{KCl} + \text{NaCl}$; $\text{MgCl}_2 + \text{NaCl}$; and $\text{CaCl}_2 + \text{NaCl}$ are plotted in another manner. The ordinates are the square root of the Na^+ ion concentration (including the NaOH added to the mother solution). The abscissas are the difference between the observed pH and that obtained with the same ionic strength with NaCl alone. In other words the curve for NaCl is taken as a standard, the deviations from which are plotted in Fig. 5.

It will be observed that the first break of each curve (except the $\text{KCl} + \text{NaCl}$ curve) comes at 0.015 M NaCl concentration *regardless of the amount of the other salt.*

in the previous paper of the series, namely, that cations in mixtures selectively inactivate the two ionic species² to degrees not proportional to the inactivation by one cation alone.

III.

EXPERIMENTAL.

0.02 molar glycine plus 0.500 equivalent of NaOH was made up in 100 cc. amounts. These were used as mother solutions. For each observation 5.00 cc. of mother solution was placed in a 10 cc. volumetric flask, together with desired amounts of salt solutions, and made up to 10 cc. (*i.e.*, 0.0100 molar with respect to glycine). The salts were added from stock solutions of three different ionic strengths (0.025 μ , 0.25 μ , and 2.50 μ). The pH of each solution was measured at 25°C. in a water-jacketed hydrogen electrode of the bubbling type.

Solutions free from salt had a pH of 9.685.

The data are given in Tables I to VIII.

² The two ionic species in the case of glycine in this pH range are, first the *neutral* (or zwitterion) form which predominates at neutral pH values, and second the *anion* form which predominates in alkaline solutions. The lowering of the pH on the addition of a single salt presumably "inactivates" the glycine anion more than the zwitterion. We do not measure the actual inactivation, but rather the ratio of inactivation of the two ionic species. It is reasonable to suppose that this ratio may be disturbed in a mixture of salts.

(Summary on page 792.)

TABLE I.
Effect of NaCl on Na Glycinate.
($\mu = C_{\text{NaCl}} + 0.005$.)

C_{NaCl}	pH	C_{NaCl}	pH	C_{NaCl}	pH
0	9.685	0.050	9.567	0.625	9.460
0.0025	9.663	0.075	9.551	0.750	9.448
0.0050	9.643	0.125	9.533	1.000	9.416
0.0250	9.587	0.250	9.510		

TABLE II.
Effect of KCl on Na Glycinate.
($\mu = C_{\text{KCl}} + 0.005$.)

C_{KCl}	pH	C_{KCl}	pH	C_{KCl}	pH
0	9.685	0.025	9.526	0.250	9.504
0.0025	9.558	0.050	9.494	0.500	9.516
0.0050	9.609	0.075	9.457		
0.0125	9.565	0.125	9.497		

TABLE III.
Effect of MgCl_2 on Na Glycinate.
($\mu = 3 C_{\text{MgCl}_2} + 0.005$.)

C_{MgCl_2}	pH	C_{MgCl_2}	pH	C_{MgCl_2}	pH
0	9.685	0.0050	9.553	0.0250	9.279
0.00050	9.638	0.0075	9.472	0.0500	9.191
0.00100	9.621	0.0100	9.433	0.100	9.049
0.00250	9.596	0.0150	9.376		

TABLE IV.
Effect of CaCl_2 on Na Glycinate.
($\mu = 3 C_{\text{CaCl}_2} + 0.005$.)

C_{CaCl_2}	pH	C_{CaCl_2}	pH	C_{CaCl_2}	pH
0	9.685	0.0083	9.574	0.083	9.394
0.00083	9.658	0.0167	9.521	0.167	9.352
0.00167	9.624	0.0250	9.472		
0.00417	9.604	0.0417	9.433		

TABLE V.
Effect of NaCl on a Glycine Solution Containing 0.0125 M KCl.
 (pH without salt = 9.685.)
 ($\mu = C_{\text{NaCl}} + 0.0175$.)

C_{NaCl}	pH	ΔpH	C_{NaCl}	pH	ΔpH
0	9.565	-0.045	0.050	9.516	-0.042
0.0025	9.609	+0.005	0.075	9.577	+0.031
0.0050	9.634	+0.034	0.125	9.594	0.064
0.0125	9.601	+0.014	0.250	9.611	0.104
0.0250	9.521	-0.056	0.500	9.628	0.155

TABLE VI.
Effect of NaCl on Glycine Solutions Containing MgCl₂.
 (pH without salt = 9.685.)

C_{NaCl}	pH	ΔpH	C_{NaCl}	pH	ΔpH
A. 0.00250 M MgCl ₂ plus various amounts of NaCl ($\mu = C_{\text{NaCl}} + 0.0125$).					
0	9.596	-0.034	0.075	9.484	-0.064
0.0025	9.616	-0.003	0.100	9.516	+0.021
0.0050	9.641	+0.029	0.125	9.550	+0.020
0.0125	9.667	+0.070	0.250	9.563	+0.056
0.0187	9.610	+0.023	0.500	9.582	+0.110
0.0250	9.577	-0.003	0.750	9.604	+0.157
0.0500	9.546	-0.016			
B. 0.0100 M MgCl ₂ plus various amounts of NaCl ($\mu = C_{\text{NaCl}} + 0.035$).					
0	9.433	-0.151	0.075	9.440	-0.099
0.0025	9.489	-0.093	0.125	9.491	-0.034
0.0050	9.530	-0.048	0.250	9.528	+0.022
0.0125	9.547	-0.025	0.500	9.548	+0.078
0.0250	9.575	+0.012	0.750	9.567	+0.122
0.0500	9.486	-0.062			

TABLE VII.
Effect of NaCl on Glycine Solutions Containing CaCl₂.
 (pH without salt = 9.685.)

C_{NaCl}	pH	ΔpH	C_{NaCl}	pH	ΔpH
A. 0.00416 M CaCl ₂ plus various amounts of NaCl ($\mu = C_{\text{NaCl}} + 0.0175$).					
0	9.604	-0.006	0.050	9.497	-0.061
0.0025	9.621	+0.017	0.075	9.458	-0.088
0.0050	9.638	+0.038	0.125	9.500	-0.030
0.0125	9.651	+0.064	0.250	9.518	+0.011
0.0250	9.575	-0.002	0.500	9.536	+0.063
B. 0.0167 M CaCl ₂ plus various amounts of NaCl ($\mu = C_{\text{NaCl}} + 0.050$).					
0	9.521	-0.046	0.050	9.443	-0.097
0.0025	9.535	-0.030	0.075	9.486	-0.047
0.0050	9.546	-0.017	0.125	9.504	-0.018
0.0125	9.567	+0.010	0.250	9.520	+0.019
0.0187	9.511	-0.021	0.500	9.532	+0.064
0.0250	9.455	-0.096			

TABLE VIII.
Effect of CaCl₂ on Na Glycinate Solutions Containing 0.0025 M MgCl₂.
 (pH without salt = 9.685.)
 ($\mu = 3 C_{\text{CaCl}_2} + 0.0125$.)

C_{CaCl_2}	pH	C_{CaCl_2}	pH	C_{CaCl_2}	pH
0	9.596	0.0083	9.476	0.083	9.447
0.00083	9.602	0.0167	9.418	0.167	9.479
0.00167	9.624	0.025	9.355		
0.00417	9.639	0.0417	9.428		

SUMMARY.

The pH of a 0.01 molar solution of glycine, half neutralized with NaOH, is 9.685. Addition of only one of the salts NaCl, KCl, MgCl₂, or CaCl₂ will *lower* the pH of the solution (at least up to 1 μ).

If a given amount of KCl is added to a glycine solution, the subsequent addition of increasing amounts of NaCl will first *raise* the pH (up to 0.007 M NaCl). Further addition of NaCl (up to 0.035 M NaCl) will *lower* the pH, and further additions slightly raise the pH.

The same type of curve is obtained by adding NaCl to glycine solution containing MgCl₂ or CaCl₂ except that the first and second breaks occur at 0.015 M and 0.085 M NaCl, respectively.

Addition of CaCl₂ to a glycine solution containing MgCl₂ gives the same phenomena with breaks at 0.005 M and 0.025 M CaCl; or at *ionic strengths* of 0.015 μ CaCl₂ and 0.075 μ CaCl₂. This indicates that the effect is a function of the ionic strength of the added salt.

These effects are sharp and unmistakable. They are almost identical with the effects produced by the same salt mixtures on the pH of gelatin solutions. They are very suggestive of physiological antagonisms, and at the same time cannot be attributed to colloidal phenomena.