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COMBINATION OF WOOL PROTEIN WITH ACID AND BASE: HYDROCHLORIC ACID AND POTASSIUM HYDROXIDE [1]

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ABSTRACT

As an initial part of a general program for the study of the acidic and basic characteristics of textile fibers, a study has been made of the dependence on pH of the amounts of hydrochloric acid and of potassium hydroxide taken up by wool from aqueous solutions. The effect on this dependence of the maintenance of a constant ionic strength by additions of a neutral salt, potassium chloride, has also been determined. Most of the measurements were made at 0° C to minimize the effects of decomposition brought about by exposure to extreme concentrations of acid or base.

The maximum acid-binding capacity, independent of ionic strength, is 0.82 millimole per gram; the maximum base-binding capacity is greater than 0.78 millimole. With salt absent, no appreciable binding of acid or base occurs in the pH interval, 5 to 10, but the amount bound increases very sharply as these limits are exceeded. When salt is present, the amount of acid or base bound changes with pH more gradually, and there is no wide region in which combination fails to occur; the point of zero combination is sharply defined and is near pH 6.4. The positions of the titration curves with respect to the pH axis are different at every ionic strength. The differences are larger than can be attributed to the effect of salts on the dissociation of acids; thus, in dilute solutions an n-fold change in the total concentration of chloride ions produces a change almost as great as would be produced by a similar n-fold change in the concentration of hydrogen ions. This approach to stoichiometric dependence of the acid bound on the concentration of anions as well as of hydrogen ions accounts for the greater steepness of the titration curve when the source of both ions is the acid alone.

The dependence of acid bound on anion concentration or base bound on concentration of cations is explained by treating the electrostatic restrictions arising from the existence of two phases as a case of partial dissociation of protein salts. A possible alternative analysis by means of the Donnan equilibrium is also presented, and factors to be considered in making a final choice between the two treatments are described in detail. Either analysis predicts that the positions of the curves with respect to the pH axis should, at high salt concentrations, approach a limit which should correspond to the titration curve of the same protein in the dissolved state. This prediction is supported by the fact that the data for wool agree very closely at high salt concentrations with those for a similar but soluble protein, egg albumin.

On the basis of this comparison, a detailed analysis is undertaken of the composition of the titration curve in terms of the constituent di-acidic and di-basic amino acids of wool. This analysis leads to the conclusion that the binding of acid and base by wool occurs at the free carboxyl, imidazole, amino, and guanidino groups, but that no combination of base with the tyrosine hydroxyl group takes place in the pH range of this investigation.

[1] The state of combination of wool with acid and base is intimately related to its ability to absorb moisture and dyes, its elastic and tensile properties, and its behavior in carbonizing, scouring, and milling. In order to form a background for researches relating to these technologically significant properties of wool, an extensive investigation on the acidic and basic properties of the fibers has been undertaken.

Part of the material in the present paper was presented at the meeting of the American Society of Biological Chemists at Toronto, Canada, in April 1939.

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CONTENTS

I. INTRODUCTION

The nature of the dependence on pH of the degree of combination of acid and base by such insoluble proteins as wool, silk, and collagen differs in certain characteristic ways from that of soluble proteins. The examination of measurements reported by Speakman and Hirst [43],[3] Lloyd and Bidder [34], and others leads to the conclusion that the titration curves of these three proteins, determined in the absence of salt, are distinguished from those of all soluble proteins so far studied [4] under the same conditions in three principal respects:

1. They show a long region of little or no acid- or base-binding capacity, more or less symmetrically placed about the point of neutrality. This is true even when, as in the case of wool and silk, they contain appreciable quantities of histidine which, because of its imidazole group, should function as a buffer in this region.

2. The amounts of acid and base bound increase sharply as the extremes of the pH scale are approached and reach their respective maximum values at about the same pH as in the case of soluble proteins. Thus, the titration curves are steeper than those of soluble proteins, or of soluble polybasic acids in general.

3. The pH values at which half of the maximum amount of acid or base is combined (a convenient measure of the position of the curve and, in simple substances, directly related to the acid strengths of the groups titrated), are shifted considerably toward the two extremes of the pH scale. Thus, the S-shaped portions of the curves found on the acid side of pH 7 are centered, in the case of almost all dissolved proteins, about a pH very close to 4.0, but in earlier published work on wool this point appears to be 2.3 [43, 44, 45]. If these curves are regarded as resulting from the titration of many groups which possess identical dissociation constants this difference in the position of the midpoints would represent a fifty-fold increase in the acid strength of the insoluble protein, although its dissociating groups are presumed to be the same as those present in proteins which may be titrated in the dissolved state.

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[3] Figures in brackets indicate the literature references at the end of this paper.
[4] Except those, such as hemoglobin, in which irreversible effects occur [10, 37].

This description of the striking differences between the titration curves of soluble and insoluble proteins is made more concrete by the comparison in figure 1 of the data for wool (obtained as described in the experimental section) with similar data of Kekwick and Cannan [31] for the soluble protein, egg albumin, both at 25° C. The latter are chosen because of their general excellence, and the close similarity in relative composition of egg albumin and wool, with respect to those amino acids (aspartic acid, glutamic acid, and histidine) which should affect the shape and position of their titration curves [5] in the acid region, although these proteins differ con-

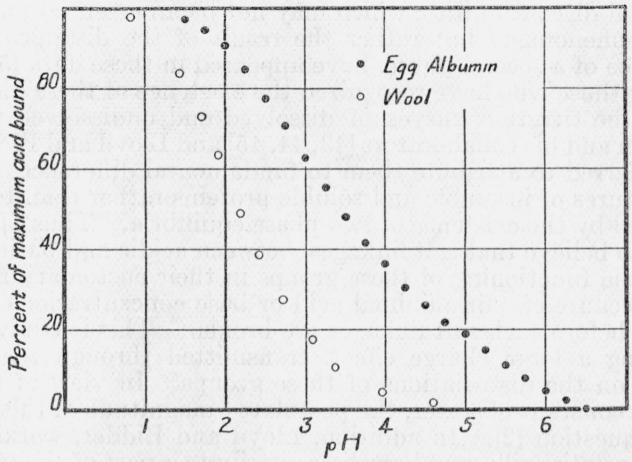

FIGURE 1.—*Comparison of the curves of acid combination of wool and of egg albumin at 25° C, in the absence of added salt.*

siderably in their combining weights. The same scale of ordinates is used for the two curves by plotting, instead of the amount of acid combined per gram by each protein at each pH, the fractions of the total amounts of acid which are required to bring them from a condition of equilibrium with a solution at pH 6.4 to one at pH 1 (the reason for choosing pH 6.4 will be apparent later). These curves are typical of those obtained over this range of pH with proteins of the two classes, and illustrate: (1) the long region, starting below pH 5 (and extending to pH 10) in which practically no acid combines with wool; (2) the much greater steepness of the curve for wool; and (3) the much greater acidity at which half the maximum amount of acid is combined.

Cannan has shown that this portion of the curve for *egg albumin* (after correction for the small amount of acid combination due to histidine) is readily described in terms of the law of mass action, using only a single dissociation constant, if allowance is made, in a semiempirical way, for the effect of electrostatic interaction between the ionized carboxyl groups on their respective dissociations [4]. Clearly, if a similar analysis is to be applied to the data for wool,

[5] The position and form of the acid titration curves are determined by the carboxyl groups of the protein rather than by the amino groups, because the latter are present as internally ionized ammonium-like salts. Thus the process of acid combination consists in replacing a proton on an ionized carboxyl group. The maximum amount of acid combined is, of course, determined by the number of amino groups [3, 7, 24].

additional factors must be taken into account. A possible clue to the nature of the difference is suggested by the fact that the steeper slope characterizing the curves for the insoluble proteins may be described to a good degree of approximation by substituting $(H^+)^2$ for (H^+) in the mass law expression for egg albumin used by Cannan.

It seems likely that these differences, characteristic not only of wool but of other high-molecular insoluble proteins as well [34], are necessarily associated with the presence of a separate phase. Nevertheless, many proteins which are relatively insoluble at their isoelectric points have hitherto been titrated in the presence of much undissolved material over at least part of the total range of the titration. Singularities and discontinuities, which may not be an essential part of the titration phenomena but rather the result of the disappearance or appearance of a second phase, have appeared in these data [5].

Among those who have recognized the existence of these differences between the titration curves of dissolved and undissolved proteins, Speakman and his collaborators [43, 44, 45] and Lloyd and Bidder [34], have preferred to attribute them to fundamental differences between the structures of insoluble and soluble proteins rather than to factors introduced by the existence of two-phase equilibria. Thus Speakman appears to believe that salt linkages, between acidic and basic groups, prevent the functioning of these groups in their customary ranges of acidity because certain minimal acid or base concentrations must be exceeded before such salt linkages are broken. This is equivalent to postulating a large charge effect, transmitted through the solvent medium, on the dissociations of these groups. In view of the high dielectric constant of water, the postulated magnitude of this effect is open to question [2]. In addition, Lloyd and Bidder, working with collagen, gelatin, silk, and horsehair, attribute a part of the inactivity of acidic and basic groups at reactions near neutrality to the existence of vaguely defined covalent linkages between adjacent polypeptide chains, which render the groups in question unavailable until these linkages are destroyed by exposure to sufficiently concentrated acid or alkali.

Nevertheless, all of these authors, and others who have been concerned with the electrochemical and osmotic behavior of insoluble proteins, have recognized that account must be taken of the presence of two phases, and have had recourse to the usual method of describing such phase equilibria by means of the Donnan equations [12, 13], at least in a qualitative way. A *quantitative* analysis of the titration curve, sufficient to decide the extent to which the existence of two phases eliminates the need to consider other factors of the kind mentioned above, has not hitherto been made. The reason for this is not far to seek: There are grave difficulties in defining, without arbitrariness, the compositions of the phases in applying the equations of the Donnan equilibrium to the cases in question. In the present paper new data on the combination of wool with both acid and base are described in terms of a more readily defined and simpler concept. This treatment does not pretend to supplant the treatment of Donnan in other cases of two-phase equilibrium for which exact information as to the composition of clearly delimited phases makes possible its proper application.

As a result of certain theoretical considerations,[6] it appeared likely that data obtained in the presence of neutral salt at constant ionic strength might shed light on the cause of the differences apparent between curves obtained with acid alone (fig. 1). The measurements to be described here, most of which were made at 0° C to avoid complications introduced by hydrolytic decomposition, have led to a simple and general way of relating these differences to the conditions under which insoluble proteins such as fibers must be titrated. It seems probable that the resulting viewpoint has consequences for many phenomena characteristic of soluble as well as of insoluble proteins. Thus, for example, in the study of the effect of pH upon protein solubility a condition necessary for measuring the phenomenon is the presence of the solid phase.

II. EXPERIMENTAL PROCEDURE

1. MATERIAL

Raw wool fibers were cut into root and tip portions, and the latter, which are contaminated and partially decomposed, were discarded. The root portion was purified by washing in cold ether, extracting for 16 hours, first with alcohol and then with ether, at temperatures below 35° C, and finally washing repeatedly in distilled water. It was carded by hand or on a sample carding machine,[7] rewashed in distilled water, air-dried, and finally conditioned at 21° C and 65-percent relative humidity before weighing. Analyses for total nitrogen, amide nitrogen, primary amino nitrogen, sulfur, and cystine gave nearly identical results with all the batches of wool used. Analyses for ash were made by combustion, and by an adaptation of the electrodialysis method of Joseph and Stadie [30, 48]. Most of the data on combination of acid at 0° C were obtained with wool containing only 0.10 percent of ash. No effort was made to remove this ash and no correction was applied for its effect (shown to be very small, see section III–1) on the amount of acid combined. The remaining data, obtained with material of higher ash content (0.26 percent), were corrected for the alkali equivalence of the ash by subtracting from or adding to the measured acid- or base-binding capacity an amount determined by direct comparison with the low-ash wool, occasionally supplemented by other methods of estimation.[8] The moisture content of the fibers used in each experiment was determined by drying representative samples for 2 hours in a vacuum oven at 105° C. The data are expressed as acid or base bound per gram of dry wool.

2. METHODS

(a) TITRATION WITH ACID

Samples of wool weighing either 1.2 or 2.0 g and containing from 13.1 to 15.0 percent of moisture, were placed in 100-ml portions of acid solutions at the temperature of the experiment (0° or 25° C). The solutions contained varying amounts of hydrochloric acid and sufficient potassium chloride to bring them, after absorption of acid by

[6] These considerations are discussed in section IV.

[7] Thanks are due to the Eavenson and Levering Co., of Camden, N. J., for carding four batches of wool used in this work.

[8] The principal alternative method used was an electrodialytic estimation of combined base, by an adaptation of the method of Joseph and Stadie [30].

the fibers, to predetermined ionic strengths between 0.005 and 1.0. Sets of solutions containing only HCl, without added salt, were also used. Rapid and complete wetting-out of the wool was facilitated by pumping off air with a filter pump. Repeated preliminary sampling showed that during immersion over a period of 48 hours at the lower temperature, there was a perceptible drift of pH in solutions from which a large fraction of the total acid was removed by combining with the wool. Therefore, in order to assure attainment of equilibrium, the fibers were exposed to the solutions at either temperature for periods of from 48 to 75 hours before samples were withdrawn. Portions of each solution, transferred to stoppered Pyrex test tubes, were allowed to come to room temperature (22° to 31° C) and the pH of each was determined. Aliquots of the original solution and of the solution in equilibrium with the wool were titrated with sodium hydroxide, using bromcresol purple as indicator. From the difference between these titers, the weight of the sample, and its content of moisture, the amount of acid taken up per gram of dry wool was calculated. Since differences in titers are involved, and since these differences do not change in direct proportion to the total concentration of acid, the measurements made in the most acid solutions are most subject to error. This limitation of accuracy was partly offset by using the larger wool samples in the most acid solutions.

The chloride disappearing from solution was determined by titrating the same aliquots, after neutralization, with 0.1 *M* silver nitrate. Sufficient potassium chromate solution to bring the chromate concentration above 0.01 *M* was used as indicator. Errors caused by end point uncertainties were reduced by matching the colors of the aliquots with one another.

In calculating the hydrogen ion and chloride ion bound by the fibers from the difference in titers of the solutions, proper account was taken of the effect of water absorption by the fibers on the final concentration of acid. With the ratios of fibers to solutions used, this correction was negligible except when the acid concentration exceeded 0.1 *M*, but the importance of this procedure has been amply demonstrated by critical experiments with ratios more favorable to the detection of effects due to moisture absorption. In calculating these small corrections, it has been assumed that the hydration of the fiber is the same in acid solutions as it is in water, and that no hydrochloric acid is dissolved in the water taken up. Recent unpublished measurements on cotton fibers in this laboratory indicate that the latter assumption may not be strictly true, but only a slight over-correction results from its use. Omission of the correction would result in an apparent decrease in the acid bound when the concentrations of acid exceeded 0.2 *M*.

In order to determine whether prolonged exposure to acid produced significant hydrolysis of amide bonds, other aliquots from each solution were tested for ammonia with the Nessler reagent of Koch and McMeekin [33]. At 0° C, only the highest acidities used (over 0.2 *M*) produced a detectable amount of ammonia (0.06 mg/g). The effect of this amount on the titration curve is practically negligible. At 25° C, concentrations over 0.1 *M* produced sufficient ammonia to account for 0.01 to 0.03 millimole of acid combined. This is equivalent to hydrolyzing from 1 to 2 percent of the amide groups present. Tests

with the phenol reagent of Folin and Ciocalteu [14] showed the presence of traces of a reducing substance in the most strongly acid solutions. This appeared to be present in the wool before treatment with acid rather than to result from decomposition, since the maximum amount found was the same at both temperatures. Negative sulfide and biuret tests on the solutions showed that no appreciable dissolution or decomposition had occurred.

(b) TITRATION WITH BASE

Portions of wool weighing either 3.0 or 4.0 g were placed in carbonate-free alkaline solutions (usually in volumes of 200 ml) at 0° C. The solutions contained varying amounts of potassium hydroxide and sufficient potassium chloride to bring the total ionic strengths to predetermined values of 0.02 or 0.2 *M*. Sets of solutions without added salt were also used. The larger samples were used in the most alkaline solutions. Although it is very desirable to limit the time of exposure to alkali to a minimum in order to minimize the amount of decomposition, control experiments at acidities at which decomposition was negligibly slow showed that periods of immersion of at least 18 hours were required to attain equilibrium. Approximately 20 hours was allowed in all the experiments reported, with the exception of those mentioned later.

In determining the amounts of base bound, the procedure of Harris and Rutherford [25] was followed except for the substitution of bromcresol purple for methyl red as indicator, and the use of nesslerization for the determination of dissolved protein. Additional aliquots were used for immediate measurement of pH at the temperature of the experiment. The use of bromcresol purple reduced the error introduced on the acid side of pH 6 by the buffering capacity of dissolved protein in those solutions in which appreciable decomposition has occurred.[9]

A slight modification of the method of calculating the corrections for decomposition was introduced. Comparisons of the ratio of dissolved nitrogen, after Kjeldahl digestion, to dissolved inorganic sulfur with the same ratio in the intact protein indicated that a small fraction (usually from one-tenth to one-fifth) of the inorganic sulfur in each solution was derived from the wool that had dissolved. Therefore, only the remaining eight- or nine-tenths of the inorganic sulfur in solution was produced by hydrolysis of disulfide bonds *in the undissolved wool*. Only the latter portion should be included in the correction for disulfide bonds broken since the base taken up by the dissolved protein, including that taken up by the sulfhydryl groups formed by hydrolysis of its own cystine, is not measured by the differential titration method used in this work.

This modified correction was shown to be justified by control measurements with several different concentrations of potassium hydroxide between 0.05 and 0.30 *M*, and with periods of immersion between 1.5 and 27 hours at 0° C. These measurements showed that with the highest concentrations there is a measurable increase in the amounts of base combined up to at least 10 hours, and that after this period,

[9] Buffering capacity on the alkaline side of the pH for zero combination is taken care of by the method of correction for the effects of decomposition used by these authors.

the amount bound does not change if the constantly increasing corrections for decomposition are properly made. If, on the other hand, the corrections are applied as in the method of Harris and Rutherford, the calculated amount of base bound decreases somewhat on prolonged exposure to strongly alkaline solutions. This would be the expected result of over-correction when the amounts of decomposition become large. The same effect is evident when results at different concentrations of potassium hydroxide, obtained after the same times of exposure, are compared. The calculated amounts of base bound tend to *decrease* at the highest concentrations, if the correction for *all* the inorganic sulfur is applied.

At 0°C the largest correction, 0.28 milliequivalent per gram, (M-eq/g), corresponded to the destruction of approximately one-half of the disulfide bonds and the dissolving of one-twelfth of the total protein. In similar controls carried out at 25°C, as much as 85 percent of the cystine was destroyed, and up to one-sixth of the protein dissolved. With these larger amounts of decomposition, the necessity for modification of the method of Harris and Rutherford was even clearer. Thus, while the modification described here gave consistent results at the two temperatures, the unmodified corrections gave lower values at high concentrations of potassium hydroxide at the higher temperature.

Since the earlier measurements of Harris and Rutherford were obtained, for the most part, after exposure of wool to alkali for 2 hours, and since the corrections subtracted were slightly high, their values for the amounts of base bound are probably a little lower than the true equilibrium values. The data of the present investigation indicate that the maximum possible amount of base has not been bound even at potassium hydroxide concentrations of 0.3 *M*. However, the protein may be modified by decomposition of bonds other than disulfide when these or higher concentrations of alkali are used, and the methods of correction applied here may no longer suffice.

In view of the magnitude of the corrections described, no additional corrections for the effect of water absorption by the fibers on the calculated amount of combined base were applied. The small effect of this correction is within the error of the values given.

(c) MEASUREMENT OF pH

Measurements of pH were made with the glass-electrode assembly developed by MacInnes and Belcher [35], connected with a thermionic potentiometer which employed a cathode-ray "eye" as null indicator. The potentiometer, arranged to read directly in pH at temperatures between 0° and 30°C, could be set reproducibly to 0.002 pH unit when measuring buffered solutions. The following solutions [29] were employed in establishing the pH scale, in terms of which the measurements at 25°C are expressed: (a) 0.1 *M* hydrochloric acid; (b) 0.01 *M* hydrochloric acid plus 0.09 *M* potassium chloride, using the values 1.085 and 2.078; (c) 0.05 *M* potassium hydrogen phthalate,

using the value 4.008; (d) 0.1 *M* acetic acid plus 0.1 *M* sodium acetate, using the value 4.648; and (e) 0.05 *M* sodium tetraborate, with the pH value 9.180. In addition, 0.05 *M* potassium hydroxide was used. A pH of 12.61 was assigned to this solution, consistent with the activity coefficient (0.824) determined by Harned and Cook [20] and the value of Harned and Hamer [22] for the ion product of water (pK_w=13.997).

Calibration at 0° C was carried out with the same solutions, omitting the sodium tetraborate. The same pH values were assigned to the hydrochloric acid and phthalate [18] solutions at both temperatures, but the value assigned to the acetate buffer at 0° C (4.673) was estimated by adding to the value at 25° C the logarithm of the ratio of the dissociation constants at the two temperatures. The negligible effect of temperature on the activity coefficients of the ions in these solutions is thus not taken into account in the values assigned. The pH assigned to 0.05 *M* potassium hydroxide at 0° C was 13.56, estimated from the value of Harned and Hamer at this temperature for pK_W (14.94) and the activity coefficient (0.829) determined by Harned and Cook.

When the pH-meter was set at the proper pH with any of these standards except the most alkaline one, each of the other four buffers could be read to within 0.01 pH unit of the values assigned to them. Readings in alkaline solutions required adjustment for the departure, at pH greater than 9, of the slope of the pH-emf function from its theoretical value. This adjustment was carried out as follows: After setting the pH-meter to read 4.008 with the phthalate buffer in the electrode vessel, the pH reading of 0.05 *M* potassium hydroxide was determined, and subtracted from the calculated correct reading. The difference (never over 0.26 pH unit at 0° C) was assumed to be uniformly cumulative over the pH interval of 8.55 units. This is equivalent to assuming that over this range of pH the proportionality between emf and pH is lower than the proper value of RT/F by about 3 percent. This assumption leads to adjustments which are subject to most error at pH 7 to pH 10. In this interval only a few readings were taken; and owing to the relatively small slope of this part of the titration curve, a small error in pH has very little effect on its shape or position. The pH values reported for alkaline solutions are less certain than those in the acid range because of these adjustments (based in part on a *calculated* pH), and because of some hysteresis of the glass membrane after exposure to concentrated alkali. The latter sometimes introduced a discrepancy of 0.03 to 0.04 pH unit, apparent when the measurements of acid standards were repeated after an alkaline series. In agreement with the work of Dole and Wiener [11], the required adjustments and the residual uncertainties are larger at the higher temperature.

The pH values of alkaline solutions were measured with the electrode assembly mounted in an air thermostat at either 3°C or 25°C. Solutions were introduced and manipulations made from the outside. The results given at 0°C are calculated from measurements made at

the lower temperature. Practically all acid solutions were measured at room temperature. Since many of the solutions were very poorly buffered, the accuracy of a large number of the measured pH values was tested by direct comparison with the calculated values based on the titer of the solutions and the activity coefficients of the acid or base [19, 20, 21, 22, 26]. With solutions more acid than pH 3.5 at ionic strengths below 0.1 *M*, agreement to 0.01 pH unit was usually found. The presence of *traces* of a soluble buffering substance derived from the wool reduced this agreement in the range pH 4 to pH 10, but in the absence of wool this agreement extended to pH 4.5, beyond which the concentration of HCl could not be accurately determined. The stability and reproducibility of the measurements below pH 6 render it very probable that the same high accuracy prevails up to this value, even in the presence of wool. Measurements between pH 6 and pH 9 required almost continuous flushing of the electrode vessel, and are less certain. pH values for solutions containing potassium hydroxide in concentrations of 0.1 *M* or more were calculated from their titers, allowance being made for the contribution to the titer of any hydrosulfide present. Acid solutions with an ionic strength of more than 0.1 required application of a small empirical correction (between 0.02 pH at ionic strength 0.2 and 0.11 pH at ionic strength 1.0) due to the difference in ionic strength between the standardizing buffers and the solutions measured.

(d) CONTROL OF TEMPERATURE

A well-stirred 200-liter water-thermostat equipped with cooling coils, 300-watt heating element, mercury thermoregulator, and time-delay relay, kept the temperature constant at 25.00°±.02°C. No thermoregulator was used at 0°C; ice was allowed to form on the coils as the result of intermittent operation of the cooling unit. With a large amount of ice present, the temperature remained constant to ±0.01°C.

III. RESULTS AND DISCUSSION

1. COMBINATION WITH HYDROCHLORIC ACID

Measurements of the combination of wool with hydrochloric acid as a function of pH at 0°C are given in table 1, in which each section, except the first, summarizes data obtained at a different constant ionic strength. In the first section, each measurement represents a different ionic strength, since no salt was added. Data obtained both by titration of acid and of chloride are given. As was to be expected from considerations of electrical neutrality, the values in the two columns agree very closely. This agreement shows that the small amount of alkaline ash in the material used in most of the experiments neutralizes only negligible amounts of acid. In those cases in which much ash was known to be present, the agreement demonstrates the adequacy of the corrections applied for the effect of this ash.[10]

[10] When alkaline ash is combined with wool, the chloride disappearing from solution rather than the decrease in titratable acidity is a measure of the acid which would be taken up by ash-free wool. Thus, curves of chloride bound as a function of pH, although of lower accuracy, require no adjustments for the presence of ash.

TABLE 1.—*Combination of wool with* HCl *at 0° C*

pH	Acid bound	Chloride bound
	(a) No added salt	
	Millimoles/g	*Millimoles/g*
0.447 [a]	0.868	
0.635	.838	
0.792 [a]	.836	0.810
0.813	.805	.804
0.814 [a]	.829	.836
0.918 [a]	.813	
1.087 [a]	.762	.771
1.125	.789	.736
1.470	.768	.754
1.663 [a]	.676	.694
1.711	.652	.654
1.741 [a]	.631	.635
1.931	.574	.585
2.061 [a]	.510	.515
2.228	.445	.440
2.340 [a]	.385	.399
2.451	.348	.353
2.726	.251	.261
3.039	.166	.169
3.272	.114	.113
3.620	.067	.071
3.844	.045	
4.198	.027	.023
4.414	.017	.023
4.740	.012	.018
5.145	.004	.009
	(b) Ionic strength 0.005	
2.340	0.385	0.399
2.681	.314	.324
2.980	.264	.263
3.469	.187	.193
3.778	.146	.143
4.130	.113	
4.403	.085	.088
4.701	.058	.073
5.108	.045	.046
5.236	.035	.039
5.670	.022	.024
5.769	.021	.019
5.96	.009	
6.5	.000	
	(c) Ionic strength 0.01	
2.061	0.510	0.515
2.442	.427	.427
2.705	.369	.368
2.982	.312	.312
3.326	.250	.266
3.593	.209	.221
3.856	.167	.167
4.412	.113	.118
4.652	.089	.095
5.072	.061	.079
5.317	.050	.059
5.649	.032	.043
5.98	.017	.021
6.41	.008	
6.5	.000	
	(d) Ionic strength 0.02	
	Millimoles/g	*Millimoles/g*
1.741	0.631	0.635
1.950	.591	.621
2.265	.525	.525
2.675	.429	.429
3.017	.365	.366
3.368	.289	.295
3.668	.240	.238
3.945	.202	.204
4.563	.121	.118
5.048	.074	.080
5.622	.042	.047
6.4	.000	
	(e) Ionic strength 0.04	
1.663	0.676	0.694
1.830	.668	.675
1.925	.650	.678
2.152	.605	.616
2.620	.505	.494
2.639	.500	.494
3.016	.406	.400
3.294	.357	.382
3.628	.280	.319
4.035	.229	.222
4.377	.171	.193
4.650	.146	.140
5.080	.102	.102
5.283	.082	.082
5.40	.064	.063
6.3	.000	−.011
	(f) Ionic strength 0.1	
1.087	0.762	0.771
1.201	.789	.794
1.380	.767	.776
1.678	.745	.740
1.988	.704	
2.269	.664	.680
2.535	.603	
2.810	.538	.510
3.206	.446	
3.650	.357	
4.210	.250	
4.617	.176	.195
5.032	.122	
5.473	.075	
5.867	.037	
6.15	.016	
6.3	.008	
6.4	.003	
6.5	.000	
	(g) Ionic strength 0.2	
0.792	0.836	0.810
0.814[b]	.829	.836
0.918[c]	.813	
1.100	.799	.788
1.130[c]	.798	.828
1.400[d]	.789	

See footnotes at end of table.

TABLE 1.—*Combination of wool with* HCl *at 0° C*—Continued

pH	Acid bound	Chloride bound
(g) Ionic strength 0.2—Continued		
	Millimoles/g	*Millimoles/g*
1.409	0.788	0.773
1.433[c]	.774	.780
1.707	.770	.756
1.751[c]	.753	.775
1.982[b]	.730	.730
2.038	.740	.756
2.061[c]	.702	.759
2.364	.701	.712
2.378[c]	.670	
2.708	.641	
2.744[c]	.595	
2.790[d]	.594	
3.172	.535	.591
3.273[c]	.487	
3.571[b]	.435	
3.670	.419	.460
3.825[c]	.372	
4.082	.320	.294
4.359[b]	.279	.300
4.662	.224	.198
4.855[c]	.177	
5.015	.168	
5.246	.131	.120
5.400[c]	.112	
5.528	.099	.085
5.564[c]	.067	
5.670	.078	
5.887	.054	
5.94[c]	.030	
6.14	.023	
6.3[c]	.002	
6.5	.002	
6.7[c]	−.027	
6.97	−.048	
7.0[c]	−.041	

pH	Acid bound	Chloride bound
(h) Ionic strength 0.5		
	Millimoles/g	
0.447	0.868	
0.832	.838	
1.130	.850	
1.438	.799	
1.745	.784	
2.048	.781	
2.780	.687	
3.750	.471	
3.910	.432	
4.304	.330	
4.887	.228	
5.211	.164	
5.472	.112	
5.870	.068	
6.10	.034	
6.5	.002	
(i) Ionic strength 1.0[c]		
0.578	0.920	
0.840	.890	
1.138	.852	
1.445	.840	
1.743	.794	
2.062	.777	
3.074	.652	
4.258	.403	
4.334	.395	
4.768	.288	
5.342	.208	
5.651	.119	
5.908	.065	
6.4	.013	
6.7	−.015	
7.0	−.048	

[a] No-salt data from experiments at constant ionic strengths in other columns.
[b], [c], [d] Supplementary control determinations on three different batches containing alkaline ash. These data are corrected for differences in ash as explained in the text.
[c] Data in this column corrected for alkaline ash of wool used.

The measurements of acid bound (table 1) are represented graphically in figure 2, in which the continuous curves serve to differentiate the sets of points from one another. All of the other curves shown are distinguished from the "no-salt" curve (at the extreme left of the figure) by their smaller slopes. When compared at a point well above the base line, as, for example, at the points at which half the maximum acid-binding capacity is found, the slopes of all of them are very nearly equal. Near the base line the slopes vary as the curves converge and finally cross.[11] The addition of even small amounts of salt suffices to produce this difference: thus, when compared at 0.05 millimole of acid bound per gram, the point on the curve obtained with a total ion concentration of only 0.005 is shifted with respect to a corresponding point on the no-salt curve by about 1.3 pH units. The magnitude of this effect may be made clearer by comparing the amounts of acid bound at the same pH. In the absence of salt, only a little more than 0.01 millimole of acid per gram is bound at pH 4.6, but the addition of salt, sufficient to bring the concentration of chloride to 0.005 *M*, increases this figure almost sixfold. At pH 5 and above, this factor is even larger. Conversely, as the part of the total ionic strength which

[11] The positions of the curves are reversed on the alkaline side of the point of zero combination and again tend to become parallel.

is contributed by salt diminishes, the factor becomes smaller, and each curve finally terminates at its intersection with the curve obtained without salt.

A simple regularity relates the curves obtained with different total ionic strengths: the distance (with respect to pH) between any two adjacent curves, taken for convenience at the point of half of the maximum combination with acid, is only slightly less than the logarithm of the ratio of the ion concentrations which the curves represent.

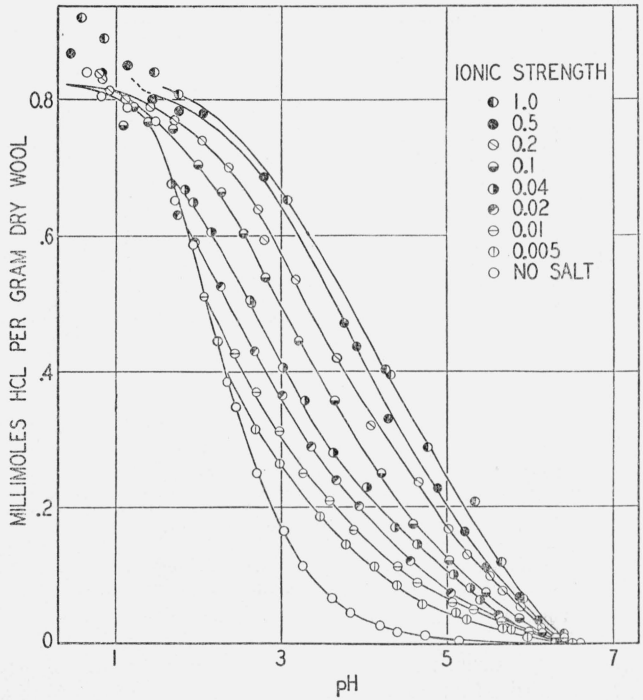

FIGURE 2.—*Combination of acid by wool as a function of* pH *and ionic strength at* 0°*C.*

(To avoid crowding the figure, only a single set of the experimental values obtained at an ionic strength of 0.2 is included. The smooth curves serve merely to distinguish the sets of data from one another.)

This implies a close approach to a direct stoichiometric relationship of the acid bound by wool to the concentration of chloride ion as well as to the concentration of hydrogen ion: Thus each time the chloride concentration is doubled, the curves are shifted to pH values approximately 0.30 unit higher than those at the lower ionic strength. As shown in figure 3, this direct relation is approximated very closely over the fortyfold range of chloride concentrations between 0.005 and 0.200 *M*. Above this range the dependence on ionic strength diminishes: It appears that the curves may approach a limiting position with respect to the pH axis, and that this limit may not be far beyond the position of the curve at 1.0 *M* ionic strength. It is significant that the pH for half maximal combination (4.2) which characterizes the position of the curve at 1.0 *M* chloride is practically identical with

the midpoint of the first S-shaped portion of the titration curves of soluble proteins measured at the same salt concentration.

The remarkably regular dependence of the positions of the curves on the total concentration of chloride ions may be interpreted as implying a certain degree of interchangeability of the concentrations of acid and salt or of hydrogen and chloride ions as factors limiting the amounts of acid bound. The function which describes the amount of acid bound must involve two independent variables, and its dependence on each of these variables must possess a degree of similarity.

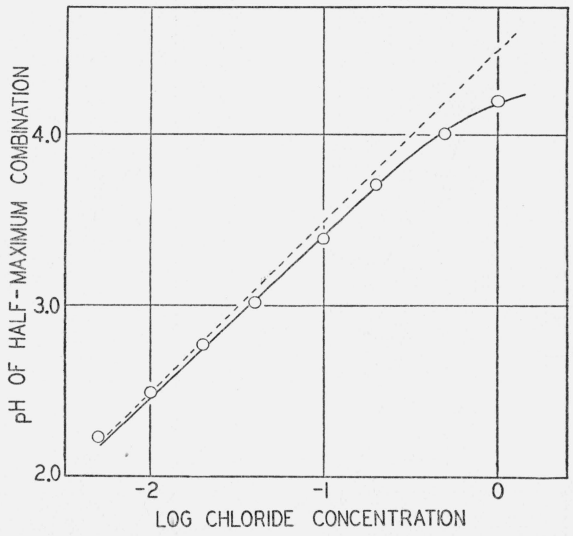

FIGURE 3.—*The dependence of the* pH *of half-maximal combination with acid on the level at which the chloride concentration is held constant.*

The broken line represents a direct stoichiometric relation.

Thus, if, instead of plotting the amount of acid bound against pH it were plotted against the negative logarithm of the total chloride concentration at a number of constant concentrations of acid, a group of curves would be obtained related to one another very much as the curves in figure 2. Several earlier studies on *soluble* proteins [4, 10] have shown the existence of much smaller effects of salt on the positions and shapes of their acid-titration curves. These effects are only slightly larger than those which characterize the common buffers, and which are explained in terms of the modern electrical-interaction theory of solutions. They cannot be compared to the much larger and nearly stoichiometric dependence which is found with wool in the range of low ionic strengths. The significance of this large and interchangeable dependence of acid and salt on acid combination is discussed in section IV of this paper.

Since the curves of acid combination as a function of pH represent the reaction of hydrogen ions with carboxylate ions (see footnote 5), and since the dissociation constants of the conjugate carboxylic acids have only a slight dependence upon temperature (between 0° C and room temperature), the effect of temperature on these curves should be small. This expectation is completely fulfilled by comparison of

the data at 0° already given with the measurements on wool at 25° C summarized in table 4 and plotted in figures 1 and 5.[12] Comparison of the data obtained in the absence of salt at either temperature with the data of Speakman and Stott [45], obtained at an intermediate temperature, is therefore not unjustified. The agreement with the data of these investigators is close over the greater part of their range, and is probably within the limits of possible differences in definition of the pH scale in the two investigations. Although different wools were used, there is also close agreement between the values for maximum acid-binding capacity. As shown in figure 2, all of the sets of points tend to level off near pH 1 at 0.80 to 0.84 M-eq of acid bound per gram.[13] Speakman obtained a maximum of 0.80 or 0.81 with wool which had been previously extracted with very dilute hydrochloric acid to remove the ash. The agreement of our maximum values with his supports the previous conclusion that the residual 0.10 percent of ash in the wool used in the present investigation is practically without effect on the measurements. This conclusion is also borne out by the amounts of acid shown to be bound in the absence of salt at pH 4.8, which Speakman considers to be the point of zero combination. This quantity, approximately 0.01 millimole per gram, is therefore the maximum amount which may be attributed to ash present in the wool of the present investigation. The effect of this ash in neutralizing acid may be even smaller, or zero, since Speakman's acid extraction and adjustment to pH 4.8 may have resulted in the combination of so small an amount of acid as 0.01 millimole with his wool. The curves obtained in the presence of salt make plain that pH 4.8 is not the point of zero combination of acid with wool at 0° C. It seems probable that at this temperature the base line is crossed, not only in the presence of salt but in salt-free solutions as well, at pH 6.3–6.5.

2. COMBINATION WITH POTASSIUM HYDROXIDE

Although fewer measurements of the combination of wool with base have been made, they suffice to show that the relationships between the data on acid combination obtained in the presence and absence of salt, and at different constant ionic strengths, also prevail in the alkaline branches of the wool titration curves. Very little modification of the foregoing description is therefore necessary. The corrected data for base combination at 0° C, at two ionic strengths and in the absence of added salt, are summarized in table 2. Parallel data for the combination of the fiber with potassium ion, obtained by electrodialysis, are omitted from this table; although less accurate than the data for base bound, they are in fair agreement with the values given. In figure 4, the data of table 2 are shown combined with the corresponding sets of measurements for acid combination; the figure thus represents the complete titration curve under these conditions over the entire pH range studied.

[12] A closer study of the effects of temperature on the combination of acid and base is in progress. These effects are of importance in the application of the low-temperature titration data presented in this paper to such related aspects of wool processing as dyeing and scouring, which usually involve high temperatures.

[13] The slight differences in the maxima shown by the various curves may represent, in part, residual differences in alkaline ash for which inadequate corrections have been made. It is more likely that they are the result of incorrect allowance for differential absorption of water, salt, and acid when wool unsaturated with water is placed in the various solutions. This absorption will affect only the values calculated for the most acid solutions. It is also possible that in the most acid solutions, the titration of the very weakly basic amide groups is begun, and that the curves are not entirely level below pH 0.7.

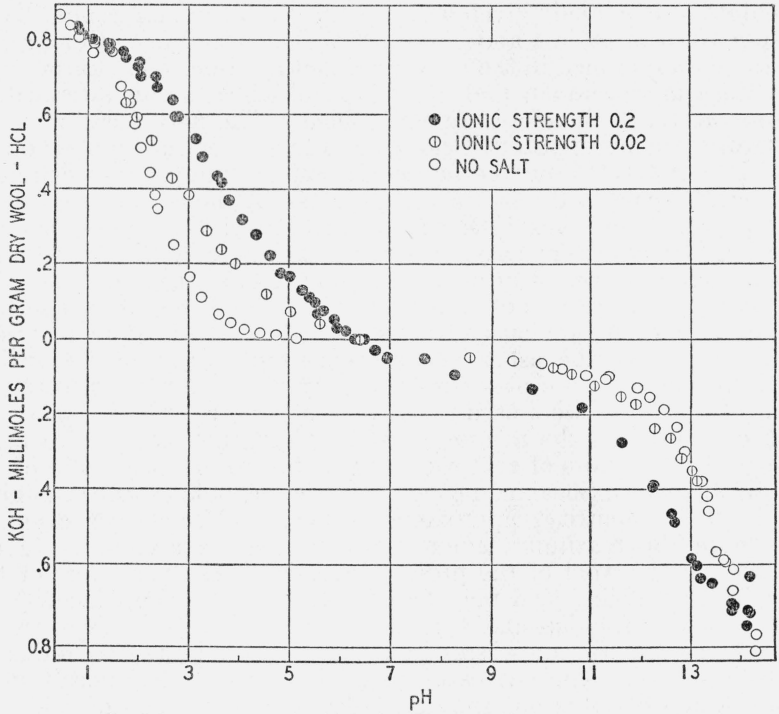

FIGURE 4.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of* pH.

Data are shown obtained in the absence of added salt, and in the presence of two different constant total ionic concentrations.

TABLE 2.—*Combination of wool with* KOH *at 0° C* [a]

pH	Base bound	pH	Base bound	pH	Base bound
(a) No added salt		(b) Ionic strength 0.02		(c) Ionic strength 0.20	
	Millimoles/g		*Millimoles/g*		*Millimoles/g*
[b] 9.45	0.055	8.59	0.047	[c] 6.95	0.050
[b] 10.01	.063	10.25	.095	7.68	.050
[b] 10.43	.077	10.62	.112	8.28	.093
[b] 10.91	.094	11.09	.142	9.84	.130
11.32	.105	11.60	.170	10.83	.180
11.37	.097	11.90	.191	11.63	.273
11.93	.128	12.27	.236	[c] 12.23	.391
12.17	.153	12.60	.262	12.25	.389
12.46	.185	12.81	.316	12.62	.462
12.72	.232	13.03	.348	12.68	.495
12.88	.297	13.22	.397	13.01	.582
13.12	.375			[c] 13.11	.600
[c] 13.22	.397			13.19	.635
13.32	.436			[c] 13.39	.649
13.35	.476			[b] 13.82	.704
13.49	.565			[d] 13.82	.722
[b] 13.65	.586			[c] 13.85	.710
13.68	.590			[f] 14.11	.764
13.85	.669			[b] 14.13	.723
[b] 13.85	.611			[c] 14.16	.630
14.11	.764			[d] 14.17	.730
[b] 14.13	.723				
[c] 14.16	.630				
[d] 14.17	.730				
14.29	.828				
[b] 14.30	.787				

[a] The data are corrected for the effects of decomposition and for the content of alkaline ash, as explained in section II.

[b], [c] Supplementary control determinations on two different conditioning batches, and with different periods of equilibration.

[d] Control determinations on wool of different ash content.

[e] Data from section (b).

[f] Data from section (a).

When the data obtained at constant ionic strengths are so presented in their entirety, the composite curves bear a close resemblance to the titration curves of dissolved proteins. The acid branch crosses the base line without discontinuity and continues downward with progressively more gradual slope until buffering capacity is practically lost over a range of 1 to 2 pH units. Approximately 0.10 millimole of base per gram has been bound at this point. As the pH increases, marked buffering capacity once more appears and large amounts of base are bound in the pH range 11 to 14. The largest amount bound in the range measured is 0.78 millimole per gram, but there is no indication from the shape of the curves that somewhat larger amounts of base would not be bound at a higher pH. This maximum value is slightly higher than that reported by Harris and Rutherford. The cause of part of this difference has already been given (see section II). Another part may be due to the alkaline ash content of the wool yarn, as pointed out in the earlier work.

Although the alkali titration curve is more complex than the acid curve, the shifts produced in its position by salt are exactly analogous to the shifts in the acid curves. Thus every point on the curve representing measurements at 0.02 *M* is shifted to the alkaline side by almost exactly 1.0 pH unit from the corresponding point at 0.20 *M*. The data obtained without salt are widely separated from the others, especially as the pH for zero combination is approached. The curve representing the data obtained in the absence of salt in both alkaline and acid solutions consists of two steep sections and shows clearly the long central region of about 5 pH units in which the amounts of acid or base bound are very small.

3. RELATIONS TO TITRATION CURVES OF SOLUBLE PROTEINS

The occurrence of a large and nearly stoichiometric dependence of the pH coordinate of the titration data for wool on the ionic concentration makes it of some interest to determine whether any of the large number of possible positions of the resulting curves corresponds with the curve which would characterize a similar protein in the dissolved state. It has been shown (fig. 1) that the acid curves of wool and of a comparable soluble protein, egg albumin, obtained in the total absence of salt, differ very widely. In figure 5, data for the same two proteins are compared at a high salt concentration, 0.5 *M*. Above this concentration the positions of the titration curves with respect to the pH axis have a lower degree of dependence on salt concentration.[14]

The data for wool shown in figures 1 and 5 were obtained at 25° C, the temperature used by Cannan, and are summarized in table 3. The points for egg albumin in figure 5 represent interpolations at equidistant points along the pH axis between the curves given by Cannan [4] for 0.27 and 0.67 *M* salt. The data for wool at 25° C differ only slightly from those at 0° C already described.

It is clear that the differences between the data for the two proteins, so marked in figure 1, have been greatly reduced in figure 5. Aside from a slightly more acid position for the curve for wool, discrepancies to which significance may be attached appear only in the region of very slight acid binding. These discrepancies are very small. Some disagreement might be expected in this region owing to small dif-

[14] The smaller shifts that occur at salt concentrations above 0.5 *M* are of practically the same magnitude as those which occur with the dissolved protein in the same range of concentrations.

ferences between the ratios of imidazole to carboxyl groups in the two proteins. Since the histidine content of both proteins is small, it is very likely that the analytical values reported are not exact.

TABLE 3.—*Combination of wool with hydrochloric acid at 25° C* [a]

(a) No added salt		(b) Ionic strength 0.50	
pH	Acid bound	pH	Acid bound
	Millimoles/g		*Millimoles/g*
0.638	0.877	0.412	0.877
0.826	.802	0.839	.810
1.448	.687	1.154	.815
1.710	.599	1.470	.824
1.915	.520	1.774	.757
2.191	.402	2.088	.762
2.406	.316	2.835	.636
2.722	.225	3.772	.426
3.093	.143	3.929	.383
3.370	.087	4.380	.281
3.962	.036	4.852	.214
4.603	.014	5.33	.140
5.257	[b]−.013	5.82	.082
6.38	[b]−.026	6.25	.023
6.82	[b]−.035	6.50	.002
7.28	[b]−.042	6.72	[b]−.026

[a] The data are corrected for water uptake by the fiber and the effect of its alkaline ash, as explained in section II.
[b] Negative values indicate loss of bound acid or combination with hydroxyl ion.

This comparison shows that proteins of comparable composition with respect to dicarboxylic amino acids and histidine, give closely similar acid-combination curves when they are compared over the same acid range in solutions containing high concentrations of ions. This

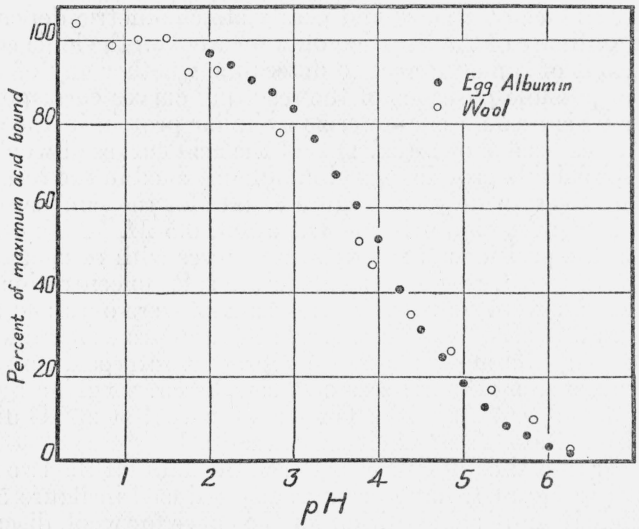

FIGURE 5.—*Comparison of the curves of acid combination of wool and of egg albumin at 25° C, in the presence of 0.5 M chloride.*

result must be taken into account in any explanation offered for the great difference in acid-binding behavior between the same proteins when neutral salts are absent. In itself, the comparison is sufficient

to exclude from consideration explanations which appeal to a fundamental difference in configuration of the acidic and basic groups in the two classes of substances. The comparison gains further significance from Cannan's demonstration that the egg albumin curves of figures 1 and 5 are very similar to those of polyvalent acids in which all of the dissociating groups have the same intrinsic strength, but in which the negative charges left by the dissociation of each hydrogen ion hinder the dissociation of the remaining groups. The present comparison indicates that Cannan's reasoning must apply to wool to the same degree as to egg albumin. The differences between the titration curves of the two proteins found in the absence of salt must find their explanation in elements of an equilibrium which occurs between two phases.

4. ANALYSIS OF THE TITRATION CURVES

By analogy with similar analyses of titration curves of soluble proteins [8, 9, 10, 27, 28], it should be possible to identify each portion of the curves for wool at high constant ionic strength with one of the several kinds of dissociating groups contributed by the constituent amino-acid residues. The acid branch is clearly attributable to the back-titrations of the ionized carboxyl groups of glutamic, hydroxyglutamic, and aspartic acids, overlapping in the pH range above 4.5 the combination with acid of the imino groups of histidine. In addition, those carboxyl groups which have not donated their protons to amino and guanidino groups (the excess of carboxyl groups over these groups) must be responsible for part of the unbroken continuation of this S-shaped curve to pH 8; another part is contributed by the removal of protons from those histidine residues which have their imino groups in the charged (acid) form. In the unbuffered region between pH 8 and pH 10 almost no groups dissociate, but in the buffered section of the curve (pH 11 to pH 14) the ϵ-amino groups of lysine are regenerated from their conjugate acid forms by loss of a proton. In addition, the hydroxyl groups of tyrosine (if they are free in wool) become ionized, and at least some of the guanidino groups of arginine, present in their conjugate acid form, give up their protons.

The foregoing partial analysis of the composition of the high ionic-strength curves may be rendered more convincing by the following quantitative comparison of its implications with the admittedly incomplete data at present available on the amino-acid composition of wool.

TABLE 4.—*Acidic and basic amino-acids in wool*

	Reference	Percent	Millimoles per gram
(1) Aspartic acid	[46]	7.27	0.545
(2) Glutamic acid	[46]	15.27	1.035
(3) Amide nitrogen	[46]	1.37	0.978
(1) plus (2) minus (3)			0.602
(4) Arginine	[16]	10.2	0.586
(5) Lysine	[39]	3.3	.226
(6) Histidine	[50]	0.66	.044
(4) plus (5) plus (6)			0.856
(1) plus (2) minus (3) plus (4) plus (5) plus (6)			1.458
(7) Tyrosine	[38]	5.8	0.320

The sum of the acid-binding and base-binding capacities of wool between pH 0.8 and pH 14.3 is 1.59 ±0.04 millimoles per gram. Table 4 lists the reported contents of aspartic and glutamic acids, histidine, lysine, arginine, amide nitrogen, and tyrosine. The sum of the acid- and base-binding equivalents, *omitting the tyrosine*, and after subtraction of the amide nitrogen to allow for the part of the dicarboxylic acids present as glutamine and asparagine, is 1.46 millimoles per gram. *With the tyrosine included* it is 1.78. Both figures are given because the experimental sum falls between them, and because there is a suggestion in certain unpublished data on wool and in published data on some soluble proteins [15, 47] that some or all of the hydroxyl groups of tyrosine in proteins may not be free to combine with base. Another explanation of the small discrepancy is possible: The figures for the carboxylic acids and histidine were obtained by isolation methods and are likely to be low. Should this be the case one may conclude that tyrosine hydroxyl groups are *not* titrated. However, it is not certain that the present measurements give the maximum base-binding capacity, and only part of the arginine may be combined with base at pH 14.3. Should this be true one may conclude that the tyrosine is titrated quantitatively.

It is possible to calculate roughly what the maximum base-binding capacity should be from the maximum amount of acid which is bound and the pH at which no combination with either acid or base occurs. Since the latter falls in the titration range of histidine, which is present in small amount, it appears that in the uncombined state of the protein practically all the carboxyl groups have donated their hydrogen ions to basic groups. Thus the sum of the basic groups (from lysine and arginine) must be equal to the sum of the carboxyl groups (from glutamic, hydroxyglutamic, and aspartic acids) within a margin of uncertainty which is smaller than the small content of histidine. The maximum base-binding capacity, *excluding tyrosine*, should therefore correspond roughly with the maximum acid-binding capacity,—which may be taken as 0.82. The sum of the two should not be far from $2\times0.82=1.64$, a figure close to 1.59, the value found. It would be necessary to assume that about half the arginine is still not titrated at pH 14.3 in order to make room for the titration of all the tyrosine. This would increase the maximum amount of base bound at higher alkalinities to about 1.18, far above the figure found at pH 14.3. The balance of probability therefore favors the conclusion that in the unhydrolyzed wool the hydroxyl groups of tyrosine are not free to combine with base, and therefore contribute no part of the large buffering effect at pH 11 to pH 14.

This conclusion entails the plausible assumption that some or all of the analytical data are low. Since the sum of the arginine and lysine listed in the table is 0.82 millimole per gram, in good agreement with the capacity of wool to bind acid, the largest part of the discrepancy must be sought in the values for the dicarboxylic acids and amide nitrogen, in both of which the potentialities for error have long been recognized. The relatively large amount of acid plus base combined between pH 6 and pH 8 (about 0.13 millimole) suggests that the histidine figure in table 3 may also be low, since only a small part of the combination in this range may be attributed to carboxyl groups. However, an increase in the estimated histidine content will not greatly affect the close agreement of the experimentally

determined sum of acid- plus base-binding capacity with the sum calculated from the acid-binding capacity alone, since only an indeterminate part of the histidine content contributes to the curve of acid combination, the balance being responsible for a part of the base combined at higher pH.

This analysis of the structure of the titration curve of wool differs in at least one significant respect from the interpretation offered by MacMahon and Speakman [36]. These authors regard the step on the curve which represents the base bound in salt-free solutions, between the pH at zero combination and the relatively unbuffered section at pH 8 to pH 10 as caused by the titration of sulfhydryl groups which are present in wool that has suffered partial decomposition. In the present investigation on undamaged wool, this step on the curve of base combination also appears; in the curves obtained in the presence of salt it clearly represents a continuation of the first S-shaped branch of the titration curve which begins its downward course near pH 1, and which has been accounted for above as an expression of the partially overlapping titration of carboxyl and imino groups. It seems unlikely that sulfhydryl groups, which are extremely weak acids, would be appreciably titrated in this range of pH. The formation of strongly acid groups by exposure to light may result in a shift of the pH at zero combination to lower values. This would have the effect of increasing the height of the alkaline step, which Speakman attributed to sulfhydryl.

IV. THEORY OF THE COMBINATION WITH ACID[15]

1. COMBINATION OF INSOLUBLE AMPHOLYTES WITH THE ANIONS OF ACIDS

It has been shown in a previous section (III–1) that the acid bound by wool is a function of the concentration of salt as well as of the concentration of hydrogen ions. Furthermore, the way in which the total anion concentration (from acid plus salt) participates in any equation defining the amounts of acid bound under various conditions must be in some measure analogous to the manner of participation of the hydrogen ion. When it is considered that practically equivalent quantities of hydrogen and chloride ions must be combined, dissolved, or adsorbed by the fiber, it is evident that the extent to which one of these ions may be taken up depends in part on the simultaneous availability of ions of the opposite sign.

Restrictions of the latter kind in two-phase acid-base equilibria have long been known. Cases involving two sharply delimited phases, separated by a membrane which is impermeable to one or more of the ions present, have been treated thermodynamically by Donnan [12, 13]. Application of the Donnan equations to the present case would involve elements of arbitrariness in the choice of volumes, concentrations, concentration-scale (because of the high concentration of dissolved substances), the distinction between acid bound by protein and acid merely dissolved by the medium present in the fiber phase, and in the allocation of the phase boundary. It will be shown later that with such an arbitrary choice permitted, a

[15] The acid branch of the titration curve has been chosen for discussion because it is composed of fewer distinct components. The treatment here proposed should be applicable, with suitable modifications, to the alkali branch as well.

good approximation to certain features of the data already described could be made. In other respects the approximation is less successful, and leads to consequences which appear to be incompatible with the comparison made above between data for wool and for soluble proteins. For these reasons, and because of the nearly complete interchangeability of hydrogen and chloride ions suggested by our results in acid solutions, it has seemed preferable to treat the restriction on the combination of wool with hydrogen ions more simply on the basis of three easily stated assumptions:

1. *The fiber protein combines with chloride ions as well as with hydrogen ions, and the combination of both is subject to the law of mass action, or to the Langmuir adsorption law.*[16] This assumption may be thought of as implying the formation of a partially dissociated linkage between RNH_3^+ groups and Cl— ions. Alternatively, the positively charged acid fiber might be thought of as forming a unimolecular adsorption layer of chloride ions. However, the observation that acid-dye anions permeate a wool fiber with a high degree of uniformity when time for full penetration is allowed shows that if the latter view be taken, adsorption does not occur merely on the outside of the fiber. The existence of stoichiometrical relations in the combination of acid and base by the wool also shows that the adsorption must occur on every molecule in the fiber.

2. *No recognition need be taken of differences in hydrogen-ion concentrations inside and outside the fiber.* This may signify either that these concentrations are identical, or that they are related to one another in a way which is practically independent of the presence of other ions. The assumption appears to be incompatible with the conventional application of the Donnan treatment to two-phase systems. Nevertheless, it represents a reasonable approximation, if postulate 1 given above is accepted: the existence of partially undissociated protein-chloride combinations would greatly minimize the differences predicted by the Donnan equations for the case of the combination of hydrogen ions alone. Since pH differences are the essential feature of the usual application of the Donnan equilibrium to fiber problems, disregard of this necessarily complicated and, in the present case, arbitrary treatment requires no further *a priori* justification.

Alternatively, postulate 1 may be regarded as a purely empirical way of evading explicit treatment of pH differences brought about by combination of the fiber protein with hydrogen ions alone. As will be shown later, both methods of calculation lead to very similar descriptions of a large part of the experimental phenomena, and a final choice between them can only be made on the basis of a wider field of observation, including comparisons with similar results with soluble proteins and with other insoluble materials, and the effects of other experimental variables which are still under investigation. In the meantime, the present treatment possesses a great advantage in simplicity of calculation.

[16] The assumption that the mass-action law may be applied implies that the fiber is a dispersed phase. In this case, the fiber must be thought of as a solid solution or as a solution of protein in imbibed water. If, on the other hand, the process of combination is one of adsorption, use of the Langmuir equation to describe it implies chemisorption at definite unsaturated points throughout the fiber; these points may be identified with the acidic and basic groups. Neither alternative involves new assumptions; the validity of one or the other is implied by the familiar experimental fact that the curves of acid combination by wool immersed in water are continuous and free of singular points. Derivations based either on the law of mass action or on the Langmuir adsorption equations necessarily lead to the same result.

The present treatment makes no assumption that the dispersed wool protein is a single chemical individual.

3. *Variation of activity coefficients in the fiber phase may be disregarded.* This is the only procedure possible when the system is not accessible to direct measurement by independent means. The postulate is required only if the law of mass action rather than the Langmuir adsorption equation is used.

It is implied in postulate 1 that an equilibrium,

$$WCl^+ \rightleftharpoons W^\pm + Cl^-, \tag{1}$$

in which $W^\pm$ represents wool in the uncombined state, occurs in addition to the more familiar acid-base equilibrium which may be written

$$WHCl \rightleftharpoons WCl^- + H^+. \tag{2}$$

If these are the only two equilibria which can occur and if, for simplicity, wool is regarded as a divalent ampholyte, that is, capable of combining a single equivalent of acid or a single equivalent of base, and two thermodynamic dissociation constants K_A' and K_H' are assigned to the equilibria 1 and 2 listed above, the law of mass action (plus postulate 3) or the Langmuir adsorption law permits us to write

$$\frac{[WHCl]}{[WHCl]+[WCl^-]+[W^\pm]} = \frac{1}{1+\frac{K_H'}{a_H}\left(1+\frac{K_A'}{a_{Cl}}\right)}. \tag{3}$$

The bracketed quantities represent concentrations, a_H and a_{Cl} the activities of hydrogen and chloride ions, respectively, and the left-hand member represents the fraction of the total wool combined with acid.

The resemblance of eq 3 to the familiar expression for the ionization of a dibasic acid is apparent. If account is also taken of the hydrogen ions removed from the external solution by absorption in the fiber in an amount equivalent to the negative charge contributed by combined chloride ions, the ratio of acid taken up to total wool becomes

$$\frac{[WHCl]+[WCl^-]}{[WHCl]+[WCl^-]+[W^\pm]} = \frac{1}{1+\frac{K_H'K_A'}{a_{Cl}(a_H+K_H')}}. \tag{3'}$$

Obviously the successive stages of combination might be formulated with equal *a priori* validity in the alternative equilibria:

$$WH^+ \rightleftharpoons W^\pm + H^+ \tag{4}$$

$$WHCl \rightleftharpoons WH^+ + Cl^- \tag{5}$$

to which the dissociation constants K_H and K_A may be successively assigned. The corresponding form of eq 3 is then

$$\frac{[WHCl]}{[WHCl]+[WH^+]+[W^\pm]} = \frac{1}{1+\frac{K_A}{a_{Cl}}\left(1+\frac{K_H}{a_H}\right)}, \tag{6}$$

and the corresponding form of eq 3′, which represents more closely the measured quantities, is

$$\frac{[WHCl]+[WH^+]}{[WHCl]+[WH^+]+[W^\pm]}=\frac{1}{1+\frac{K_H K_A}{a_H(a_{Cl}+K_A)}}. \qquad (6')$$

The four dissociation constants which appear in these equations are obviously interrelated as follows:

$$K_A K_H = K_A' K_H'.$$

Equations 3′ and 6′ differ from one another by the omission of different single terms in a complete expression for the sum of all the ionic states of the wool. This omission leads them to describe quite different relations between the hydrogen-ion and chloride-ion concentrations at which equal amounts of acid are taken up. Since there is no compelling reason for choosing between them, it is necessary to take both sets of equilibria into account in deriving the most general expression for the fraction of the maximum amount of acid bound as a function of a_H and a_{Cl}. This may be done in two ways, according to whether $[WH^+]$ is larger or smaller than $[WCl^-]$, since only the larger one of these two terms must be included in the numerator of the left-hand fraction, which expresses the part of the wool which binds acid. When $[WH^+]$ is the larger, the positive charge on the fiber is partly neutralized by the smaller term $[WCl^-]$ and partly by chloride ions in an adsorption layer.[17] Within the range of pH, and salt concentrations to which attention is here confined, and with any assignment of physically reasonable values to K_H' and K_A', $[WH^+]$ will be larger than $[WCl^-]$ and should therefore be chosen for inclusion in the numerator. This choice, implying $K_H \ll K_A'$, leads to the relation:

$$\frac{[WHCl]+[WH^+]}{[WHCl]+[WH^+]+[WCl^-]+[W^\pm]}=\frac{1}{1+\frac{K_H'}{a_H}\left(\frac{a_{Cl}+K_A'}{a_{Cl}+K_A}\right)}. \qquad (7)$$

The right-hand member of eq 7, like that of eq 3, which it closely resembles, differs from the expression for the dissociation residue of a monobasic acid only by the presence of the term in parentheses. In the presence of a constant concentration of chloride ions, this term is constant. Under these conditions the amount of undissociated acid varies with pH exactly as in the case of a monobasic acid, but the apparent strength of the acid (the position of the curve on the pH axis) varies with the magnitude of the bracketed term, which in turn depends on the concentration of chloride ions. With high anion concentrations the term in parentheses approaches a limiting value of unity, and the dependence of the acid bound at any given pH on further increases in the anion concentration disappears. Thus the limiting position of the titration curve is determined solely by K_H' and should correspond with the position of the titration curve of a

[17] With dye anions of high affinity for wool fibers, such as those used in substantive dyeing, it would be necessary to substitute in the numerator a term analogous to $[WCl^-]$ in place of $[WH^+]$. When more than one kind of cation is present, however, (in the presence of salt), this simple substitution does not suffice. The considerably modified relations which are applicable to cases in which $K_A' < K_H$ are beyond the scope of the present paper.

similar *dissolved* acid. It has already been shown that these properties of eq 7 correspond to experimental facts.[18]

The quantitative dependence of the positions of the titration curves described by eq 7 on the level at which the anion concentration is held constant may be demonstrated by determining the pH for half-maximal combination as a function of the anion concentration. This may be done by equating the right-hand member of eq 7 to 0.5. The resulting relation is

$$pH_{\frac{1}{2}}=pK_H'+\log\left(\frac{a_{Cl}+K_A}{a_{Cl}+K_A'}\right) \tag{8}$$

In addition to the relation $K_H \ll K_A'$ already discussed, electrostatic considerations obviously require that $K_A'>K_A$ (and $K_H>K_H'$). Thus, in the range $K_A' \gg a_{Cl} \gg K_A$, the logarithmic term approaches $\log \frac{a_{Cl}}{K_A'}$, and the pH for half-maximal combination, which characterizes the position of the entire acid-combination curve, shows a close approximation to direct dependence on the logarithm of the concentration of chloride ions. This range of direct dependence extends down to the lowest possible values of a_{Cl} (equal concentrations of hydrogen and chloride ions). The upper limit of this range is determined by K_A', which determines the concentration of anions at which a limiting position of the curve is approached.

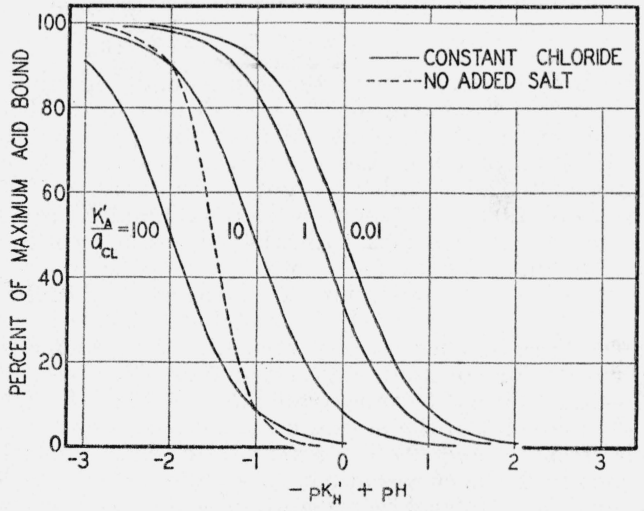

FIGURE 6.—*Theoretical curves of acid combination described by equations 7 and 7'.*

The effect of variations in ionic strength is demonstrated by representation of curves for four different values of K_A'/a_{Cl}.

The properties of eq 7 are illustrated in figure 6, which shows the theoretical curves of acid combination with respect to pH at several different values of K_A'/a_{Cl}. For purposes of calculation, K_A has been

[18] Thus, it is K_H' rather than K_H which corresponds to the dissociation constant of the acid in solution. This seems to imply that (in dilute solutions) K_H' is as little dependent on the nature of the anion as K_H. If this is true, the absolute values of K_A and K_A' can depend on the nature of the anion, but the ratio K_A/K_A' cannot. The conclusion is a reasonable one if the difference between K_A and K_A' or K_H and K_H' is attributed almost entirely to an electrostatic effect due to a single charge.

taken as vanishingly small, since $K_A' \gg K_A$. It is noteworthy that the assignment of an infinitely small value to K_A would make the right-hand members of eq 7 and 3 identical.

It is clear that the positions of the curves are determined by K_A'/a_{Cl}, although their shapes are independent of this ratio. The dependence of the positions on $\log K_A'/a_{Cl}$ is at first linear, but becomes less marked as the ratio diminishes; finally, at very low values of the fraction (a_{Cl} large), the positions of the curves approach a limit, determined by K_H'. By utilizing eq 8, K_A' may be roughly evaluated from the pH differences at the midpoints of curves at two or more different salt concentrations sufficiently high to be beyond the region of simple stoichiometric dependence.

In solutions containing no added salt, a_{Cl} is not constant but equal to a_H. Equation 7 then becomes

$$\frac{[WHCl]+[WH^+]}{[WHCl]+[WH^+]+[WCl^-]+[W^+]} = \frac{1}{1+\frac{K_H'}{a_H}\left(\frac{a_H+K_A'}{a_H+K_A}\right)}. \qquad (7')$$

Since K_A' has already been assumed to be large, and K_A very small, there is a wide range of values of a_H in which the bracketed expression reduces to K_A'/a_H. The curve in the absence of salt is then described, up to moderately high acidities and high degree of combination, by the relation

$$\text{Fraction of maximum acid bound} \doteq \frac{1}{1+\frac{K_H'K_A'}{a_H^2}}, \qquad (7'')$$

which is entirely consistent with the approximate description mentioned earlier: that the curve for the case of no added salt could be related to the curves at constant ionic strength by substitutions of $[H^+]^2$ for $[H^+]$ in functions describing the latter. The curve in figure 6 labelled "no added salt" is drawn to represent eq 7'. K_A' has been taken to be 1.000 K_H' (approximately 0.1) and K_A is considered negligibly small. In contrast with the curves for constant ionic strengths, the value of K_A' affects not only the pH value at which a given amount of acid is bound, but also the exact form of the relation between acid bound and pH. If K_A' is taken sufficiently small, the no-salt curve loses its characteristic steepness and becomes identical with the curves for constant ionic strength. Thus two additional means of estimating K_A' become available: (1) The difference in pH between the midpoint of the no-salt curve and any one curve at constant ionic strength, and (2) The extent to which the no-salt curves differ in slope from the curves at constant ionic strength. The value of K_A' selected for graphical representation in the no-added-salt curve of figure 6 (approximately 0.1) was chosen because it gives very nearly the proper separation from the other curves when the same value is substituted into the value of K_A'/a_{Cl} attached to each of them.

The most exacting of the remaining tests which could be applied to the consequences developed from the postulates would be to attempt superposition of the several sets of data with the theoretical curves described by eq 7 or 7'. These equations, however, were derived for the simplest case of monovalent acids; and wool, like other proteins, is highly polyvalent. This difference can have either

of two results: (1) If all the dissociating groups have identical *intrinsic* dissociation constants and if they are far enough removed from one another in the molecule to exclude appreciable electrical interaction between them, the acid-combining curves, as Simms [40], Weber [51], von Muralt [49], and others have shown, should be identical with those of a monobasic acid, and eq 7 and 7′ should be applicable. (2) If the groups are of unequal intrinsic strength, or if they are of equal intrinsic strength but are close enough to one another so that changes in distribution of electric charges, caused by partial dissociation, weaken the dissociating tendency of the remaining groups, the resulting acid-combination curve would be the sum of a number of smaller curves distributed along the pH axis. Consequently, it would be broader with respect to the pH axis than those predicted by the equations above.

In the direct comparison presented in figure 7, the estimated contribution of the imidazole groups (0.03 millimole) has been subtracted

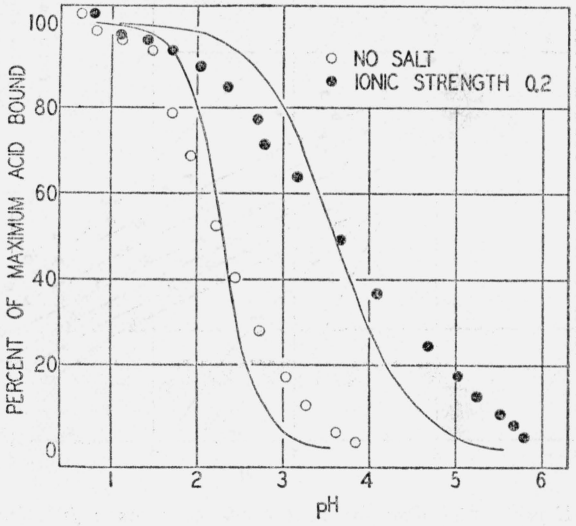

FIGURE 7.—*Experimental data for acid combination as a function of* pH *compared with theoretical curves for a mono-acid base (eq 7 and 7′).*

An estimated contribution from the histidine content of 0.03 millimole/g has been subtracted from the experimental values, as described in the text.

from the acid-combination data in an effort to have them represent as nearly as possible only the dissociation of carboxyl groups.[19] As the figure makes clear, the corrected experimental curves, both in the presence and absence of salt, are broader than the theoretical ones, and the second of the two alternatives stated above must prevail.

The high degree of symmetry of the differences between the experimental and the theoretical curves suggests, however, that the experi-

[19] The subtraction of a constant quantity is justified only at pH values more acid than those at which imidazole groups begin to give off hydrogen ions. In 0.2 *M* salt solutions, the correction should diminish appreciably at pH 5.4 and become zero at pH 6.4. Since the pH at which the correction would diminish in solutions low in salt or containing no salt cannot be estimated, recourse has been had to the subtraction of a quantity independent of pH. This results in varying degrees of overcorrection at the low end of each set of data, but the effect is small.

mental curve is not the sum of a small number of distinct groups of curves characterized by *widely different* dissociation constants, but is the result of a very regular kind of electrostatic interaction between ionic charges affecting the dissociation of groups which are all intrinsically similar. Earlier analyses of the acid-titration curves of other proteins [5, 8, 9, 10, 17] have been based on the opposite assumption of distinct groups characterized by widely different constants.

The view suggested above is based on considerations similar to those which led Cannan to the same conclusion for the titration curve of egg albumin; it receives added support from the fact that the midpoint of the experimental curve (at high salt concentrations) occurs at a pH (4.0 to 4.1) which may be expected to characterize the dis-

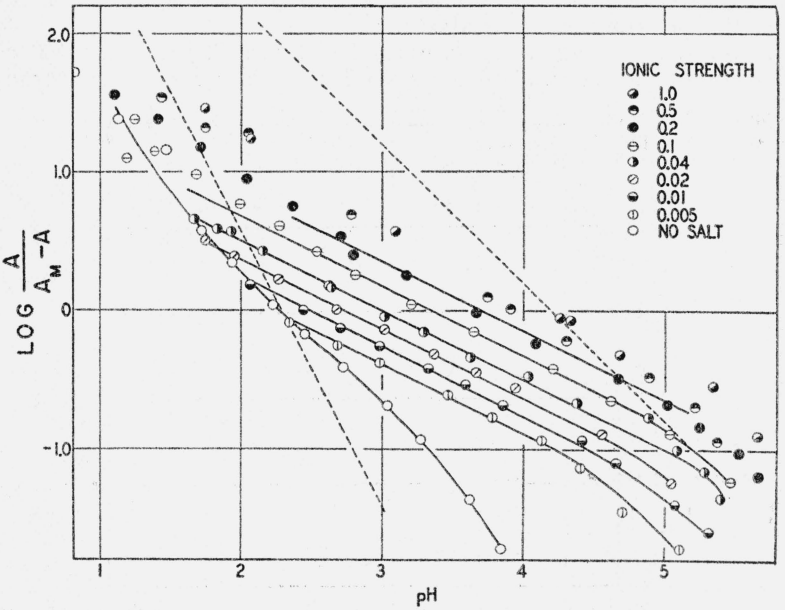

FIGURE 8.—*Experimental data for acid combination as a function of* pH *and chloride concentration (corrected by subtraction of the estimated contribution of histidine) compared with the linear relations described by equations 7 and 7′.*

The straight lines through the experimental points over the range of approximately 5 percent to 90 percent of maximal combination were drawn with a slope of 0.5. The broken lines represent the theoretical equations for no-salt (7′) and for infinite salt [7], respectively.

sociation of carboxyl groups in large peptides [6], and by other considerations discussed below.

The regularity of the difference between the theoretical curve for a monovalent acid and the experimental curve is shown more clearly if the ordinates of all the curves, after subtraction of the estimated contribution of histidine, are changed from the acid bound to log $A/(A_m - A)$, where A signifies the amount of acid bound at any pH and A_m is the maximum acid-binding capacity. The resulting graphs of the measurements at constant ionic strength, shown in figure 8, are close approximations to straight lines which are roughly parallel over a wide part of their total range. Deviations from this regularity appear only where the maximum and zero values are approached,

but at both these extremes the quantity plotted is extremely sensitive to experimental error. In addition, the data may well deviate from this simple theoretical relation for acid combination as zero acid combination is approached, since the base combination function which begins to be of importance in this region has been left out of account. As a result of this additional factor, the curves must intersect at the base line within a narrow interval of pH, and cannot remain parallel.

The slope of the theoretical curve in figure 8 for constant ionic strength is −1.0. The slopes of the lines through the experimental points for ionic strengths up to 0.2 are exactly −0.50. Thus a very simple relation exists between the experimental data and the theo-

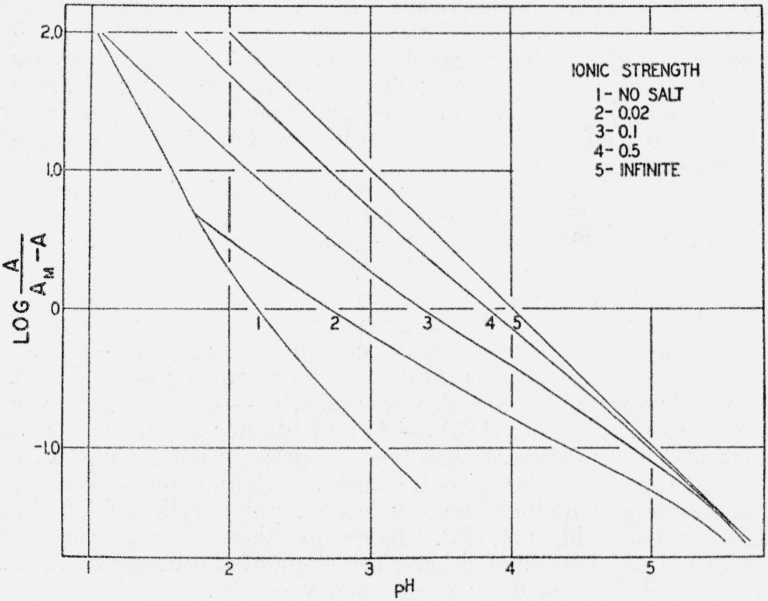

FIGURE 9.—*Effect of different constant anion concentrations on the acid bound by a nondiffusible base as a function of* pH.

The parameters employed in the application of the Donnan equations are given in the text.

retical curves, which were derived on the assumption of a single dissociation constant for all the dissociating groups. The simplicity of this relation appears to support the view that the curve is the sum of a *continuous* series of curves for the individual dissociating groups, which differ in strength from one another by slight increments determined by some law of electrical interaction between their charges, rather than that the curve is the sum of a few groups of identical curves with widely different constants.

The slope of the theoretical curve for no added salt (eq 7′) depends on the ratio of K_H' to K_A'. When $K_A' \gg K_H'$ (the more probable case with HCl), the slope approaches −2. When K_A' is only 10 times larger than K_H' the slope is still nearly −2 for small amounts of acid bound, but its absolute value becomes considerably smaller for larger amounts. However, the data obtained in the absence of salt, shown in figure 8,

have a slope of very nearly -1 when the amounts of acid bound are less than three-quarters of the maximum. For larger amounts bound, the slope approaches -2. It is not surprising that the discrepancy between the simple theory and the measurements should be more serious and less regular in the absence of salt than at constant ionic strength, since the dependence of acid bound on hydrogen ion concentration in eq 7′ or 7″ is essentially of higher degree than in eq 7. The transition (involving a summation) from the simple equation for a monovalent acid to the proper polyvalent equation is, therefore, more complicated.

The fact that over a great part of the pH range the slope obtained in the absence of salt is also just one-half of the value required by the equations has the fortuitous result that the experimental curve somewhat resembles the curve of the simple law of mass action for a monobasic acid [44]. This approximate resemblance must be regarded as the resultant of the opposing effects of the steepening of the curve caused by simultaneous variation of hydrogen and chloride ions, and the broadening effect of electrostatic interaction between many identical dissociating groups.

The hypothesis that the departure of the individual experimental curves from the slopes predicted by eq 7 and 7′ is caused solely by the high polyvalency of the wool protein, does not depend exclusively on the considerations just discussed. It is very strongly supported by the almost exact correspondence of the experimental curves for wool at high constant ionic strengths with the corresponding curves for egg albumin. When similarly plotted as $\log A/(A_m-A)$ against pH these data (at least for dilute salt solutions) also yield straight lines, with slopes which are also very nearly -0.5. The same linear relationship between $\log A/(A_m-A)$ and pH has appeared in investigations of widely different dissolved substances which have in common only the properties of polybasicity and high molecular-weight. These substances include, among others, polyacrylic acid [32], and certain nucleic acids [23, 41]. Kern has shown that, over a wide range of polymerization numbers, the slope of the linear relationship for polyacrylic acid is also almost exactly -0.50.

2. ALTERNATIVE ANALYSIS BY THE DONNAN EQUILIBRIUM

The difficulties inherent in application of the Donnan equations to the combination of wool fibers with acid have already been detailed. However, if a somewhat arbitrary selection of parameters is permitted, an alternative treatment for the restrictions on combination of acid due to the presence of two phases is possible, and a number of features of the present data can be described as direct consequences of the Donnan equations. In application of the Donnan equilibrium to fibers, a choice must be made between regarding the solid phase as a dispersed phase (in imbibed solvent), permeable to dissolved acid and salts, and the alternative view that it is a true solid phase in which the phenomena of acid combination take place in an external adsorption layer. The first of these models has been chosen as a basis for calculation, since the second offers no obvious basis for the assignment of any but arbitrary numerical parameters.

The result of this choice is given in figure 9, which shows calculated curves of acid combined by protein as $\log A/(A_m-A)$. In this cal

culation a single dissociation constant, 1×10^{-4}, and a protein concentration of 0.8 equivalent per liter have been used. The latter value bears a reasonable relation to the equivalent weight of wool, which is approximately 1,250, and its moisture content at saturation which is approximately 30 percent by weight. Although the relations of the curves calculated for no added salt and for different constant ionic strengths would be appreciably altered by the choice of other numerical values, their positions on the pH axis and the value of the maximum fix the relation of the dissociation constant to the equivalent concentration within fairly narrow limits. Activity coefficients have been neglected. The ordinates of the curves represent only the acid reacting with the protein and do not include the acid which may be merely dissolved in the fiber phase. The latter should be practically negligible except in the immediate vicinity of the top of the curve.

It is immediately evident that the curves in figure 9 satisfy the requirements imposed by the data in three outstanding respects: (1) The curve for the case of no added salt is very similar to the experimental one. (2) The curves for constant ionic strengths are less steep than the curve for no salt, and their average slopes, although still too high, are nearer the experimental ones than the slopes given by eq 7, which were twice as large as those required by the data. (3) When measured in the upper half of the figure, the dependence on ionic strength of the pH values at which a given amount of acid is combined is approximately logarithmic when the ionic strengths are low; when the ionic strengths are high, the positions of the curves with respect to the pH axis approach a limit.

In another important respect, however, the curves of figure 9 are entirely different from those obtained by experiment or calculated from eq 7 (shown in fig. 8); the experimental curves are roughly parallel except at very high ionic strengths, and as they approach closely to the base line. The curves of figure 9, on the contrary, converge continuously, and this convergence is inherent in any simple analysis by means of the Donnan equilibrium.

Another consideration must be taken into account, before accepting as significant the partial agreement of the Donnan equations with experiment. It has already been shown that there is a detailed agreement between the experimental data for wool and for dissolved egg albumin at moderate ionic strengths. If significance were to be attached to the fact that the Donnan equations reduce the slope of the theoretical monobasic acid curve to a value considerably closer to its experimental value for wool, then it would seem necessary to conclude that the Donnan treatment must be applied also to egg albumin *in solution*. The further requirement that the application of the Donnan equations to the *dissolved molecules* must involve nearly identical numerical values for the parameters of the equations in the two physically quite distinct cases is even less acceptable.

The model which has been used as a basis for the application of the Donnan equations to the present experiments is intrinsically unreal. It is unlikely, for example, that all the water contained in the immersed fibers can be regarded as solvent water. Not only is it comparable in magnitude with the water of crystallization of proteins in general, but it corresponds closely with the hypothesis of definite and limited hydration of the polar groups or bonds of the wool molecule.[20]

[20] Unlike such cases as swollen gelatin to which the Donnan equations are more legitimately applied.

Such bound water can be regarded as a solvent medium only in a very restricted sense, and it is hardly likely that acids or salts will distribute themselves between it and the external solvent medium in a simple one-to-one ratio. It has not proved possible to detect experimentally any combination with neutral salts when fibers containing half of the maximum amount of water which they can absorb are placed in 0.2 *M* KCl at pH 6.4. Thus half or nearly half of the maximum water content is not free to dissolve ionized acids or salts. This conclusion also results from a recent detailed study of moisture absorption of cotton fibers in this laboratory [42].

The principal distinction for practical purposes (such as the interpretation of acid dyeing) between the mechanism of acid combination proposed in this paper and the alternative treatment in terms of the Donnan equilibrium, lies in the expectation that the specific affinities ($1/K_A'$) of different anions for wool may vary considerably. Conversely, the experimental study of such a variation and its dependence on such variables as temperature and dielectric constant has considerable bearing on the views discussed here, and is at present in progress.

The authors are indebted to Charles H. Fugitt for many of the experimental measurements reported in this paper.

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