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COMBINATION OF WOOL PROTEIN WITH ACID AND BASE: THE EFFECT OF TEMPERATURE ON THE TITRATION CURVE

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ABSTRACT

Equilibrium measurements are reported of the amounts of hydrochloric acid and potassium hydroxide bound by wool, as a function of pH and salt concentration at 0°, 25°, and 50° C. In order to eliminate from the measurements of base combination effects due to decomposition, a study was also made of the effect of temperature on the rate of decomposition of the disulfide bonds in wool and on the rate of solution of the fiber in alkaline solutions. The data support the assumption made in accounting for previously reported titration measurements at 0° C, that the carboxyl and amino groups of wool in the uncombined state are completely ionized. Thus changes in the pH coordinates of the titration curves brought about by changes in temperature are small in the pH range in which acid is combined, which indicates that combination with acid is equivalent to back-titration of the carboxyl groups, but are large in the pH range in which base is combined, which indicates that combination with base is equivalent to back-titration of amino groups.

The heats of dissociation calculated for the two kinds of groups, approximately 2,500 and 14,000 calories, respectively, are in good agreement with values for these groups in comparable compounds and in soluble proteins. The value obtained in the acid range also agrees with the results of calorimetric measurements on the combination of acid with wool.

It is shown that approximately equal parts of the total heat changes in the acid range are associated with the dissociation of hydrogen ions and chloride ions from the fiber. An appreciable part of the total heat effect may be ascribed to a heat of transfer of the ions between the two phases of the heterogeneous titration system. Neither titrimetric nor calorimetric estimations of the heats of dissociation provide evidence for or against the existence of "salt linkages" in wool.

CONTENTS

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I. INTRODUCTION

Measurements of the dependence on pH and salt concentration of the amounts of hydrochloric acid and potassium hydroxide bound by wool at 0° C. have been described in an earlier publication by two of the present authors [29].[2] The proposed theoretical analysis of these measurements was based on the assumptions: (1) That in the uncombined state the carboxyl groups and basic groups are completely ionized, i. e., the hydrogen atoms of the carboxyl groups have been transferred, with their positive charges, to the amino and guanidino groups, leaving negatively charged carboxylate groups and positively charged alkylammonium groups; (2) that when the protein combines with hydrogen ions it also forms partially dissociated stoichiometric complexes with negative ions, (e. g., Cl^-), and when it combines with hydroxyl ions, it forms similar complexes with positive ions (e. g., K^+). The first assumption is equivalent to postulating for the protein the same zwitterion [3] structure originally proposed for amino-acids and certain other ampholytes by Adams [1] and by Bjerrum [2], and now almost universally adopted as the result of the researches of Bjerrum [2], Harris and Birch [15], Wyman [35], Cohn and his collaborators [7], and others. The purpose of the present paper is to adduce evidence justifying application of the dipolar ion concept to wool protein. Experiments designed to further justify and extend the second and less familiar assumption are at present in preparation for publication.

It was pointed out by Bjerrum that the effects of temperature on the dissociation constants of the α-amino-acids furnished an indication of their existence as dipolar ions. Since the publication of Bjerrum's paper this criterion has been made use of in the study of proteins as well as of amino-acids by Pertzoff and Carpenter (casein) [24], and by Wyman (hemoglobin) [36]. Changes in temperature were observed to have very small effects on the parts of the titration curves of these proteins which lie on the acid side of neutrality, but the effects on the alkaline side were very considerable. These results may be understood if these proteins are, like their constituent amino-acids, dipolar ions, since then the addition of alkali to their solutions serves mainly to remove hydrogen ions from amino groups, the dissociation constants of which are very dependent upon temperature.

Measurements of the effects of temperature on the titration curves of wool keratin, presented in the present paper, lead to the same conclusion. They also offer a means of evaluating the energy changes accompanying the associations of anions and cations, postulated in assumption 2. It is a consequence of this assumption that the anions of different acids should be characterized by different affinities for wool (i. e., this affinity is inversely related to the degree to which they dissociate under standard conditions).[4] The existence of these postulated differences in affinity would be manifested by different positions with respect to the pH coordinates of the titration curves of wool obtained with different acids.[5] The differences in affinity, if sufficiently large, would be accompanied by varying degrees of sensitivity

[2] Figures in brackets indicate the literature references at the end of this paper.
[3] In the remainder of this paper the term "dipolar ion" is substituted for "zwitterion" in conformity with recent American usage [6, 7].
[4] It has been demonstrated [28, 30] that very wide differences do indeed exist between the affinities of the anions of various acids for wool and for other proteins.
[5] In the case of acid dyes this affinity is directly related to fastness to washing and to alkalies.

of the respective titration curves to changes in temperature. While an inverse relation between the magnitudes of dissociation constants and the heats of the corresponding dissociations is not regular or exact, it is so general that the demonstration of an increased temperature dependence of the titration curve with ions of high affinity should be an essential part of the experimental validation of the second assumption. Because of the wide use of hydrochloric acid by all investigators of the acidic and basic properties of wool as well as of other proteins, and because the data with this acid are unusually complete, it is convenient to refer all comparisons of the titration curves of wool with other acids to the common basis of its titration curve with hydrochloric acid, and to express the affinities of the anions of other acids in terms of the affinity of wool for chloride ions. For this purpose one needs not only titration data with hydrochloric acid at a single temperature, such as have already been described [29], but also measurements of the effect of temperature on the same equilibrium, as given in the present paper. In order to obtain accurate data in solutions of alkaline pH, at which decomposition of the disulfide bonds occurs very rapidly at room temperature, it has also been necessary to investigate the lability of this bond as a function of temperature as well as of pH and concentration of ions.

Irrespective of what views may be entertained as to the nature of the equilibria involved in the titration of wool with acid or base, it is desirable to appraise any effect which may be produced on the normal temperature dependence of a protein titration curve by the circumstance that the titration system exists in two phases. With this information at hand, it should be possible to determine the relationship of the detailed titration measurements previously obtained at 0° C to the phenomena of combination of acid and base by wool at the elevated temperatures which prevail when wool is exposed to acid or base during carbonizing, acid-dyeing, scouring, and milling. If an effort is to be made to relate the equilibria in such a process as acid-dyeing to the acid-base equilibrium between wool and an inorganic acid, the latter must be defined at the temperature at which the process in question is normally carried out.

II. EXPERIMENTAL PROCEDURE

Details of the preparation of materials and of most of the methods used in the present investigation have already been described [29]. Modifications in the procedures, or considerations bearing on their applicability to measurements made at high temperatures, are listed in the following sections:

1. MATERIALS

In order to assure that data obtained at different temperatures might be legitimately compared, most of the measurements were made on a single large batch of root wool fibers, purified as previously described [29] and carefully mixed before the final carding to insure homogeneity. Thus, certain of the measurements previously made at 0° C with a different batch of wool have been repeated, and use has been made of the earlier measurements at this temperature only in certain cases in which a number of duplicate measurements with the new batch of wool showed the essential identity of the two sets of

results. On incineration at 700° C the wool used left an ash of 0.26 percent; the hydrogen-ion equivalence of its cation content, as shown by electrodialysis [26], was 0.036 millimole per g. Measurements of the amounts of acid or base bound have been corrected for the alkali equivalence of the ash by subtracting from or adding to the measured acid or base bound an amount, 0.032 millimole per g, determined by direct comparison with the wool containing only a negligible amount of ash used in the previous investigations [29], as well as by subtracting the average amounts of chloride removed from solution in a large number of measurements from the average amount of hydrogen ion taken up in the same series. The closeness of the empirical correction, 0.032, to the equivalence of the cationic ash, 0.036, shows that the larger part of the cations are combined with the fibers as base, and are not present as adsorbed or entrained inorganic salts. Although the amount of ash is small, and due allowance has been made for its alkali equivalence in calculating the amounts of acid or base bound by wool free of ash, it must be recognized that the presence of an alkaline ash in the fibers results in the introduction of small amounts of salt into all the acid solutions, including those to which no salt was intentionally added. Thus, curves obtained with acid in the absence of added salt may nevertheless show small effects characteristic of the presence of traces of added negative ions [29], especially in the pH region where very small amounts of acid are combined.

2. METHODS

(a) TITRATION WITH ACID

Hydrolysis of amide groups from glutamine and asparagine residues contained in the protein was considerably more rapid at 40° and 50° C than at the lower temperatures previously reported. The extent of hydrolysis was minimized by limiting the time of immersion of the wool to approximately 24 hours, an interval which proved sufficient for attainment of equilibrium at 25° C and at higher temperatures. Even in this shorter time, however, sufficient hydrolysis occurred to require the application of corrections for the ammonia produced. Thus, at 50° C, 0.5 *M* hydrochloric acid liberated approximately 0.12 millimole per g of wool per day, and 0.2 *M* acid liberated 0.035 millimole per g of wool per day. The presence of salt (potassium chloride) increased these rates. In addition to ammonia, very small amounts of other soluble products were formed at 40° and 50° C, as evidenced by the weak positive biuret test given by the solutions. With the concentrations of acid used, the biuret test was always negative in experiments at temperatures below 25° C, even after periods of immersion up to 4 or 5 days. The appearance of soluble, peptide-like decomposition products at higher temperatures is probably not accompanied by any appreciable increase in the number of basic groups in the insoluble residue, since no increase in the maximum combining power of wool for acid is found at 50° C over the value for 0° C previously reported, when corrections are properly applied for the effects of the hydrolytic production of ammonia.[6] The presence of

[6] The reported increase in the capacity of wool to bind hydrochloric acid at high temperatures [10] may be explained by the failure to take into account the production of ammonia and its effect on the titers of the aliquots used in obtaining the measurements. The causes of the effects observed by the same investigators with dye acids are undoubtedly more complex.

dissolved fragments of protein in the aliquots titrated should be without effect on measurements of the acid taken up by the protein when indicators having end points well on the acid side of neutrality are employed in the necessary titrations.

(b) TITRATION WITH BASE

Harris and Rutherford have shown [16] that when wool is exposed to alkaline solutions, disruption of its disulfide bonds results in the more or less rapid formation *in the fiber* of groups which combine with base (tentatively identified as sulfhydryl), and that the number of acid groups which are formed in this manner approaches as a limit the number of cystine residues initially present. At the same time half of the sulfur originally contained in the cystine is found in solution as inorganic sulfide. By determining the dissolved inorganic sulfide and applying additional small corrections for the wool dissolved [29] an estimate can be made of the extent of decomposition of the disulfide linkages. It is an empirical fact that at all temperatures reported in the present paper, increases in the amounts of base bound, as a function of time of exposure to alkaline solutions, closely parallel the production of dissolved sulfide. This finding, originally reported for 0° C by Harris and Rutherford, provides a basis for distinguishing between the additional base bound by acid groups, formed as the result of decomposition at any temperature, and the base that is bound by the residual unmodified protein. The application of this correction merely requires subtraction of the number of equivalents of sulfur split off per gram of wool from the total amount of base neutralized per gram in the same experiment.[7] This procedure clearly involves the assumption that the newly formed acid groups are sufficiently strong to combine *quantitatively* with base at the pH values at which disulfide break-down occurs. At 0° C and at 25° C the range of pH in which appreciable quantities of inorganic sulfide are found in solution is sufficiently high, especially when data are obtained in the absence of added salt, so that this assumption is not called into question, even when it is postulated that the groups formed are the very weakly acid sulfhydryl, formed by hydrolysis of the disulfide bonds [8, 16]. The dissociation constant at 25° C of the sulfhydryl group of cysteine itself is not far from 10^{-10} [4], and practically quantitative combination of this acidic group with base can be expected at pH values above 12. In the presence of 0.2 *M* potassium ions at 25° C little sulfur is split out within 24 hours at pH values below 12, and in the absence of added salt practically no decomposition occurs at pH values below 12.5. At lower temperatures the pH range in which decomposition occurs is even more favorable to the concept of quantitative combination with base by sulfhydryl groups having dissociation constants not far from those of the sulfhydryl in cysteine. Nothing is known of the acid strength of sulfhydryl groups in proteins, but recent work with peptides of cysteine [11] indicates that they may be expected to be stronger rather than weaker than in cysteine itself; thus the quantitative combination with base of this group in proteins at the pH values at which the decomposition of wool occurs at low temperatures is a virtual certainty.

[7] A slight modification of this correction, required when large amounts of wool are dissolved, takes account of the contribution of the dissolved wool to the total inorganic sulfide in solution [29].

At 50° C the evidence also indicates practically quantitative combination of the newly formed acid groups with base, but the lowest pH at which extensive decomposition is found at this temperature is very much lower than when low temperatures are used. In the presence of 0.5 *M* potassium ions, appreciable decomposition within 24 hours occurs at pH values only slightly higher than 8, and even in the absence of salt appreciable loss of sulfur within this time is found at pH values below 10. Unless the sulfhydryl groups which may be formed are more than one thousand times more acidic in the protein than in the amino-acid cysteine, it may not be assumed that stoichiometric combination between these groups and base would occur in this range of pH. Nevertheless, the evidence for the occurrence of such stoichiometric combination is as strong in the measurements at 50° C. as it is for the experiments at lower temperatures.

One possible alternative to attributing the necessary relatively large acid dissociation to sulfhydryl groups in the protein lies in the possibility that the newly formed acid groups may be other than sulfhydryl. Thus, for example, the sulfhydryl groups produced by the hydrolytic reaction postulated by Harris and Rutherford might be readily oxidized by air in alkaline solutions to stronger sulfur acids. No evidence at present available demonstrates conclusively the presence of appreciable quantities of free sulfhydryl in wool from which almost one-half of the initial content of sulfur, has been removed by severe treatment with alkali at temperatures between 0° and 50° C. Such wool gives little or no reaction with the nitroprusside reagent, and treatment with *p*-chlorobenzyl chloride fails to introduce significant amounts of chlorine into the fiber.[8] The present incomplete evidence may thus be interpreted as favoring the view that if sulfhydryl groups are initially formed, they are rapidly transformed to other radicals with more strongly acidic properties than those usually attributed to sulfhydryl groups in familiar compounds. Application to the titration data of the correction for the formation of new acid groups does not depend on speculation as to the nature of these groups but rests upon the experimental demonstration that such groups are actually produced. Details of this demonstration, already furnished by Harris and Rutherford in work at 0° and 22° C, have been extended to measurements made at 50° C and are indicated in part in the section that follows.

It is noteworthy that at 50° C the corrections for the protein dissolving in alkaline solutions became almost negligible because the proportion of the total wool which dissolves at a given degree of decomposition of the disulfide bonds diminishes sharply as the temperature is increased. Thus, for example, under conditions causing the splitting of 25 percent of the disulfide bonds after a day of exposure to alkali at different temperatures, it is found that approximately 3.0 percent of the fiber has dissolved at 0° C, only 1.5 percent has dissolved at 25° C, and less than 0.8 percent has dissolved at 50° C. This effect, which might appear contrary to the usual effect of temperature on solubility, is readily understood on considering the pH values at which the same given degree of decomposition of the disulfide bonds takes place in 24 hours at the three temperatures—pH 14.1 at

[8] It has been shown [23] that when sulfhydryl groups are formed by *reduction* of the disulfide groups in wool they may be quantitatively alkylated or aralkylated with alkyl or aryl halides. By using *p*-chlorobenzyl chloride as the aralkylating agent the extent of the aralkylation may be determined by subsequent analysis of the fibers for chlorine.

0° C, pH 12.4 at 25° C, and pH 9.3 at 50° C. It thus appears that the dissolving of wool in alkaline solutions does not depend solely on the combination of the newly formed acid groups with base, but that a large proportion of the preexisting groups in the fiber which undergo acidic dissociations in alkaline solutions (largely RNH_3^+ groups from lysine and arginine) must react with base before dissolution occurs. (The removal of a hydrogen ion from RNH_3^+ groups increases the net negative charge of the protein as effectively as the ionization of a carboxyl group.) This conclusion is supported by the way in which the amount of wool dissolved depends on pH at the three temperatures. At each temperature the amount dissolved per disulfide bond broken is practically constant (at the level indicated by the preceding figures) until at least one-quarter of the disulfide bonds have been disrupted. Beyond a certain pH characteristic of each temperature this constancy no longer prevails, and the amount dissolving per additional disulfide bond broken increases rapidly. At 0° C this critical pH is approximately 14, at which value between 30 and 40 percent of the cystine has been destroyed; at 25° C the rapid increase in solubility begins at about pH 13, at which value, however, over 60 percent of the cystine has broken down; at 50° C the sharp rise in solubility occurs near pH 11.6 after 80 percent of the intial content of cystine has been lost. The differences between these pH values, *which do not correspond to the decomposition of a definite proportion of the disulfide bonds in the protein*, is approximately the same as the differences between the position of the titration curves along the pH axis at the same temperatures. Since in these ranges of pH, the curve is largely determined by the equilibrium $RNH_3^+ + OH^- \rightleftharpoons RNH_2 + H_2O$, it seems probable that the dissolution of wool requires the combination with base of a large proportion of the preexisting, as well as of the newly formed acidic groups. It should thus be possible, by treating wool at elevated temperatures with solutions only slightly to the alkaline side of neutrality, to destroy practically all the disulfide bonds in its structure without dissolving an appreciable amount of the fibers.

(c) MEASUREMENT OF pH

The accuracy of the pH measurements between pH 7 and pH 10 was improved by calibrating the glass electrode with 0.05 *M* sodium tetraborate at all of the temperatures at which measurements were made, in addition to employing the other pH standards already enumerated [29]. The value, 9.180, given by Hitchcock and Taylor [17] for 25° C was used as the basis for calculating the pH values of this buffer at 50° C from the ratio of the dissociation constants of boric acid at 50° and 25° C as given by Owen [21, 22]. The same procedure was used in determining the pH value of this buffer for 0° C, but an extrapolated value of the dissociation constant was employed, since Owen's data do not extend below 5° C. The values employed in standardizing the electrode were 9.39 at 0° C, 9.18 at 25° C, 9.07 at 40° C, and 9.04 at 50° C.

By employing a standard in this range of pH, in which the glass electrode starts to deviate from the theoretical behavior of a perfect hydrogen electrode, it was possible to obtain more nearly exact correction terms for the pH measurements made in solutions above pH 9 than when the correction was averaged over the long interval between the pH of phthalate buffer and that of the dilute potassium

hydroxide solution previously described. All determinations of pH values above 5.5 were made at the temperature at which the titration curves were measured. Control measurements showed that pH measurements at room temperature could be substituted for measurements at the temperature of the experiment at pH values below 5.5.

(d) CONTROL OF TEMPERATURE

The thermostatic equipment previously described kept the temperature constant within $\pm 0.01°$ at 0° C, within $\pm 0.02°$ at 25° C, within $\pm 0.1°$ at 40° C, and within $\pm 0.5°$ at 50° C.

III. RESULTS AND DISCUSSION

Measurements of the combination of wool with both hydrochloric acid and potassium hydroxide as a function of pH were made at 0°, 25°, and 50° C. Sets of measurements were made with solutions which contained no added salt, and with solutions to which a neutral salt, potassium chloride, had been added in quantities sufficient to maintain predetermined constant concentrations of chloride ions in the experiments with acid, and constant concentrations of potassium ions in the experiments with base. Two constant ionic concentrations, 0.2 and 0.5 *M* were used. A fourth temperature, 40° C, was included among those at which measurements of combination with acid were made in the series of solutions to which no salt was added, and 25° was omitted from the temperatures at which measurements were made in solutions of 0.5 *M* ionic strength. The results obtained in the three different sets of measurements are summarized in table 1. The measurements are represented graphically in figures 1, 2, and 3, in each of which results obtained at different temperatures in a given ionic environment are directly compared. In each figure the points on the alkaline side of neutrality connected by the broken curves represent measurements of base bound, calculated in the accustomed way [29], from determinations of changes in the titer of alkaline solutions in which samples of wool have been immersed for approximately 24 hours. The points immediately above each of these, connected by the solid curves, represent the same measurements after application of the corrections for the effects of disulfide breakdown discussed in section II. The necessity of making corrections for disulfide hydrolysis when working at temperatures near 0° and 25°C has already been demonstrated [16, 29]. The present measurements show that the errors which would be introduced by neglecting to take disulfide hydrolysis into account are even more serious at 50°C than at the lower temperatures. Thus the positions of the broken curves, representing measurements made after 24 hours, are in part fortuitous and depend in a high degree on the length of time during which the wool is immersed in the experimental solutions. Points representing the corrected measurements, however, always fall on or near the appropriate solid curve, regardless of the time elapsing before the solutions are sampled, provided that sufficient time has been allowed for attainment of equilibrium. These statements are illustrated by sets of measurements, represented by crosses, made after the wool has been immersed in the experimental solutions for 3 or 4 hours in place of the longer period. The lower of each pair of two crosses at the same pH, shows the calculated amount of base

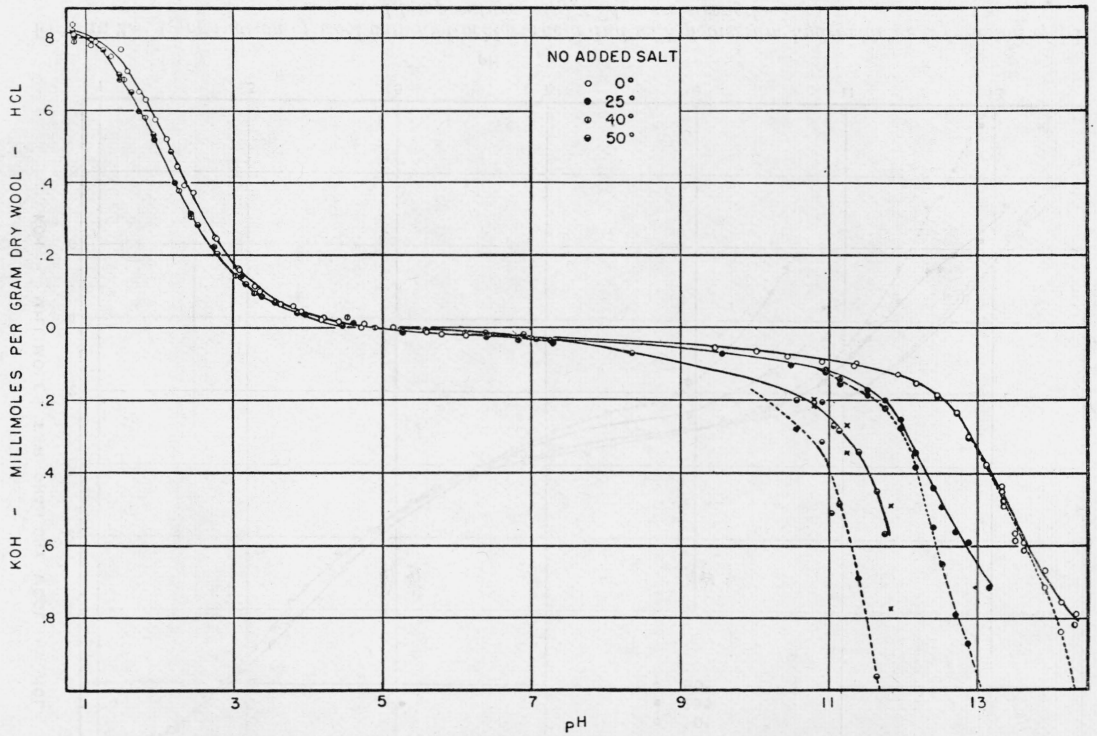

FIGURE 1.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of* pH *and temperature, in the absence of added salt.*

The difference between the solid lines and the broken lines is explained in the text.

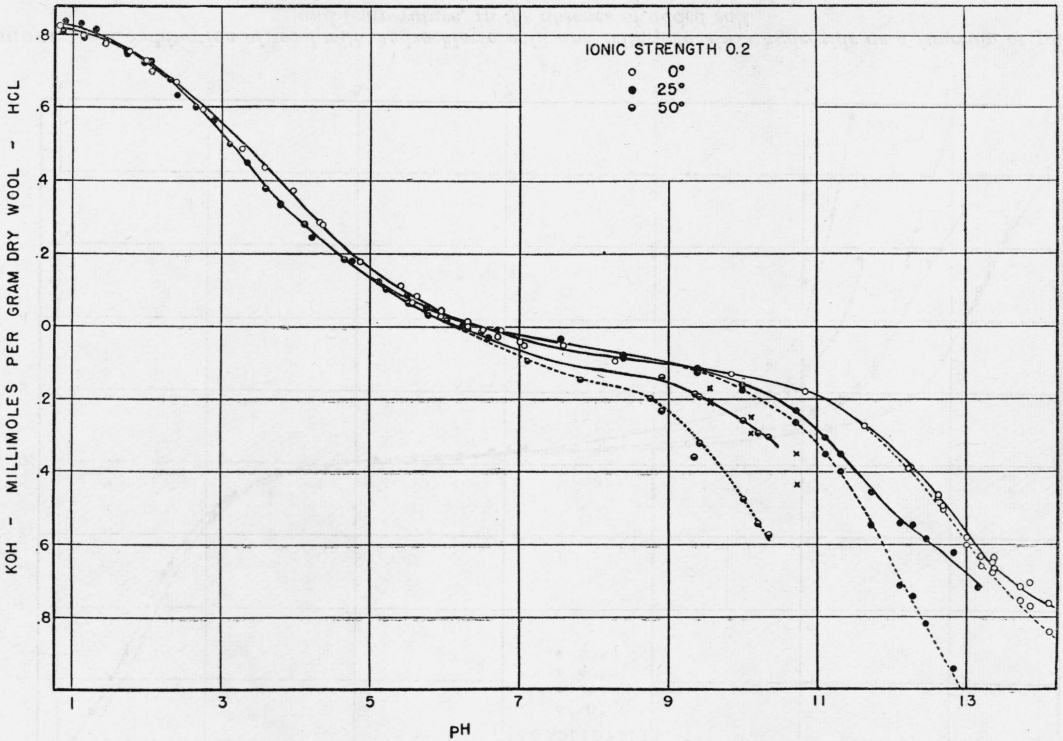

FIGURE 2.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of* pH *and temperature, at 0.2 M ionic strength.*

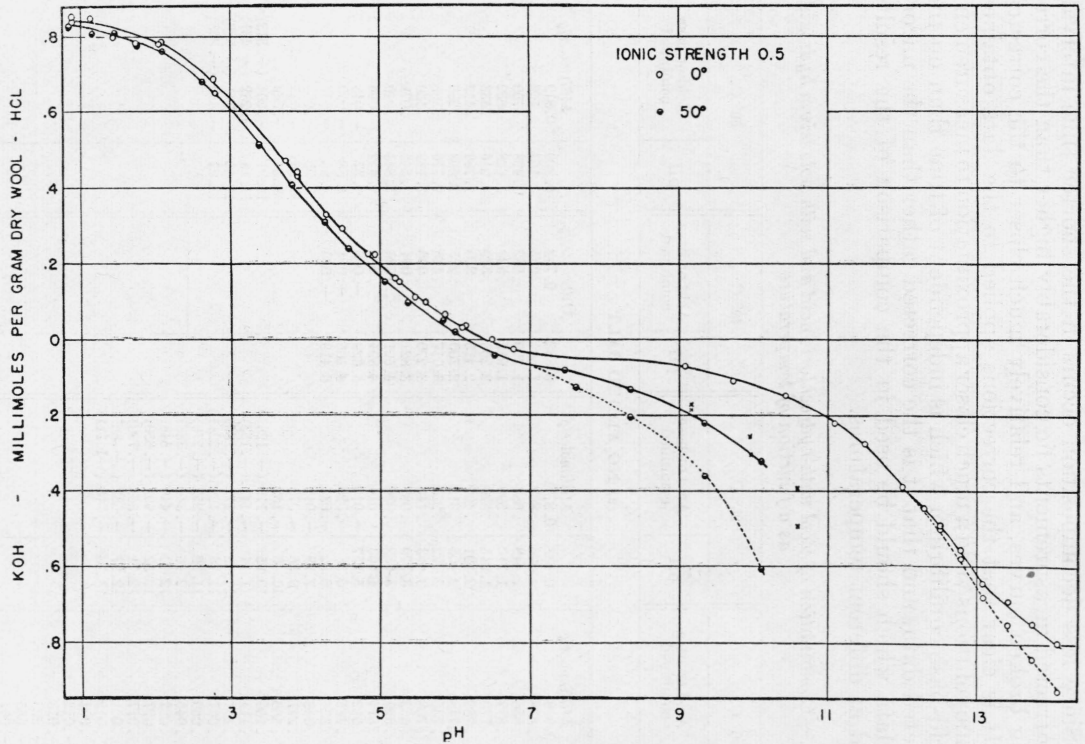

FIGURE 3.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of* pH *and temperature, at 0.5 M ionic strength.*

combined when no account is taken of the base bound by acid groups formed in the fiber as the result of hydrolysis of disulfide bonds; the upper cross represents the same measurement properly corrected. The crosses representing the corrected measurements all fall very slightly above the solid curves, which indicates that the time allowed was somewhat short of that required for the attainment of equilibrium. Since less decomposition occurs in the shorter time interval, the uncorrected measurements lie considerably higher than the corresponding broken curves, and relatively much closer to the corrected data. It is clear that the corrections applied to the data obtained in alkaline solutions yield a much closer approximation to measuremen t of an acid-base equilibrium which is independent of time than do the uncorrected data, and that it is the corrected rather than the uncorrected data which should be used in the comparison of the results obtained at different temperatures.

TABLE 1.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of temperature*

0° C.		25° C.		40° C.		50° C.	
pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined
(a) NO ADDED SALT							
	Millimoles/g		*Millimoles/g*		*Millimoles/g*		*Millimoles/g*
0.447	0.868	0.638	0.877	0.832	0.792	0.800	0.819
.635	.838	.826	.802	1.504	.685	1.103	.769
.813	.836	1.448	.687	1.790	.582	1.438	.700
.822	.812	1.710	.599	1.917	.529	1.608	.652
1.062	.779	1.915	.520	2.263	.379	1.916	.533
1.125	.789	2.191	.402	2.415	.310	2.189	.412
1.327	.748	2.406	.316	2.700	.225	2.503	.286
1.470	.735	2.722	.225	3.017	.145	2.770	.205
1.553	.708	3.093	.143	3.270	.096	3.149	.120
1.711	.652	3.370	.087	3.821	.044	3.545	.071
1.797	.629	3.962	.036	4.521	.030	3.843	.040
1.931	.574	4.603	.014	4.894	.003	4.452	.006
2.085	.522	5.257	−.013	5.629	−.006	6.37	−.013
2.228	.445	6.38	−.026	6.105	−.014	6.88	−.020
2.320	.394	6.82	−.035	6.418	−.020	6.89	−.017
2.451	.348	7.28	−.042			7.05	−.033
2.748	.247	9.56	−.070			7.24	−.035
3.039	.166	10.46	−.102			8.34	−.069
3.072	.160	10.95	−.115 (−.123)[a]			10.91	−.204 (−.312)[a]
3.272	.114	11.14	−.141 (−.153)			11.14	−.282 (−.481)
3.348	.101	11.52	−.171 (−.185)			11.40	−.341 (−.686)
3.620	.067	11.76	−.198 (−.221)			11.64	−.449 (−.958)
3.783	.060	11.96	−.252 (−.276)				
3.844	.045	12.16	−.344 (−.383)				
4.169	.022	12.40	−.440 (−.548)				
4.198	.027	12.52	−.491 (−.650)				
4.414	.017	12.71	−.561 (−.790)				
4.688	.001	12.87	−.587 (−.867)				
4.740	.012	13.16	−.716 (−1.111)				
5.140	.004						
5.58	−.012						
5.79	−.018						
6.10	−.023						
9.45	−.055						
10.01	−.067						
10.43	−.077						
10.91	−.094						
11.32	−.105						
11.37	−.097						

Footnote at end of table.

TABLE 1.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of temperature*—Continued

0° C.		25° C.		40° C.		50° C.	
pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined
(a) NO ADDED SALT—Continued							
	Millimoles/g		*Millimoles/g*		*Millimoles/g*		*Millimoles/g*
11.93	−.128						
12.17	−.153						
12.46	−.185 (−.187)[a]						
12.72	−.232 (−.236)						
12.88	−.297 (−.301)						
13.12	−.375 (−.379)						
13.32	−.436 (−.450)						
13.35	−.476 (−.488)						
13.49	−.565 (−.586)						
13.63	−.590 (−.610)						
13.90	−.669 (−.719)						
14.12	−.757 (−.838)						
14.24	−.702 (−.788)						
14.29	−.820 (−1.023)						
14.30	−.787 (−1.019)						
(b) IONIC STRENGTH 0.2							
0.814	0.829	0.837	0.836			0.822	0.809
1.130	.800	1.100	.830			1.128	.792
1.433	.773	1.310	.815			1.436	.780
1.751	.753	1.725	.755			1.724	.746
1.982	.730	2.042	.729			1.953	.724
2.061	.702	2.392	.639			2.297	.677
2.378	.670	2.762	.565			2.638	.601
2.744	.595	3.333	.448			3.096	.499
2.767	.607	3.782	.334			3.615	.378
3.273	.487	4.210	.244			4.112	.279
3.571	.435	4.747	.181			4.635	.184
3.825	.372	5.107	.123			5.206	.103
4.235	.269	5.477	.085			5.511	.063
4.318	.285	5.742	.053			5.766	.032
4.359	.279	5.94	.032			6.01	.017
4.855	.177	6.23	.004			6.30	−.006
5.400	.112	6.58	−.028			6.46	−.015
5.564	.067	7.55	−.034			7.10	−.093
5.618	.083	8.34	−.077 (−.085)[a]			7.82	------- (−.146)[a]
5.94	.045	9.36	−.115 (−.125)			8.75	------- (−.197)
5.94	.030	9.98	−.162 (−.175)			9.41	−.193 (−.321)
6.29	.015	10.71	−.232 (−.264)			10.00	−.259 (−.475)
6.3	.002	11.10	−.307 (−.352)			10.22	−.294 (−.542)
6.7	−.007	11.31	−.352 (−.399)				
6.7	−.027	11.72	−.457 (−.545)				
6.75	−.010	12.11	−.541 (−.714)				
6.84	−.024	12.28	−.547 (−.743)				
7.00	−.041	12.45	−.585 (−.819)				
7.05	−.050	12.83	−.624 (−.944)				
7.68	−.050	13.16	−.720 (−1.102)				
8.28	−.093						
9.84	−.130						
10.83	−.180						
11.63	−.273						
12.23	−.391 (−.391)[a]						
12.25	−.389 (−.389)						
12.62	−.464 (−.471)						
12.68	−.495 (−.506)						
13.01	−.582 (−.602)						
13.19	−.635 (−.660)						
13.39	−.649 (−.678)						
13.41	−.640 (−.670)						
13.73	−.720 (−.760)						
13.85	−.710 (−.770)						
14.11	−.764 (−.840)						

Footnote at end of table.

TABLE 1.—*Combination of wool with hydrochloric acid and with potassium hydroxide as a function of temperature*—Continued

0° C.		25° C.		40° C.		50° C.	
pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined	pH	Acid or base combined
(c) IONIC STRENGTH 0.5							
	Millimoles/g		*Millimoles/g*		*Millimoles/g*		*Millimoles/g*
0.477	0.868					0.436	0.947
.828	.854					.830	.828
.832	.838					1.156	.807
1.130	.850					1.458	.808
1.438	.799					1.760	.774
1.461	.810					2.090	.760
1.745	.784					2.628	.680
2.048	.781					3.388	.513
2.071	.786					3.825	.408
2.781	.687					4.266	.310
2.808	.652					4.575	.240
3.750	.471					5.069	.154
3.905	.442					5.417	.099
3.910	.432					5.843	.051
4.304	.339					6.003	.020
4.503	.294					6.53	−.042
4.887	.228					7.47	−.079
4.933	.227					7.67	−.125
5.211	.164					8.35	−.131 (−.202)[a]
5.26	.155					9.34	−.218 (−.360)
5.472	.112					10.11	−.320 (−.605)
5.61	.101						
5.870	.068						
6.10	.034						
6.14	.039						
6.5	.002						
6.78	−.024						
9.08	−.068						
9.73	−.108						
10.43	−.048						
11.09	−.220						
11.50	−.275						
12.01	−.388						
12.30	−.443 (−.443)[a]						
12.52	−.488 (−.498)						
12.80	−.552 (−.576)						
13.09	−.642 (−.678)						
13.42	−.691 (−.752)						
13.76	−.752 (−.844)						
14.09	−.792 (−.927)						

[a] Uncorrected for the effect of disulfide bond decomposition, as explained in the text.

It is apparent that the rate of decomposition increases sharply as the temperature is raised. Thus it can be seen in figure 1, representing measurements made after 24 hours, that only negligible amounts of decomposition occur at 0° C at pH values up to 13.5. At 25° C practically all the disulfide bonds are hydrolyzed at this pH within the same interval of time. At this temperature, considerable decomposition within 24 hours cannot be detected at pH values below 12.1, at which pH value decomposition is again practically complete at 50° C within the same period of time. Comparable large differences between the rates of decomposition at each of the same temperatures can be seen in figures 2 and 3.

The principal qualitative features of the comparison of the data obtained at different temperatures are common to all three sets. They are most readily distinguished in figure 1 in which the steepness of the titration curves obtained in the absence of salt results in a sharp distinction between the positions on the pH axis of the curves obtained at each temperature. It is apparent on inspection that the curves represented are relatively well separated with respect to position on

the pH axis at the alkaline end of the scale (reference is here had to the solid curves), but that their positions differ only very slightly in neutral and acid regions of pH. Indeed, the separation between the curves of acid-combination is so small in the temperature interval, 25° to 50° C, that only a single curve has been drawn through the points representing measurements made at 25°, 40°, and 50° C. Between 0° and 25° C, however, an appreciable difference in the position of the curves is found; it is in the same direction as the shift recorded in the alkaline range of pH, but it is a good deal smaller. In the intermediate range of pH values the differences in the positions of the titration curves are more difficult to evaluate, because of the very large change in pH which is required to produce a small increment in acid or base bound, and because pH measurements are least accurate in the extremely unbuffered solutions in this region of pH. The best estimates which can be made from the figure indicate a shift in the abscissas of the points in the same direction as in the other parts of the curves, as the temperature is increased. This shift is intermediate in magnitude between the small effect in the acid region and the much larger effect in the most alkaline range. Its exact magnitude is so uncertain, however, that no effort is made to evaluate it in the sections that follow.

The flatness of the titration curve determined in the absence of salt also renders the precise pH value at which neither hydrochloric acid nor potassium hydroxide is bound rather uncertain. In an earlier paper the opinion was ventured that this value was probably very nearly the same in the absence of salt as in its presence, approximately 6.4. More numerous determinations in this region now make it appear that *in the absence of salt* the pH at zero combination is in the range 4.7 to 5.1, where it has been placed by Speakman and others.

The well-marked differences in the relative magnitudes of the effects of temperature on the three main parts of the titration curves appear to be as characteristic of proteins as of their constituent amino-acids, as the recalculation of the data of Hoffman and Gortner on casein by Pertzoff and Carpenter [18, 24] and the work of Wyman on horse hemoglobin [36] have shown. They are entirely consistent with the assumption previously made that the carboxyl and amino groups of the uncombined protein are completely ionized. The effect of temperature on the acid branch of the titration curve is small because the heats of dissociation of carboxyl groups are, in general, very small. The values for different carboxylic acids are distributed about zero, so that the sign of the heat may be either positive or negative, depending on the identity of the carboxylic acid in question, and on the temperature. The changes in heat content accompanying the dissociation of hydrogen ions from the conjugate acids of imidazole and substituted ammonium bases are, on the contrary, very considerable: some 6,000 calories in the case of imidazole groups, and from 9,000 to 14,000 calories in the case of amino groups [5]. The values of the heats of dissociation of certain of the groups in wool may be estimated from the differences in the abscissas of the curves represented in the figure. In the discussion that follows this is done for the two main regions of the titration curve on either side of neutrality. No effort is made to treat separately the effect of temperature in the neutral region for the reasons already given and because the amount of imidazole groups (from histidine) in wool is, known to be very small [31].

1. DATA OBTAINED IN THE ABSENCE OF SALT

(a) COMBINATION WITH ACID

The existence of an appreciable difference between the positions of the acid combination curves at 0° and 25° C, combined with the absence of any clearly distinguishable differences between the curves at 25°, 40°, and 50° C, probably is an indication that the dissociation constants of the carboxyl groups of the protein attain a maximum value in the temperature interval 25° to 50° C, and thus probably diminish at higher temperatures. The existence of a maximum value of the dissociation constant in the temperature range of this investigation is a common characteristic of carboxylic acids [12, 14]. For these acids the curve relating the dissociation constant to temperature has a very flat maximum but becomes steeper (the dissociation constant acquires an increasing dependence on temperature) at temperatures farther from the temperature of the maximum [12]. The temperature of the maximum is about 25° C for formic acid and acetic acid but diminishes as the hydrocarbon chain attached to the carboxyl group lengthens; it is not far from 10° C in the case of *n*-butyric acid [14]. A more cogent comparison is with the constants characterizing the dissociations of the carboxyl groups of the amino-acids. No maxima have been found with glycine and alanine, which have been investigated at temperatures up to 40° and 45° C, respectively, but Harned and Embree have shown that with these amino-acids the variation of the dissociation constant with temperature at temperatures down to 10° C closely parallels the variation of the constants characterizing the simple aliphatic acids at lower temperatures, and indicates a maximum value of their dissociation constants at temperatures just above 40°. This is very close to the temperature of the maximum suggested by the present data.

Since the degree of dependence of the dissociation constants of carboxylic acids on temperature itself depends on temperature, little is gained by comparing the heats of dissociation of these groups in wool within any one temperature interval with similar quantities for related substances, such as the amino-acids. In the interval of temperature 0° to 25° C the average heat of dissociation of the carboxyl group of glycine is practically zero, of alanine is −450 calories, of glycylglycine and of glycylalanine is −600; in aspartic acid, ΔH_0^{25} (the average heat of dissociation between 0° and 25° C) or the carboxyl group next to the amino group is +1,600; for the same group in glutamic acid it is +1,900. The values of ΔH_0^{25} of the γ and δ carboxyl groups, of these amino-acids are +2,100 and +1,040 calories, respectively [5]. Since the latter are the carboxyl groups which are presumably free in proteins to react with base, the acid branch of protein titration curves should be determined by the number of these groups and their properties.

Certain complications enter into the calculation of ΔH from titration data in the case of proteins because they are polybasic. ΔH may be calculated from the shift in the abscissas of the titration curves of a monobasic acid or base, or of a polybasic acid with dissociating groups which have widely different strengths, because for such acids the difference in pH at which a given amount of base or acid is bound at two different temperatures represents the logarithm of the ratio of

the dissociation constants of a single acidic or basic group ($\Delta pH = -\log K_2/K_1 = -\Delta \log K$).[9] From this change in K produced by a change in temperature it is possible to calculate the average value of ΔH in the same interval of temperature by means of the integrated form of the Van't Hoff equation:

$$\Delta H_{T_1}^{T_2} = 4.5787 \frac{T_2 T_1}{T_2 - T_1} \Delta \log K. \quad (1)$$

The values cited above have been so calculated. It has been shown by Simms [25], Weber [33], von Muralt [32], and others that the titration curves of polybasic acids which contain identical dissociating groups far enough apart from one another in the molecule to have no influence on the dissociation of one another may be described by an expression which is functionally identical with the law of mass action for a monobasic acid. For such hypothetical acids, ΔH per mole of dissociating groups may be calculated exactly as in the simpler cases described above, by means of the relationship $\Delta pH = -\Delta \log K$. This can be done even in certain other cases when no restriction is placed on the interrelationships of the values of all the K's, as Wyman has shown in a general and mathematically rigorous analysis of the effect of temperature on the titration curves of polybasic acids. Wyman came to the conclusion that in any region of pH in which ΔpH is practically independent of pH, ΔpH can be equated to $-\Delta \log K$ for some one dissociating group or set of groups with a good degree of approximation.[10] Calculation of ΔH from ΔpH in regions of transition from one constant value of ΔpH to another does not correspond to any one set of similar dissociation equilibria, and gives values intermediate to those characterizing the homogeneous sets of dissociating groups in the pH regions on either side of the region of the transition.[11]

Thus by making the reasonable assumption that the titration curves of proteins are determined by the relative acid strengths of distinct, essentially nonoverlapping sets of similar dissociating groups, one may obtain values of $\Delta \log K$ in each of the two main regions of pH values of the present data for wool and compare them with similar results obtained by Pertzoff and Carpenter with casein, and by Wyman with hemoglobin. The results of this comparison, and a comparison of the corresponding values of ΔH, are given in table 2.

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[9] The use of pH differences and of ratios of constants avoids practically all of the theoretical difficulties that arise in attempting to evaluate the constants themselves from titration data alone. Thus ΔH can be calculated more simply and more certainly than any single value of K. The only factor left out of account in this method of calculation is the change of activity coefficients with temperatures of ions other than the hydrogen ions, but the temperature dependence of such activity coefficients is known to be very small.

[10] Unless groups possessing distinct values of ΔH chance to possess the same dissociation constants. In this case, the average heat of dissociation for all the groups would be given.

[11] Kern [19, 20], Cannan [3], two of the present authors [29], and others, have shown that the interaction between many like dissociating groups in highly polybasic acids, such as proteins, often introduces an empirical fractional exponent (equal, or nearly equal to 0.5 and practically independent of temperature) to the hydrogen-ion activity term in the simple law of mass-action equation which would characterize their behavior in the hypothetical simple case. At first sight, this might appear to indicate that the term $\Delta \log K$, used in calculating ΔH by means of eq 1, is not equivalent to $-\Delta pH$ (the difference in the pH coordinates of the titration curves obtained at T_2 and T_1) but is equal to ΔpH multiplied by the same empirical exponent. Two considerations, however, show that this does not follow as a rigorous consequence of the use of the exponent: (1) The constant in the modified equation is not to be identified with the true dissociation constant of any one group or set of groups; it is a function of all the constants of all the groups, and these are widely distributed in magnitude as a result of mutual interaction. (2) It is a matter of observation that the numerical value of the empirical constant corresponds very well to the square root of the unmodified mass-law constant that might be expected to characterize the dissociation of the groups if no interaction occurred (in proteins the midpoint of the carboxyl curve comes at pH 4.2, close to the pK of carboxyl groups in polypeptides, while $-\log K$ in the empirical equation containing the exponent is 2.1). Thus the equation might well be written $K = k_{\text{monobasic}}^{0.5} = \frac{[H^+]^{0.5}[A^-]}{[HA]}$. From this it would follow that $\Delta \log K_{\text{monobasic}} = -\Delta pH$ and not $-0.5\ \Delta pH$.

TABLE 2.—*Calculation of average heats of dissociation of carboxyl groups in wool and other proteins, in the absence of salt*

Protein	pH range studied	Temperature range	$-\Delta$pH	ΔH, if $-\Delta$pH$=\Delta \log K$
		°C		cal/mole
Wool	1 to 5	0 to 25	0.16	+2,380
		25 to 50	±.03	±500
Horse hemoglobin	4.3 to 6	6.5 to 25	----------	−2,000 to −3,000
		25 to 37.5	----------	
Casein [1]	2 to 5	22 to 35	±.1	±3,000

[1] The data on casein are subject to considerably larger uncertainties than the data for the other 2 proteins.

It is apparent that the heat of dissociation of the acid groups of wool in the lower temperature interval is very close to the values that characterize carboxyl groups in dibasic amino-acids, albeit slightly higher. In the higher temperature interval the heat of dissociation is very much smaller, as it must be in the region of a maximum value of K, where ΔH approaches zero. The values found with casein agree with those for wool as closely as should be expected, considering the low intrinsic accuracy of the casein data and the fact that different ranges of temperature were used. The data of Wyman for horse hemoglobin do not extend below pH 4.3, and thus cover only a small fraction of the range of the present comparison. Over this range the sign of ΔpH is opposite to that of the values of ΔpH characterizing the data for wool and indicates a *negative* heat of 2,000 to 3,000 calories. This difference in sign is not as important for the purpose of the present comparison as is the small magnitude of ΔH. This qualitative agreement acquires further significance when the comparison is extended to other regions of pH where the heats of dissociation of all three proteins are much larger. A possible explanation of the difference in sign between the results with wool and with hemoglobin is suggested later in this paper.

The fact that the dissociation constants of the carboxyl groups of wool have a maximum value at a temperature near 40° has an important consequence in relating the titration data reported in this paper to the acidic properties of wool at the high temperatures, near the boiling point of water, to which wool is exposed in dyeing. At these high temperatures the position of the acid titration curve on the pH coordinates is probably near its position at 0°, the temperature at which the influence of various factors has been studied in greatest detail [29].

(b) COMBINATION WITH BASE

On the alkaline side of neutrality, the titration curves of wool at the different temperatures are widely separated and show every indication of being nearly, if not exactly,[12] parallel at pH values above 10. Two nearly equal intervals with respect to the pH axis separate the curves for the three different temperatures.

In calculating values of ΔH for the acid branch of the titration curve, it was possible to proceed on the approximately valid assumption that only carboxyl groups, from aspartic and glutamic acids, determined the course of this part of the curve. On the alkaline side of the curve dissociation equilibria of two widely dissimilar basic groups, the ε-amino groups of lysine and the guanidino groups of

[12] The curves were drawn freehand without attempting to make them parallel.

arginine, as well as the dissociation of one acid group, the phenolic radical of tyrosine, may all be involved within a narrow range of pH. The relation of ΔpH to Δ log K for any one of these sets might therefore be fairly complex. Because the curves appear to be nearly parallel over a wide range, however, one may follow the procedure justified by Wyman, substituting values of $-\Delta pH$ in place of Δ log K in eq 1, for each interval of temperature. The results of this treatment of the data are represented in table 3, in which it is apparent that a fairly uniform separation (probably within experimental error), and therefore a fairly constant value of ΔH, is found for the entire alkali combination curve. The values in which the greatest discrepancies appear are obtained in the region of pH in which very small amounts of base are combined. Here the curves are so nearly flat that a very small error in the measurements, or in the corrections for ash, results in large discrepancies in the pH coordinates at which these quantities of base are bound. For this reason, and because the number of imidazole groups in wool is so small as to account for only a very small amount of acid or base bound, no effort is made to evaluate ΔH in the intermediate range of pH values near neutrality.

TABLE 3.—*Calculation of average heats of dissociation of groups in wool dissociating at* pH *values above 6*

0° to 25° C			25° to 50° C		
Base bound per gram	−ΔpH	ΔH_0^{25}	Base bound per gram	−ΔpH	ΔH_{50}^{25}
Millimoles			*Millimoles*		
0.1	0.75	11,190	0.1	1.47	25,820
.2	.81	12,070	.2	1.04	18,310
.3	.85	12,640	.3	0.84	14,800
.4	.88	13,100	.4	.78	13,720
.5	.89	13,240	.5	.84	14,800
.6	.83	12,380	----------	--------	----------
.7	.81	12,030	----------	--------	----------
Average----	----------	12,380	----------	--------	----------

The numerical values of ΔH tabulated are very close to those, ranging from 10,000 to 13,000 calories per mole [5], which would be expected to characterize the dissociation of hydrogen ions from the conjugate acid forms of amino or guanidino groups in amino-acids. They are about twice as great as the values to be expected if the acid dissociation of the hydroxyl group of tyrosine were being measured in any part of this region [9, 34], and appear to support the suggestion made earlier [29] that the hydroxyl groups of tyrosine may not be titrated by base in the region of pH covered by this investigation. The values, although consistently slightly higher, are in fair agreement with the figure, 11,500 calories, found by Wyman for horse hemoglobin, and with an approximate value of 14,000 calories which may be deduced from the figures given by Pertzoff and Carpenter, for casein.

The fact that the values for wool and for casein are so nearly equal to the heat of dissociation of water (13,481 calories at 25° [13]) shows that the effect of temperature on the amounts of base bound at any constant *hydroxyl-ion* activities (as for example in a given solution of sodium hydroxide) would be very small. Thus it is only because

the amounts of base bound are compared on a scale of *hydrogen-ion* activities that the very considerable temperature effect appears. It is the latter basis of comparison which has significance in terms of *acidic* dissociation constants, used consistently throughout this paper.

The degree of agreement with the results for other proteins may be construed as furnishing additional support for the validity of the corrections for alkaline hydrolysis of disulfide bonds included in the calculations of the amounts of base bound. Thus if ΔH were calculated from the difference in the pH coordinates of the broken curves, representing the uncorrected measurements, very high values of ΔH (20,000 to 25,000 calories), entirely without precedent in investigations of amino acids and proteins, would result. These values would not be independent of time, but would increase with increasing periods of exposure of the wool to alkali. Thus only the values calculated from the corrected measurements and tabulated in table 2 may be interpreted thermodynamically.

Regardless of what detailed significance may be attached to the values shown in table 2, they are, in combination with the much smaller values characterizing the acid branch of the curve, entirely consistent with the view that the process of titration with acid consists in the replacement of hydrogen ions on the ionized carboxyl groups of the uncombined protein while the process of titration with alkali consists predominantly in the removal of hydrogen ions from the conjugate acids of the amino and guanidino groups.

2. DATA OBTAINED AT CONSTANT IONIC STRENGTH

The data obtained in the presence of constant concentrations of chloride or potassium ions show a different functional relationship between the amounts of acid or base bound and pH [29]. However, the effect of temperature upon this relationship is so similar to its effect on the data obtained in the absence of salt that one may without further discussion tabulate representative values of ΔpH taken from figures 2 and 3 and also the values of ΔH calculated from them. This has been done in table 4.

It will be seen at once that the average of the values of ΔH calculated for the acid region of the curve for 0.2 *M* ionic strength in the temperature interval 0° to 25° C (table 4) is very close to the value 2,380 given in table 2 for this quantity in the absence of salt. The individual values fluctuate considerably, but this is largely a consequence of the manner in which the freehand curve through the data has been drawn. In the higher temperature interval, 25° to 50° C, there is practically no effect of temperature, just as in case of the data obtained with acid alone. The resemblance between these sets of data extends into the alkaline region, although here again there is great fluctuation among the individual values. It is possible that the drift from 11,000 to 12,000 calories, characterizing the middle portion of the alkaline curves, to 7,000 to 8,000 calories at their most alkaline end is a real phenomenon and represents transition at 0.5 to 0.6 millimole of base bound from the titration of amino groups to the titration of tyrosine hydroxyl groups when more base is combined.[13]

[13] Recent work in this laboratory on the combination of base by silk, in which most of the acidic groups are phenolic, indicates that the hydroxyl groups of tyrosine dissociate hydrogen ions in this region of pH values.

However, this supposition is not easily reconciled with the failure of a differential effect to appear in the measurements made in the absence of salt nor with the expected relative strengths of tyrosine hydroxyl and arginine guanidino groups. An extreme fluctuation, resulting in a change of sign of ΔH, is found in the region of very small amounts of combined base. As in the data obtained without salt, this may be ascribed to the small slope of the titration curve in this region, and the resulting large effect of ΔpH which slight changes in the drawn curves, or slight errors in corrections for ash, would produce. Wide fluctuations are also found in the data listed for the interval 25° to 50° C, but the average of the values given is 15,060 calories, in good agreement with most of the individual values in table 3 for this temperature interval.

TABLE 4.—*Average heats of dissociation of acidic and basic groups in wool calculated from titration data obtained at constant ionic strength*

IONIC STRENGTH 0.2

ACID REGION			ALKALINE REGION		
0° C to 25° C			0° C to 25° C		
Acid bound	−ΔpH	ΔH_0^{25}	Base bound	−ΔpH	ΔH_0^{25}
Millimoles/g		*Calories*	*Millimoles/g*		*Calories*
0.1	0.17	2,540	0	0	0
.2	.17	2,540	.1	−.13	−1,940
.3	.28	4,170	.2	+.64	+9,540
.4	.26	3,880	.3	.75	11,180
.5	.17	2,540	.4	.82	12,200
.6	.11	1,640	.5	.82	12,200
.7	.06	890	.6	.58	8,650
			.7	.50	7,450
Average		2,600			
25° C to 50° C			25° C to 50° C		
Acid bound	−ΔpH	ΔH_{25}^{50}	Base bound	−ΔpH	ΔH_{25}^{50}
Millimoles/g		*Calories*	*Millimoles/g*		*Calories*
0.1	0.03	530	0	0.34	5,990
.2	0	0	.1	1.31	23,100
.3	0	0	.2	0.95	16,710
.4	0	0	.3	.82	14,430
.5	0	0			
.6	0	0			
.7	0	0			

IONIC STRENGTH 0.5

0° C to 50° C			0° C to 50° C		
Acid bound	−ΔpH	ΔH_0^{50}	Base bound	−ΔpH	ΔH_0^{50}
Millimoles/g		*Calories*	*Millimoles/g*		*Calories*
0.1	0.17	1,370	0	0.325	2,620
.2	.17	1,370	.1	1.84	14,820
.3	.15	1,210	.2	1.77	14,240
.4	.16	1,290	.3	1.67	13,470
.5	.15	1,210	.325	1.61	12,900
.6	.15	1,210			
.7	.16	1,290			
Average		1,280			

The data obtained at an ionic strength of 0.5 exhibit smaller fluctuations and are numerically in good agreement with the values for ΔH obtained in the other sets of experiments. The average value of ΔH_0^{50} in the acid region is only 1,280 calories, but it has already been shown that practically all of the effect of temperature in the acid region is manifested in the lower half of this large interval of temperature. It is justifiable, therefore, to use double this figure, or 2,560 calories, as the value of ΔH_0^{25} for comparison with the other sets of data. In the alkaline region the individual values of base bound between 0.1 millimole/g and 0.325 millimole/g are constant within the error of the measurements and are close to those found in the other sets of data. Owing to the large amounts of decomposition at this temperature, the measurements do not extend sufficiently far into the alkaline region to determine whether the apparent fall in the values of ΔH found at 0.2 ionic strength would be found in these experiments also.

3. COMPARISON WITH CALORIMETRIC DATA

It is of interest to compare the present estimates of 2,380, 2,600, and 2,560 calories for the heat of dissociation of the carboxyl groups of wool in the temperature interval 0° to 25° C with the experimental value for the heat evolved at 0° per equivalent of hydrochloric acid combined by wool, as determined directly by a calorimetric method by Speakman and Stott [27]. The value obtained by these authors was 3,560 calories, significantly higher than our own figure. In calculating this quantity, Speakman and Stott assumed that there were only negligible differences between the titration curves of wool at 22.2° and 0°; this was done in order to estimate the amount of acid combined at each pH from a titration curve obtained at 22.2°. The error introduced by this approximation may easily approach 1,000 calories in the calculated result when less than half the maximum amount of acid is bound. However, this source of error should be practically negligible when high concentrations of acid are used, since then the amount of acid combined is independent of small changes in pH and temperature. Under these conditions, the heat of dilution of the acid is also relatively large, and the heat of reaction, calculated as the difference between the heat of dilution and the total heat evolved, is subject to a larger uncertainty. Nevertheless, the values obtained with high concentrations of acid are probably not subject to an error of more than two or three hundred calories. They appear entirely consistent with the value reported here; the higher value of Speakman and Stott refers to 0°, while the value obtained by the titrimetric method of the present paper is the average of a very low value at 25° and an appreciably higher value at 0°.

It should be pointed out that the measurements made by Speakman and Stott were not interpreted by these authors as heats of dissociation of the carboxyl groups, but rather as the heats of reaction of the *basic* (amino) groups with acid. Since the value obtained was very much lower than the value which usually characterizes the neutralization of a base by an acid, Speakman and Stott concluded that the value was so low as to indicate that the basic side chains of wool were not free but were combined with acid side chains to form salt linkages, as these authors had previously suggested. However, since wool is an ampholyte and in the uncombined form a dipolar ion as well, it is

clear that the heat measured was not analogous to the heat of neutralization of a base by an acid, but to the heat of association of hydrogen ions with carboxylate ions. Hence the results obtained provide no evidence either for or against the existence of salt linkages.[14] It is evident that calorimetric measurements of the heat of combination of wool with *base*, had they been made, would have led Speakman and Stott, by the same process of reasoning, to a different set of conclusions.

4. SIGNIFICANCE OF THE HEATS OF DISSOCIATION

The estimated values of ΔH reported in this paper have been calculated by assuming that when the pH values at which a fixed amount of base is bound at two different temperatures are compared, under certain conditions $\Delta pH = -\Delta \log K$. The justification for relating ΔpH to a change in the logarithm of a hydrogen-ion dissociation constant, characterizing a single set of dissociating (acid) groups in the conventional formulation of the dissociation equilibria for a dissolved protein, has already been cited and discussed. It remains to enquire whether this method of analysis is equally valid for the theory of the titration curve of wool presented in an earlier paper [29], which assumed that anions of the acids used, as well as hydrogen ions, are only partially dissociated from the protein.[15] In terms of this theory, the positions of the curves of acid combination, with respect to the pH axis, are determined not by $\log K$, where K is a conventional hydrogen-ion dissociation constant, but, to a high degree of approximation, by $\log K_H' K_A'$, where K_H' is the hydrogen-ion dissociation constant characterizing the equilibrium,

$$WHCl \rightleftarrows WCl^- + H^+, \tag{2}$$

and K_A' is the constant characterizing the dissociation equilibrium,

$$WCl^- \rightleftarrows W^+ + Cl^-, \tag{3}$$

in which W^+ represents wool in the uncombined state, and the other symbols have their familiar chemical significance. The position of curves obtained in the absence of salt is determined approximately by $1/2 \log K_H' K_A'$ rather than by $\log K_H' K_A'$ [29].

It is apparent therefore that in the presence of a constant anion concentration, ΔpH represents the sum of $\Delta \log K_H'$ plus $\Delta \log K_A'$. In the absence of salt it is approximately equivalent to one half of this quantity. Thus, the heat of dissociation calculated by inserting $-\Delta pH$ in place of $+\Delta \log K$ in the Van't Hoff equation is actually the sum of two heats of dissociation, associated with reactions (2) and (3).

It is of interest, therefore, to determine what part of the total heat effect is to be attributed to each of the two constituent equilibria. An estimate of the distribution in the case of combination with acid may be made as follows: It has been shown that at high concentrations of

[14] The values obtained by Speakman and Stott with monochloracetic acid are essentially the same as with hydrochloric acid over the part of the titration curve in which the amounts of monochloroacetic acid combined at a given pH are the same as the amounts of hydrochloric acid combined. Much lower values of ΔH are obtained at high concentrations of the partially dissociated acid when large excess amounts of acid are bound. This indicates that the heat of combination of wool with the *excess* acid is very low or zero. Direct measurements of the heat of combination of this excess acid now in progress in this laboratory show that this is indeed the case.

[15] This theory, originally proposed to explain the large scale effects of the concentration of added neutral salts on the position of acid-combination curves for wool on the pH axis, has since received additional support [30].

chloride the position of the titration curve with respect to the pH axis is practically independent of K_A'. The effect of temperature on the midpoint pH of curves obtained under these conditions must therefore be attributed in its entirety to an effect upon K_H'. This effect has been estimated (table 4) as some 2,500 calories. Since the effect of temperature upon K_H' must be invariant, regardless of whether or not salt is present, a contribution to ΔpH in the absence of salt of approximately one-half the amount found at high concentrations must be ascribed to the temperature effect on this constant. This contribution to ΔpH, about 0.08 unit, also happens to be just half of the total effect of temperature (expressed as ΔpH) found when salt is absent. Since the remaining half of the pH shift produced by a change in temperature under these conditions must be ascribed to the effect of temperature upon K_A', it is clear that the heats of dissociation of hydrogen ions and of chloride ions are approximately equal, and that the sum of the heat changes associated with the two sets of equilibria (2) and (3) is roughly double the values calculated from ΔpH under either set of conditions.

In drawing conclusions from a comparison of the numerical results obtained in this investigation, or by the calorimetric method of Speakman and Stott, with those reported for amino-acids and proteins *in solution*, a certain caution is desirable. The reaction of dissolved acid with a dissolved ampholyte involves only changes in heat content which may be described as heats of reaction and heats of mixing (the latter is small). The reaction of dissolved acid with wool involves in addition a heat change equivalent to a heat of adsorption or a heat of condensation in that quantities of acid are transferred from a large volume, that of the aqueous phase, to a small volume, that of the fiber.

Thus the total reaction may be separated into two steps:

$$(H^+)_{aq} \rightleftharpoons (H^+)_{wool} \tag{4}$$

$$(H^+)_{wool} + WCOO^- \rightleftharpoons WCOOH, \tag{5}$$

in which the first equation represents the transfer of ions to the wool phase, and the second represents the reaction of the ions with the groups in wool which combine with them. The heat effect estimated by substituting ΔpH for Δ log K in the Van't Hoff equation represents the sum of the heat changes involved in the two equilibria represented in eq 4 and 5 rather than the heat of dissociation corresponding to eq 5 alone. The latter can only be obtained by estimating, if possible, the heat change corresponding to eq 4 and subtracting it from the total heat. The magnitude of the heat of the transfer represented in eq 4, which is brought about at the expense of the chemical potential energy of the reactants, may be estimated by calculating the entropy change at equilibrium from purely geometrical considerations, if it is assumed that there is no important difference between the two phases with respect to the degree of interaction within them of the ions with one another, or with the solvent. This calculation may be made with sufficient exactness for the present purpose from the approximate relation

$$\Delta S = nRT \ln \frac{V_{aq}}{V_w}, \tag{6}$$

in which n is the number of moles compressed into the volume of the wool phase, V_w, from the volume of the aqueous phase, V_{aq}, and ΔS is the entropy change. This simplified equation leaves out of account the entropy-change contribution from the dilution of the hydrochloric acid remaining in the aqueous phase at equilibrium, but when the acid is initially more dilute than 0.01 M, under the conditions of the present experiments, the final concentration is so low as to make this contribution small.[16] No distinction is made in this method of treatment between the molal entropy change between the initial and final states and the molal entropy change for an infinitesimal change at equilibrium, although it is only the latter which, multiplied by T, may be equated to ΔH in any actual system. In the present case, only the entropy changes inherent in the dimensions of the system, and therefore only those characteristic of an ideal gas, are under consideration; therefore, the two molal entropies cited will be identical.

Substituting in eq 6 numerical values from representative experiments in which low initial concentrations of acid were used, and which therefore correspond fairly closely to the standard state conditions at which ΔH is defined, the ratio V_{aq}/V_w becomes very nearly 100, and $T\Delta S(=\Delta H)$ at 0° C is approximately 2,500 calories per mole of acid transferred. This figure is close to the figure calculated from the data in the acid region. Thus the heat of dissociation of a hydrogen ion from a carboxyl group in wool in the temperature interval 0° to 25° C, *exclusive of the heat of transfer of acid between phases*, may be very much less than the value reported here, and may even be negative, as in the corresponding values obtained by Wyman with horse hemoglobin. The fact that the heats of dissociation obtained from the alkali-combination curves of wool are also somewhat higher than the corresponding values reported by Wyman is entirely consistent with this suggestion. A similar contribution to the effect of temperature on the pH coordinates of titration measurements must be expected in all heterogeneous systems, whether they are of such a nature as to be amenable to treatment by means of the Donnan membrane equilibrium equations or require the type of stoichiometric anion-association analysis applied to wool protein by the present authors.

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