THE SORPTION OF

HYDROCHLORIC ACID AND POTASSIUM HYDROXIDE

BY MOHAIR AND WOOL

Being a thesis submitted
in part fulfilment of the requirements for the degree of

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of

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By

GRAEME REGINALD ERNEST BAMFORD

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CONTENTS.

1. AIM AND SCOPE OF PRESENT STUDY.

The main object of the present investigation has been to establish a titration curve for mohair keratin and to compare it with similar data for wool, to determine whether the differences in physical and chemical properties could be attributed in any way to the acidic and basic character of these fibres. As shown in subsequent discussion such measurements provide extremely useful information regarding the chemical structure of proteins in general, and in the technical fields involving processes such as wool scouring, carbonizing and dyeing. The study has been extended to include certain modified wools, i.e. photochemically damaged, and oxidized keratin.

The most successful contribution to the titration data of wool keratin is the work of Steinhardt and Harris and subsequent authors have tended to adopt their procedures without modification. In the present study attempts have been made to obtain a clearer understanding of the fundamental processes. New techniques and analytical methods have been introduced to improve the accuracy of the measurements.

2. BRIEF OUTLINE OF THE COMPOSITION OF PROTEINS.

Wool and mohair fall into the class of protein materials known as the keratins, which includes substances such as horn, nails, feather and hair in general. The members of this group are characterised by their long filament-like molecules and insolubility in water or dilute acids and alkalis. However, the ~~most~~ unique feature of the keratins is their high sulphur content, relative to other proteins.

When proteins are hydrolysed by strong acids or bases they are decomposed into fundamental units, which have been identified as amino acids, twenty of which have been detected in the hydrolysate of wool. These acids are similar in chemical structure, and follow the general formula

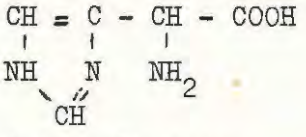
$$\underset{\text{(basic group.)}}{NH_2} - \overset{R}{\overset{|}{C}}H - \underset{\text{(acidic group.)}}{COOH}$$

where R is the group which characterises particular amino acids, and by which they may be classified into the following groups:-

(a) Amino acids containing non-reactive side groups, e.g. glycine, alanine and leucine which yield hydrocarbon residues, and proline and tryptophan which contain polar groups which are not very reactive.

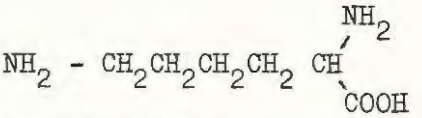
(b) Amino acids containing basic side groups. The dibasic mono-acidic amino acids provide the free basic groups in the protein, e.g.

<u>Histidine</u>



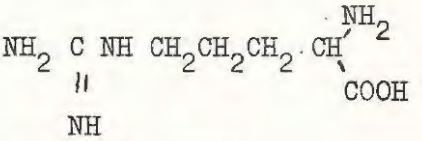
imidazole group

<u>Lysine</u>



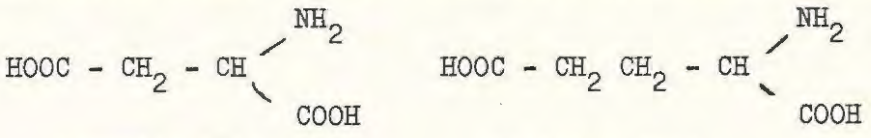
ammonium group

<u>Arginine</u>



guanidine group

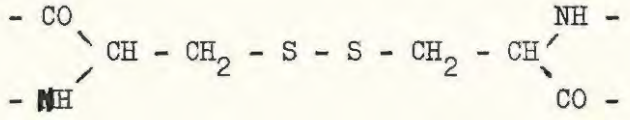
(c) Amino acids containing acidic side groups are the mono-basic dicarboxylic acids, i.e. glutamic, hydroxyglutamic and aspartic acid.



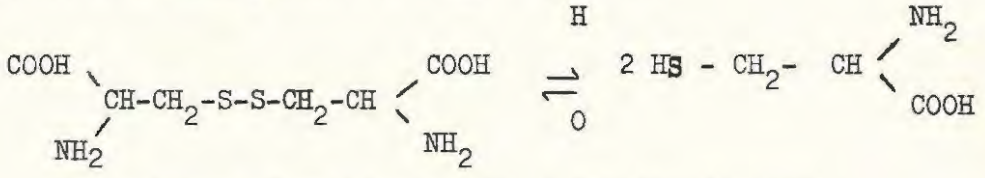
Aspartic Acid Glutamic Acid

A certain fraction of the free carboxyl groups are in the form of amides — $CONH_2$. Tyrosine contains an acidic hydroxy group which is considerably weaker than the carboxylic groups.

(d) Sulphur-containing amino acids: Cystine, cysteine and methionine. Cystine has the unique ability of forming covalent cross-linkages between polypeptide chains, and thus plays a most important rôle in the structure and chemistry of the protein, especially in the keratin group:

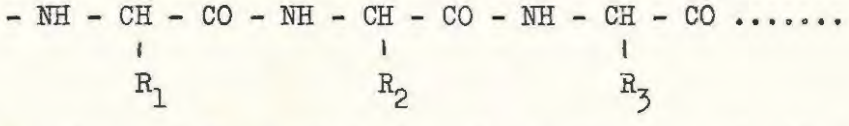


Cystine is readily reduced to give cysteine:



The sulphur of methionine $CH_3 S CH_2 CH_2 CH(NH_2)COOH$ is not alkali labile, and thus is readily distinguishable from cystine and cysteine sulphur.

In the protein molecule the amino acids are combined together by peptide linkages which may be visualized as the condensation of an α-amino group of one acid with an α-carboxyl of another, forming an imide. When this process is repeated indefinitely, long chain molecules or polypeptide chains are obtained:



The nature of the protein depends on the type of side chains, R_1, R_2, R_3 etc., their spatial configuration and arrangement, and the length of the polypeptide chains. In the case of wool keratin conclusive evidence has

been found to indicate that the polypeptide chains are folded in such a manner as to give the fibre considerable elastic properties.

The dibasic and dicarboxylic amino acids in the protein provide free basic and acidic groups respectively, and thus proteins exhibit amphoteric properties. Equations (1.1) and (1.2) illustrate the original concept of acid and base-binding by salt formation:

$$NH_2 - \overset{R}{\underset{|}{CH}} - COOH + H_2O \rightleftharpoons NH_2 - \overset{R}{\underset{|}{CH}} - COO + H_3O \quad \ldots\ldots\ldots\ldots \quad (1.1)$$

$$NH_2 - \overset{R}{\underset{|}{CH}} - COOH + H_2O \rightleftharpoons \overset{+}{N}H_3 - \overset{R}{\underset{|}{CH}} - COOH + OH^- \quad \ldots\ldots\ldots\ldots \quad (1.2)$$

The amino acid, or protein, acts as a weak base in the presence of acid, and as a weak acid in the presence of alkali, forming highly ionized salts in either case.

From equations (1.1) and 1.2)

$$Ka = \frac{a_{H+}\, a_{p-}}{a_p} \text{ and } K_b = \frac{a_{OH^-}\, a_{p+}}{a_p} \quad \ldots\ldots\ldots\ldots \quad (1.3)$$

where a = activity, and the p term refers to the various charged or uncharged forms of $(NH_2\text{-}\underset{|}{\overset{R}{CH}}\text{-}COOH)$

It would therefore be expected that K_a for glycine should be of the same order as that of acetic acid, whereas experiment has revealed an appreciable difference:

Acetic acid	$K_a = 10^{-4.8}$	CH_3COOH
Glycine	$K_a = 10^{-10}$	NH_2CH_2COOH
"	$K_b = 10^{-12}$	

Similarly K_b for glycine should be of the same order as that of ammonia ($K_b = 10^{-4.7}$) since the amino acids may be regarded as substituted derivatives of ammonia and acetic acid.

These great differences in ionization constants between the organic and the amino acids led Bjerrum to formulate the theory that amino acids exist in the form of zwitterions (dipoles) by simultaneous ionization of both amino and carboxyl groups. Measurements of dipole moments of the

amino acids give results far too high for the classical formulation and agree with the Bjerrum concept, i.e. NH_3^+ CHR-COO^-.

The ratio $$\frac{\text{Charged molecules}}{\text{uncharged molecules}} = \frac{\left[NH_3^+ \text{ CHR } COO^-\right]}{\left[NH_2 \text{ CHR } COOH\right]} = K_z \quad \ldots\ldots\ldots\ldots \quad (1.4)$$

shows for ordinary amino acids and peptides, where K_z has a value of 10^5 to 10^6, the almost exclusive presence of zwitterions. Rewriting equations (1.1) and (1.2) according to the Bjerrum concept:

$$NH_3^+ - \underset{\displaystyle R}{\underset{|}{CH}} - COOH \overset{H_2O}{\rightleftharpoons} NH_3^+ - \underset{\displaystyle R}{\underset{|}{CH}} - COO^- + H_3^+O \quad \ldots\ldots\ldots\ldots \quad (1.5)$$

$$NH_2 - \underset{\displaystyle R}{\underset{|}{CH}} - COO^- \overset{H_2O}{\rightleftharpoons} NH_3^+ + \underset{\displaystyle R}{\underset{|}{CH}} - COO^- + OH^- \quad \ldots\ldots\ldots\ldots \quad (1.6)$$

Sörenson has defined the iso-ionic point of an ampholyte as the pH at which it combines equally with acid and base. The iso-ionic point is identical to the iso-electric point only if the ampholyte does not combine with ions other than H^+ or OH^-. The zwitterion concept has been extended to the polypeptides and proteins, and titration data has been successfully interpreted on this basis.

In the field of fibrous proteins, Speakman and Hirst[1] have made a thorough investigation into the changes in mechanical properties of fibres treated with acid and alkali. Typical results of a load-extension curve for wool are shown in Fig. 1a.

The reversibility of the change is illustrated by extending the fibre first in water, then in formic acid, and then after prolonged washing in water again. Furthermore, the authors have shown the linear relationship between the amount of acid sorbed, and the decrease in the work required to extend the fibre by 30% as in Fig. 1b.

Measurements in alkaline solutions give similar results but at higher pH values the fibre suffers alkaline degradation of the disulphide bonds, causing a decrease in strength. The reduction in work for a deaminated fibre has been found to be independent of pH between 1 and 5.

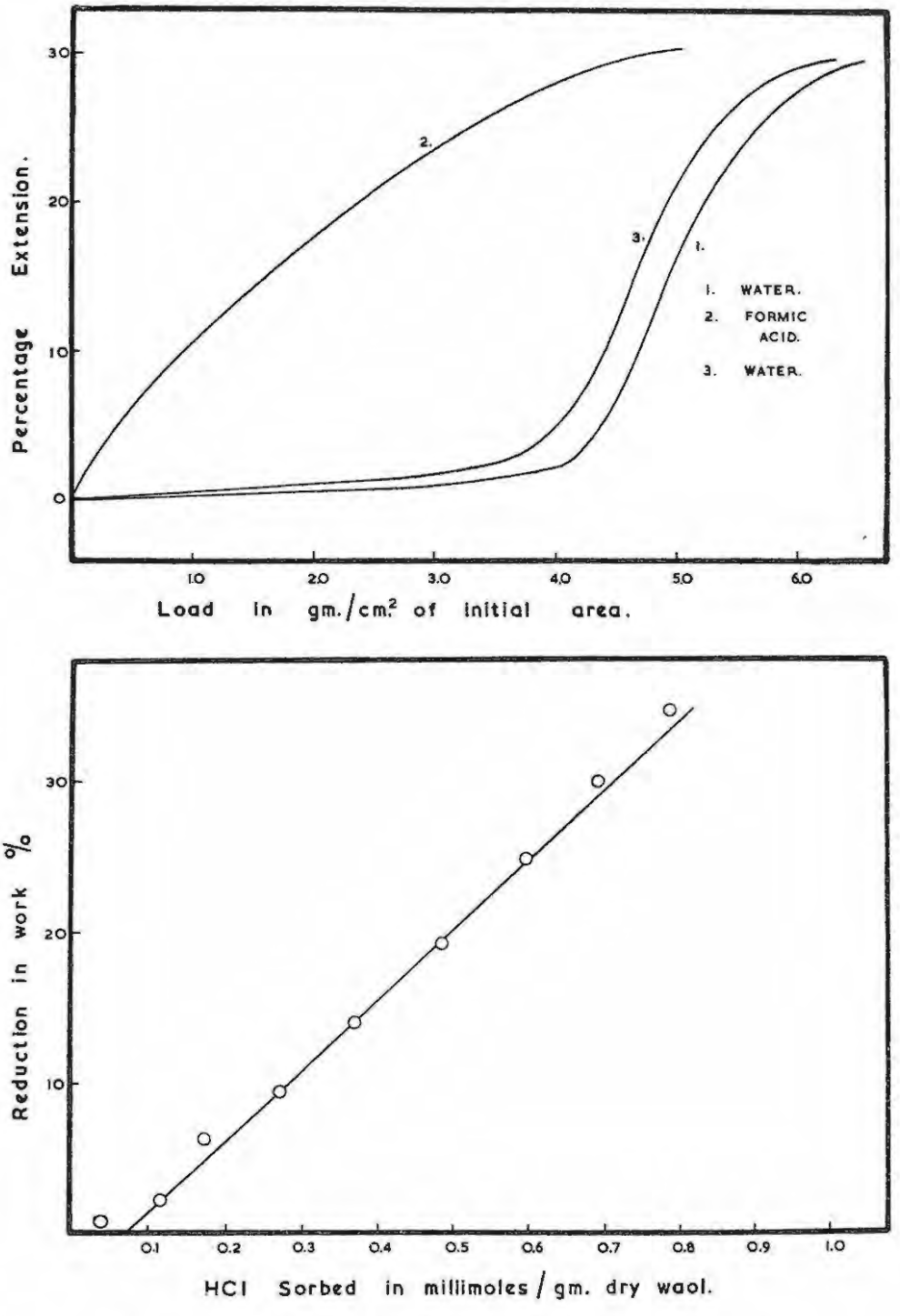


Fig. 1. (a, b). *The mechanical properties of wool fibres treated with dilute acid solutions* [1].

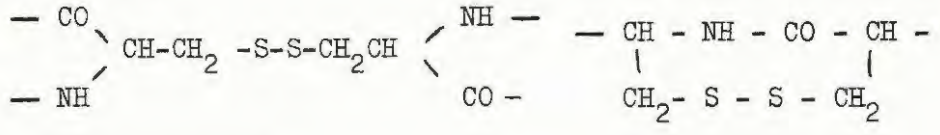
From these experimental facts, it has been concluded that the charged amino and carboxyl groups have a strong electrostatic attraction for one another. Speakman[1] has also found that swelling is a minimum in the iso-electric region, i.e. when the ampholyte has no nett charge and all the acidic and basic groups are ionized. Titration with acids or bases is accompanied by rupture of these electrostatic links, or as they are more commonly called, salt linkages:

$$-\mathrm{COO^-}\ {}^{+}\mathrm{H_3N} \xrightarrow{\mathrm{HCl}} -\mathrm{COOH}\ {}^{+}\mathrm{H_3N}\ \mathrm{Cl^-} - \quad \ldots\ldots\ldots\ldots \quad (1.7)$$

$$-\mathrm{COO^-}\ {}^{+}\mathrm{H_3N} \xrightarrow{\mathrm{NaOH}} -\mathrm{COO^-}\ \mathrm{Na^+}\quad \mathrm{H_2N} - \mathrm{H_2O} \quad \ldots\ldots\ldots\ldots \quad (1.8)$$

Measurements of the heat of reaction for wool and hydrochloric acid by Steinhardt and Harris 1940[2] are in agreement with these mechanisms. The heat of reaction in acid solution is small, and its variation with temperature is characteristic of the carboxyl group. On the other hand in the alkaline region the heat of reaction is high and corresponds with the large heat of dissociation of hydrogen from imidazolium, ammonium and guanidinium groups.

The chief characterizing feature of the keratins is their high sulphur content, nearly all being incorporated in the amino acid cystine. This unit is capable of forming covalent cross-linkages between adjacent polypeptide chains (intermolecular link) or across a chain itself (intramolecular):

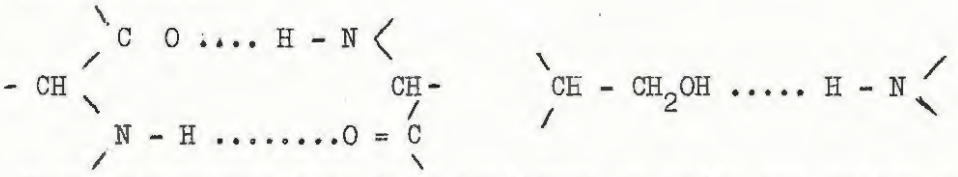


(a) Intermolecular (b) Intramolecular

The amount of each link is uncertain, but experiment has revealed that certain fractions of the cystine react differently from others. The disulphide bond is responsible for the high tensile strength, the insolubility and resistance to enzymatic degradation of the keratin.

The cystine residue is unaffected by acid solutions, but is reactive towards oxidizing and reducing agents, and alkaline solutions.

In addition to the sulphur link and the salt-linkages, cross-linkage is also provided by hydrogen bonding between hydroxyl or keto groups and imide groups:



These structures have been confirmed by infra-red analysis. Although authors have often neglected their effect, the hydrogen bonds play a most important rôle in the structure of proteins. In the case of silk, where the composition (mainly glycine and alanine, but no cystine) and structure are relatively simple, the only forces between adjacent polypeptide chains are provided by hydrogen bonding and van der Waals' forces; yet silk has a high tensile strength and is not soluble, unless treated with a hydrogen bond breaking reagent.

3. TITRATION CURVES.

(a) Introduction.

With the introduction of the concept of pH by Sörenson (1909), titration curves of amino acids, polypeptides and proteins were placed on a firm basis for comparison and theoretical examination. Measurements of acid and base combination were established by electrometric methods using cells consisting essentially of the following type:

$Pt.H_2$(p atm.)/HCl(x) protein //HCl(x)/ H_2(p atm.) Pt.

or from cells without liquid junctions:

$Pt.H_2$(p atm.)/HCl(x) protein AgCl(s) /Ag / AgCl(s) HCl(x)/H_2(p atm.) Pt.

Greenstein[3] has examined the dissociation constants of amino acids and simple di-and tri-peptides, as shown in Fig. 2. He has shown that free polar groups exert an influence upon the ultimate dissociation constants of a polypeptide, and thus theoretical titration curves cannot be constructed successfully from the dissociation data of constituent amino acids. A comparison of the acidic dissociations of proteins with those of its constituent amino acids often yields evidence of their mode of linkage and the modifications they undergo when incorporated into the higher structure. Conversely investigation of the titration curves of proteins may indicate the content of particular amino acids. Originally, this was the principal use of titration data and values of maximum acid and base binding were the only satisfactory estimate of the acidic and basic groups in the protein. This has been largely superseded by the accurate methods for amino acid analysis which are available today. Apart from these applications, a great many properties of proteins such as physiological activity, enzymic function, and denaturation by acid or base are dependent to some extent on pH, and examination of acid-base titration data is often of special interest.

(b) General Aspects of Titration Curves.

The appearance of the titration curve of a protein depends primarily upon the relative numbers and dissociation constants of the dissociating groups of the individual amino acids. As previously mentioned, the shape differs from that predicted by a summation of dissociations of the constituents, extending over a larger pH range.

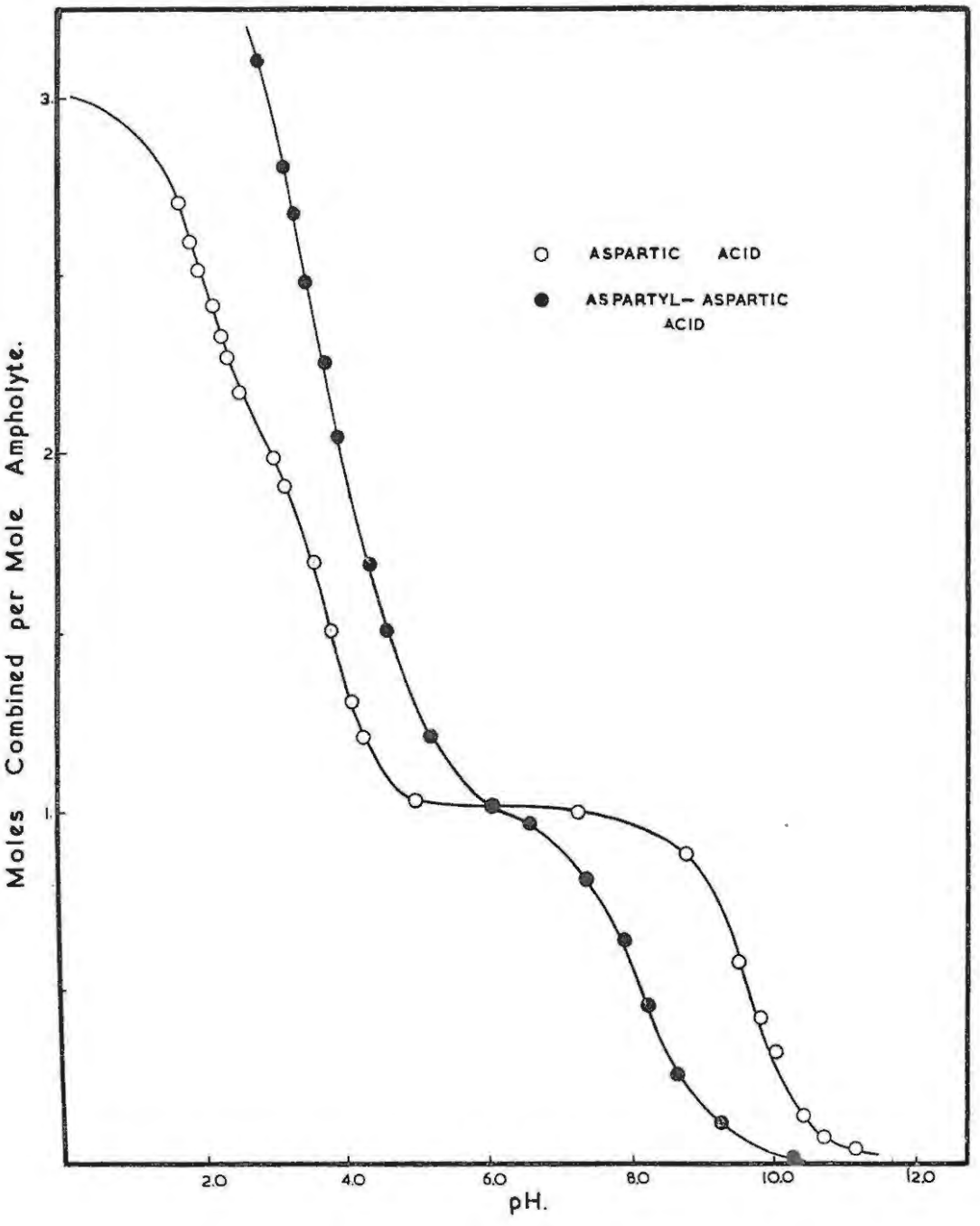


Fig. 2. *Typical titration curves of a dicarboxylic amino acid, and a simple di-peptide derivative* [8].

This spreading of the titration curve results from the electrostatic repulsion (or attraction) between the increasingly positively (or negatively) charged protein and the hydrogen ion as the amount of the latter combined increases (or decreases).

The maximum acid combination at low pH occurs when the protein reaches its maximum nett positive charge. All the dissociating groups exist in the acid form, either electrically neutral (carboxyl, hydroxyl) or charged (imidazolium, ammonium and guanidinium.) As the pH increases, these groups dissociate in order of decreasing acidity, the carboxyl from about 2.0 to 5.5, the imidazolium 5.5 to 8.0, followed by ammonium, phenoxyl, sulphydryl and guanidium. In practice a maximum in dissociation of hydrogen ions (maximum base binding) is never observed, because the weakly acidic guanidinium groups are not completely dissociated even at pH 13.5. At this stage all the acidic groups exist in the form of their conjungate bases: carboxylate, imidazole, amino, and phenoxylate.

(c) Theoretical Analysis of Titration Curves.

(i) Soluble proteins.

Dissociation curves for soluble proteins have been satisfactorily analysed by Lindström - Lang[4] who applied the theories of Bjerrum and Debye-Hückel to describe quantitatively the dissociation of a highly polyvalent ampholyte in terms of the number of dissociations, the characteristic pk values and their electro-static interaction with one another and with the environment. This treatment has been successfully applied and extended by Cannan et al[5]. The theory requires two assumptions: that the dissociation groups may be divided into a small number of classes, each of which may be characterized by a single dissociation K_{int} constant; and that the protein molecule be regarded as a sphere, with its charged groups randomly distributed over its surface. These assumptions are reasonably justified, as the molecules are relatively small, and since they are capable of forming unimolecular films on air/water interfaces they are flexible enough to orientate all the ionizable groups towards the bulk of the solution, i.e. they are freely accessible to both hydrions and anions. That the charged groups are located on the surface is supported by the fact that the electro-

phoretic mobility (proportional to the external charge of the particle) and titration curves show a similar dependence on pH.

If the molecule gains a proton its potential is raised by an amount $\frac{e}{Dr}$, and its electrostatic potential energy is increased by an amount $\frac{e^2}{Dr}$ where e = electronic charge, D = dielectric constant of the medium and r = radius of the molecule. As acid is added to an iso-electric soluble protein, the ion dipoles give rise to a nett positive charge:

$$— NH_3^+ \quad OOC^- — + H^+ \longrightarrow - NH_3^+ \quad HOOC —$$

As a result of repulsion, subsequent protons are taken up with decreasing affinity as sorption increases. Thus comparison with a mono-carboxylic acid indicates that sorption is spread over a considerably wider pH range.

If n_i denotes the total number of groups of class i, r_i of these are dissociated at a given pH, and z = average nett charge on the molecule, then

$$\log \frac{r_i}{n_i - r_i} = pH - p\,(K_{int})_i + \frac{22w}{2.303} \quad \ldots\ldots\ldots\ldots \qquad (1.9)$$

where w is an electrostatic factor depending on the radius of the hydrated protein molecule, the absolute temperature and the ionic strength of the medium.

Sets of values for $p(K_{int})_i$ and n_i are chosen, and the titration curve for the protein is constructed from a summation of such curves for the various types of dissociating groups. The theoretical analysis is held to be satisfactory if the experimental data are reproduced by a set $p(K_{int})_i$ and n_i values which are consistent with those obtained from other sources.

The electrostatic term w is dependent on the ionic strength of the medium, and the theory agrees satisfactorily with the increase in slope of the curves with increasing salt concentration.

(ii) Insoluble proteins.

Titration curves of insoluble proteins have been determined by Speakman and Stott[6], Harris and Rutherford[7] and Steinhardt and Harris[8] on wool, Harris and Gleysteen[9] on silk, and Bowes and Kenten[10] on collagen. In all cases considerable difference in behaviour from the soluble proteins has been observed. For insoluble proteins the techniques

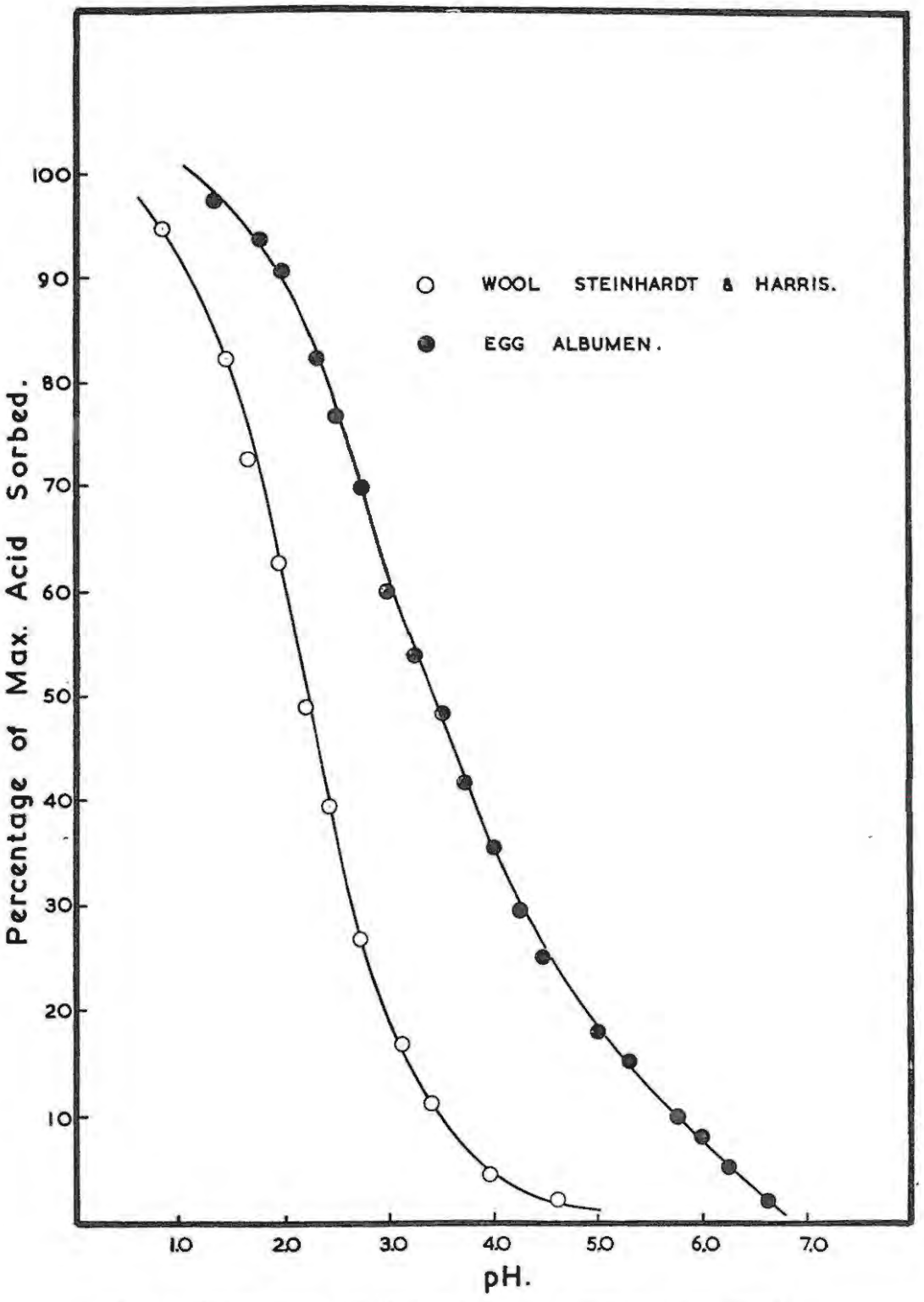


Fig. 3. *Comparison of the titration curves of a soluble (egg albumen) and an insoluble (wool) protein.*

may be modified because the definite phase difference allows an aliquot to be removed for titration. The titration curves of egg albumen and wool are compared in Fig. 3. The ionizing groups of the insoluble protein appear to be far more acid; also the effect of salt on the titration curve is entirely different.

It is obvious that these discrepancies arise from the great difference in molecular dimensions between the soluble and insoluble proteins. As before, the increase in potential produced by sorption of a proton is $\frac{e}{Dr}$ which decreases with increasing r. However, the number of ionizable groups which the molecule contains increases as r^3. In fact, if r is of microscopical instead of molecular dimensions, the fraction of sites which may be occupied before an appreciable charge is produced on the molecule, is very small. After this hydrogen ion combination can only proceed with the simultaneous entry of the anions into the fibre molecule. Thus the apparent increase in strength of the acid groups is not caused by any change of the pk values, but by the potential which develops on the surface of the protein molecule.

Although the theoretical approach of Lindström - Lang has been successfully applied to soluble proteins, it fails in the interpretation for insoluble proteins. This is to be expected because the basic concept, that of a small, spherical and completely flexible molecule, cannot be extended to the large rigid molecules of insoluble fibrous proteins.

In recent years several theories have been proposed to explain these anomalous titration curves. In all cases, the general picture of the water-swollen fibre is the same, i.e. that of a mass of interlacing polypeptide chains containing imbibed water and forming an equipotential volume. In some parts of the fibre the polypeptide chains are closer and well-ordered, forming crystalline regions as apart from the amorphous parts, although it seems likely that both are permeable to the small hydrogen ions. It is the properties of the internal solution that have been a source of disagreement in these theories. Steinhardt and Harris[8] and Gilbert and Rideal[11] have predicted in their theories that both hydrogen ions and anions are absorbed, whereas the proponents of the Donnan Theory[12] maintain that only hydrogen ions are sorbed, and that the anions remain dissolved in the internal solution. A description of

these theories, their applications, merits and shortcomings will be discussed later.

(d) Stoichiometric Relationship of Maxima of Acid and Base combination.

The number of equivalents of hydrogen ion which are bound when a protein is brought from its isoionic point to a state in which all the dissociable groups are in their acidic form is equal to the number of cationic acid groups (equivalent to maximum acid binding.) Similarly the equivalents of hydrogen ion dissociated from the isoionic point until the fully basic condition is the number of anionic basic groups (maximum base binding.) The interpretation of these limits is not always straightforward, as measurements in concentrated solutions are subject to large experimental errors unless necessary precautions have been taken. Moreover, the maximum combination and dissociation of hydrogen ions must be tested for reversibility by back-titration before the values can be ascribed to native protein. Such irreversible side reactions are greatly accelerated in concentrated acid or alkaline solution. If denaturation results in an increase in dissociablegroups or if amide or peptide hydrolysis occurs, the maxima will refer to partially denatured or to modified protein.

In the past the maximum hydrogen ion capacity of the protein has provided a more reliable value for the total cationic groups than amino acid analyses (these were generally too low, based on isolation procedures). Since the introduction of accurate methods for amino acid assay, agreement has been found for most proteins. Where the amino acid analyses exceed the values from maximum hydrogen ion combination it has been found that some of the dissociable groups are not available in the protein.

(e) Differentiation of Reactive Groups.

(i) Assignment of segments of the curve to specific groups.

The carboxyl group dissociates between pH 2 and 5.5, thus the number of equivalents of hydrogen ion which are dissociated in this region is equal to the number of free carboxyl groups, i.e. the sum of aspartic and glutamic acid residues plus terminal α-carboxyl groups minus the amide groups. For some proteins agreement with free carboxyl group content is found, but in other cases the titration data indicates more carboxyl groups.

Because of the diverse nature of the ionizable groups of the basic amino acid residues, the titration curves of most proteins are far too complex for individual analysis, except in the case of histidine. The amount of hydrogen ion dissociating between pH 5.5 and 8.0 generally results from the imidazolium groups of histidine, with perhaps a small contribution from α-ammonium groups. Where comparison with amino acid analyses has been possible, agreement has been found to be satisfactory.

(ii) Modification of dissociation constants.

Ionization constants are substantially altered by the dielectric of the solvent medium, and titration curves have been performed in alcohol-water, and acetone-water mixtures. Their use is limited however, since proteins tend to precipitate in organic solvents and may even undergo denaturation. Moreover the observed changes in titration curves are not easy to interpret.

Many reagents have been employed to alter the dissociation constants of the reactive groups of proteins, but few are really specific or even quantitative, i.e. reaction of iodine which enters the benzene nucleus of tyrosine, but also reacts with other groups.

Formaldehyde reacts rapidly and quantitatively with amino groups to give addition products which are far weaker bases:

$$R.NH_2 + CH_2O \rightleftharpoons R\,NH\,CH_2OH \quad \ldots\ldots\ldots\ldots \qquad (1.10)$$

$$R\,NH\,CH_2OH + CH_2O \rightleftharpoons R\,N\,(CH_2OH)_2 \quad \ldots\ldots\ldots\ldots \qquad (1.11)$$

In the Formol titration (Sörenson)(1907) the apparent dissociation equilibrium of the ε-ammonium groups of lysine are displaced towards acid pH by 3 units. Differential titration in the presence and absence of formaldehyde gives the number of ε-ammonium groups. Steinhardt and Fugitt[13] found that the effect of formaldehyde on the titration curve of wool keratin was consistent with the combination of this reagent with ε-ammonium groups of lysine. At higher pH the guanidinium groups interfere.

S-methyl iso-thio urea has been used by Christensen[14] to convert the ε-ammonium groups of lysine to guanidine, and results in a substantial elevation in the pk value. The process is similar to the use of formaldehyde. Carboxylation has been successfully achieved with sodium

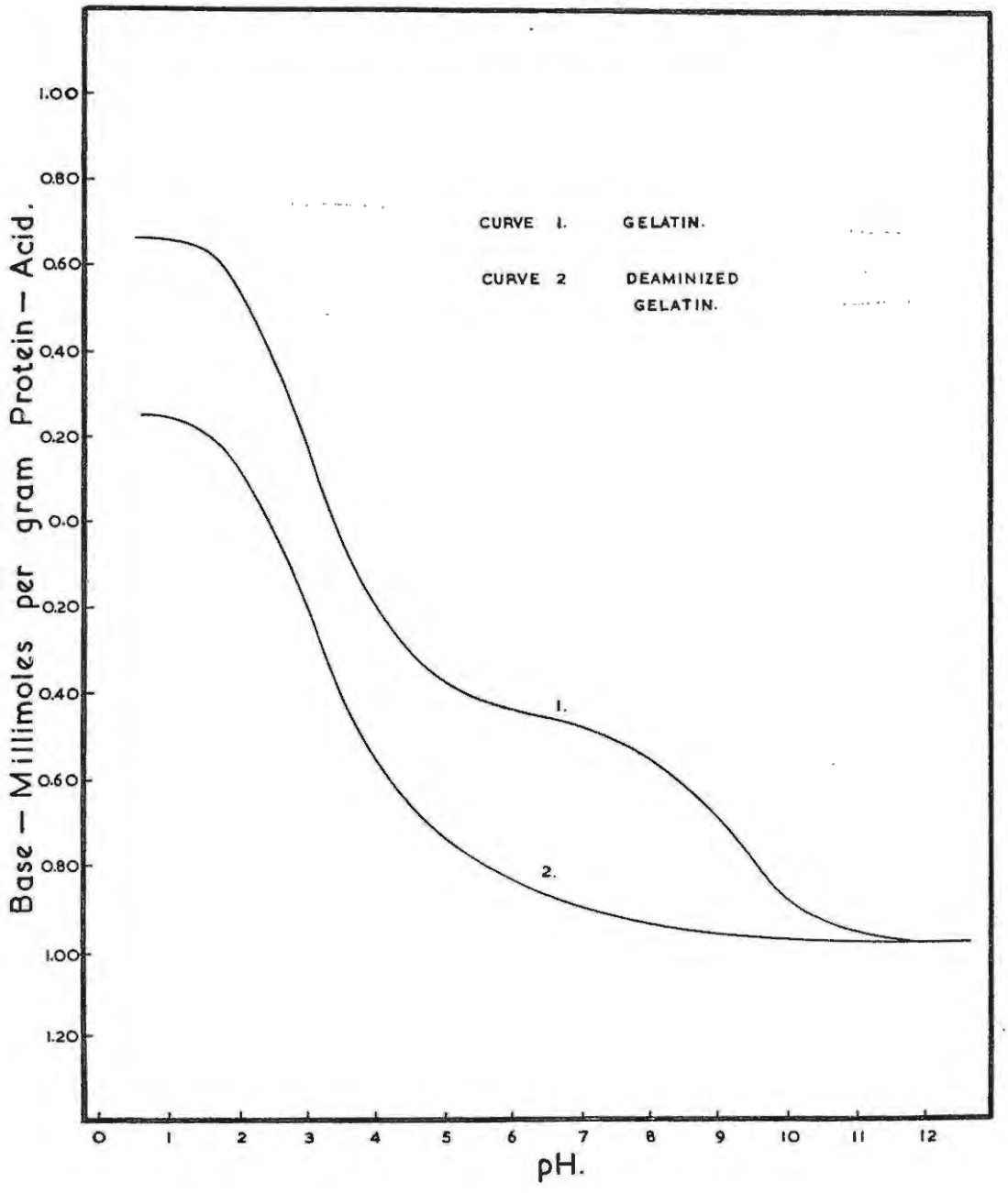


Fig. 4. *Titration curves of normal and deaminized gelatin*[16].

bromo-acetate by Sykes[15] on collagen:

$$-NH_2 \xrightarrow{2\ BrCH_2COO^-} -N(CH_2COO^-)2 \qquad \ldots\ldots\ldots \quad (1.12)$$

Some of the more complex reactions which proteins undergo often result in an alteration in pk or number of dissociable groups, and differential titrations may provide useful information. Such studies on haemoglobin have contributed considerable knowledge relating to the structure and reactivity of their heme-linked acid functions.

(iii) Inactivation of specific groups.

Unfortunately most of these chemical modifications are either non-specific or can only be effected under drastic conditions which destroy or alter the native protein.

A few reactions have been found suitable to eliminate partially or totally the contribution of various groups. Methylation and acetylation of amino and phenoxyl groups have been used effectively, and their ability to dissociate hydrogen ions is eliminated. Lichtenstein[16] has de-aminized gelatin (see Fig. 4), showing by changes in titration curves that the free ammonium groups had been removed. Carboxyl groups may be blocked by esterification (these compounds tend to decompose in alkaline solution). Decarboxylation may be effected by reduction with phenyl magnesium bromide Fox,[17], or lithium aluminium hydride - Nystrom and Brown[18]: both reactions lead to the formation of a tertiary alcohol.

(iv) Spectrophotometric methods:

The ionization of phenoxyl groups of tyrosine is accompanied by a change in ultraviolet absorption spectrum. Crammer and Neuberger[19] have used this to measure the number and pk value of these groups in proteins. In some cases the spectrum accounts for all the tyrosine present, whereas with ovalbumin none of the phenoxyl groups are dissociated until the protein had been denatured by heat, acid or chemical treatment.

(f) Interpretation of Titration Curves.

Although the experimental procedure for acid-base titration curves is relatively simple, the interpretation of the data requires extremely cautious analysis. Many proteins are extremely sensitive to

acid, even in dilute solutions, but the determination of maximum combination or dissociation of hydrogen ions involves working near the extremes of the pH scale. Many investigators have acknowledged the possibility of denaturation of the protein during this procedure, whereas others have analysed titration data without considering this factor. Denaturation or even chemical modification of the proteins may occur during preparative stages. This neglect has led to the impression that the titration curves of native and denatured proteins differ only slightly, except where the denatured protein is precipitated during the sorption reaction.

Titration data of modified proteins must be thoroughly investigated before differences are interpreted as specific effects of a modified group. Chemical modifications are seldom specific, and unless the protein is thoroughly purified after the treatment, erroneous results will be obtained. Analysis of the data of insoluble proteins is even more complex as these curves cannot be analysed by the Lindström - Lang treatment. Although amino acid analyses may be of assistance, hydrolysis of the modified protein may result in the formation or elimination of certain reactive groups.

SECTION II : ACID SORPTION.

4. EXPERIMENTAL PROCEDURE FOR ACID SORPTION.

(a) Purification of Samples.

In order that wool and mohair could be compared, a standard sample of each was carefully selected. The wool sample consisted of a blend of Merino 64's and the standard mohair was a random selection of Super Summer Firsts. Both samples were large and were thoroughly mixed during the preparative stages to prevent or minimise sampling errors. A series of mohair samples (No's A - J) were chosen so that the influence of various factors such as age, fibre diameter and degree of weathering on the titration curves could be studied. The tips of the raw fibres, which are usually damaged by exposure to sun, rain, etc. were removed. Tip portions of the standard wool and mohair samples were set aside for later investigation.

All samples were purified according to the following procedure:

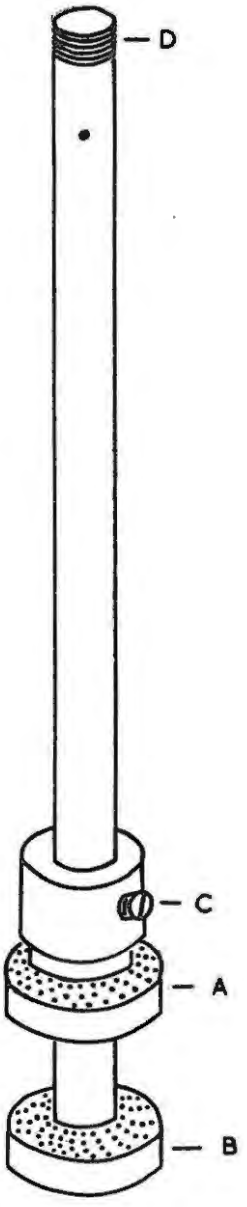
(i) Scour by hand, using a non-ionic detergent in water at 40° - 50°C. Most of the "yolk" was removed in the form of grease, suint and sand. The excess detergent was removed by several washings in cold water.

(ii) After drying in a stream of air at 55° - 60°C, the samples were soxhletted for 12 hours with ether to remove the final traces of grease. The remaining suint was extracted from the fibres by soxhletting for 4 hours with ethyl alcohol, keeping the temperature of the extracting liquor below 40°C. The samples were air-dried.

(iii) Since carding tends to damage the fibre surface, vegetable matter was removed by hand.

(iv) After a short immersion in distilled water, the samples were steeped in a large volume of $^{N}/1000$ hydrochloric acid (liquor ratio approximately 10:1). After 48 hours they were removed and washed repeatedly with distilled water till the pH rose to 5.0 to 5.2. The fibres are now at their iso-electric point.

(v) After air-drying at 55°C, the samples were again opened and carefully cleaned of any remaining vegetable matter or dried skin. The fibres were cut into approximately $\frac{1}{2}$ inch lengths to facilitate mixing. Finally the samples were placed in the conditioning room at 68°F and 65% relative humidity. Samples were analysed regularly for moisture content by drying 1 gm. of conditioned fibres to constant weight at 105°C.



A. ADJUSTIBLE PERFORATED DISC.

B. FIXED PERFORATED DISC.

C. LOCKING SCREW.

D. COUPLING THREAD.

Fig. 5. *Ebonite plunger designed for the rapid sorption procedure.*

(b) Equilibration Techniques.

It has been the practice of previous authors[6,8] to determine acid and alkali sorption of wool, silk, etc. by immersing known weights of fibres in a fixed volume of acid or alkali for periods between 24 and 75 hours. After equilibrium has been established, the solution is analysed either by titration or pH measurement, and comparison with the original solution gives the amount of acid or alkali sorbed.

Unfortunately this method has several disadvantages. As the rate of sorption is slow, long periods of immersion are required before equilibrium has been attained. At pH 1 to 2 these long exposures to acid cause considerable hydrolysis of the amide groups, with the liberation of ammonia. This combines with an equivalent amount of hydrochloric acid, and necessitates the introduction of a correction term. On the alkaline side, however, the side reactions become a major problem. The disulphide bonds are particularly susceptible to alkaline attack, a reaction which complicates the determination of alkali sorption since alkali is consumed in the process. It is therefore of the utmost importance that the sorption period be as short as possible in order that side reactions be minimised. This is particularly true at high concentrations of acid or alkali.

A method has therefore been sought whereby the sorption rate could be increased without detracting from the accuracy of the determination. The plunger device of Dickenson and Palmer[20] was found to be most suitable for this purpose. In this apparatus a small sample of fibres (usually 1 gm.) is compressed between perforated discs, and pumped back and forth in the solution contained in a close-fitting tube. The solution at the fibre surface is constantly renewed, and thus the sorption rate is dependent only on diffusion in the fibre phase and not diffusion in the solution. The apparatus is shown in Fig. 5. In early experiments the metal tube and plunger (high-grade stainless steel) were found to dissolve slightly in solutions stronger than $^{N}/_{1000}$ HCl.

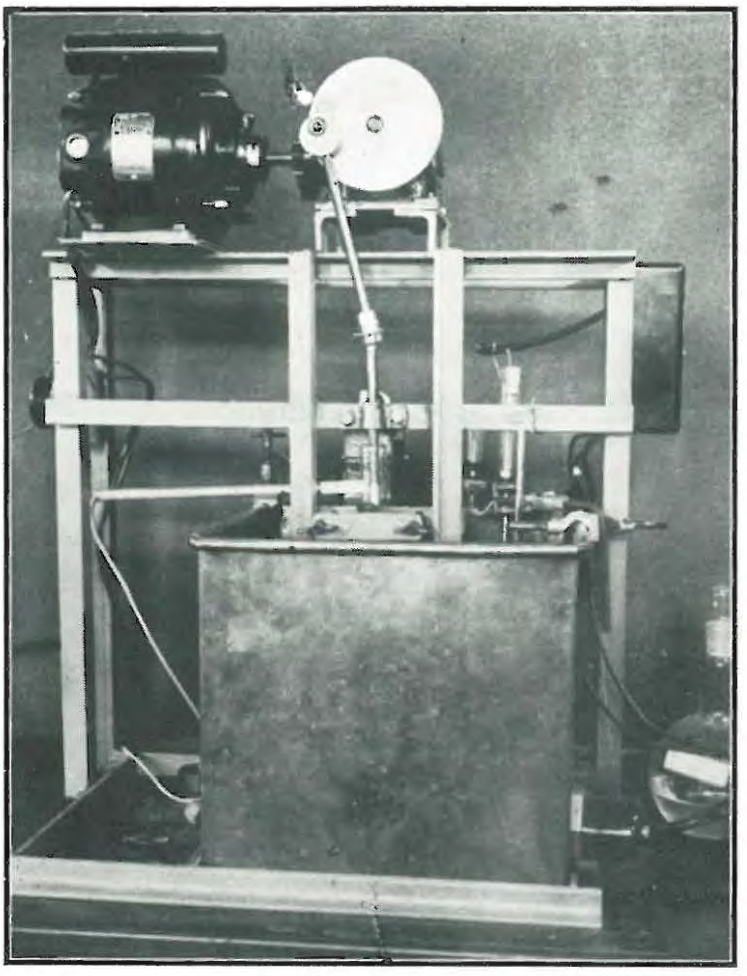


Fig. 6.. *Automatic pumping machine constructed to operate at 20 strokes/minute.*

Apart from the objectionable formation of ferric hydroxide during the subsequent titration, the presence of foreign ions influences the sorption measurements. After much experimenting, ebonite was found to be a suitable material for the plunger as it is unaffected by acid or alkaline solutions. The metal tube was replaced by a thick-walled glass tube of uniform bore, giving a clearance between tube and plunger of approximately 0.5 m.m.

The advantages of this type of system are:-

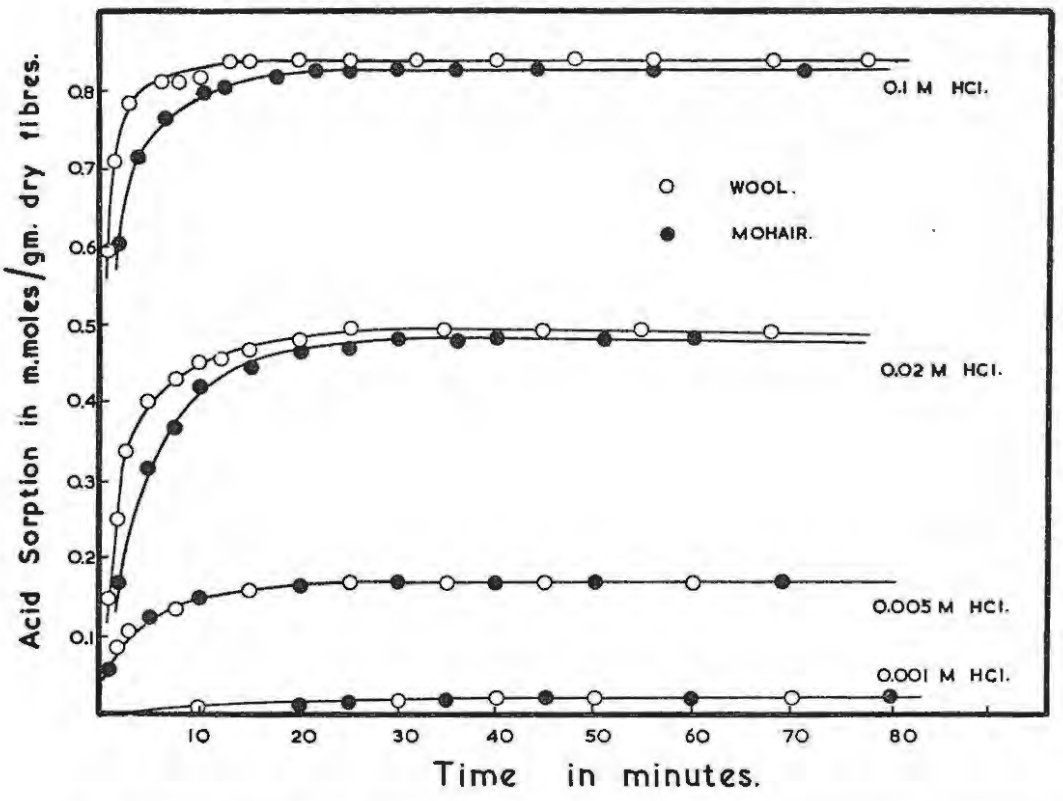
(i) A rapid sorption rate is obtained.

(ii) A small liquor:wool ratio may be used. 50:1 ratio was found to be most suitable. In an ordinary soaking experiment, it is difficult to get good mixing with a low liquor ratio. Clearly the accuracy of the titration is increased if a low liquor ratio is maintained.

Pumping by hand in preliminary experiments indicated that a rate of 20 to 30 strokes per minute was most suitable. An automatic pumping apparatus was designed and constructed to perform this operation (Fig. 6). An $\frac{1}{8}$th H.P. motor working on a 40:1 reduction gear drives a cam giving a 10 cm. vertical movement at a rate of 20 strokes/minute. Adjustable jaws with rubber pads were designed to clamp the glass tubes in position. Breakages of tube were few, usually resulting from excessive pressure from the clamps or incorrect alignment of plunger and tube. The dimensions of the plunger and tube were chosen such that the fibre plug remained in the solution at all phases of the stroke.

Sorption measurements were conducted at 25°C. The reaction tubes were immersed in a well stirred waterbath (10 litres). Two heaters were employed, a 500 watt element for rapid warming, and a 60 watt frosted bulb immersed in the bath. The small heater was sufficient to maintain the temperature of the bath which was well lagged. Temperature was thermostatically controlled at 25 $\pm$ 0.05°C with a mercury toluene thermoregulator and a Sunvic relay device.

Before measurements of acid sorption were commenced, equilibrium rates were determined over a wide range of concentrations, and under the same experimental conditions as set down for the measurements of acid and



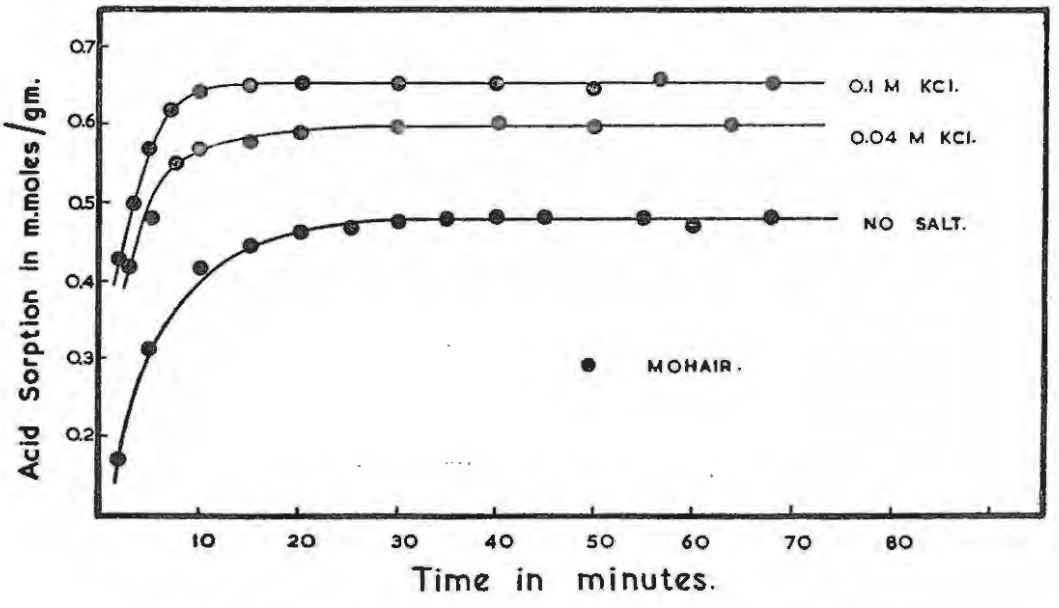


Fig. 7a (above). *The sorption rate of HCl by wool and mohair at 25°C using the automatic pumping apparatus.*

Fig. 7b (below). *The sorption rate of HCl in the presence of added electrolyte.*

base sorption,

i.e. Temperature 25°C,
Liquor ratio 50:1,
Pumping rate 20 strokes/minute.

The following procedure was used. Aliquots of 0.5 ml. were removed from the reaction chamber at various intervals after pumping had commenced, and titrated with standard alkali using a 2 ml. microburette. When appropriate corrections for the amount of acid removed were applied, the acid sorbed could be calculated. These values depend on small volumes and consequently are rather inaccurate, giving only an indication of the sorption rate. Results are shown graphically in Fig. 7a and the effect of salt addition is illustrated in Fig. 7b.

(c) Titration Procedure and pH Measurement.

(i) Preparation of solutions:

A.R. grade hydrochloric acid was used throughout. Stock solutions were prepared by diluting the concentrated acid with glass-distilled water. These solutions were found to keep well in polythene bottles, and were regularly standardized against re-crystallized borax. Dilute acid solutions were prepared from these stock solutions using accurate volumetric flasks.

A.R. grade potassium chloride was used for varying the ionic strength of solutions. After drying for 6 hours at 105°C, stock solutions were prepared.

(ii) Titration procedure.

A 50 ml. aliquot of standard acid solution was introduced to the reaction tube with a pipette; 1.000 gm. of conditioned fibres was carefully transferred to the plunger, with the perforated discs apart. After placing the plunger and sample in the tube, the perforated discs were forced together; screw c (Fig. 5) maintains the sample in this position.

The tube was then carefully mounted in the waterbath, and allowed to equilibrate for 10 to 15 minutes. The pumping mechanism was started, and the sample pumped for the prescribed

period. This value was obtained from the sorption rate data in Fig. 7; an extra 10 to 15 minutes was allowed to ensure that equilibrium had been attained.

After pumping, the reaction tube was removed, and cooled down to room temperature. A 25 ml. aliquot was removed and titrated with standard alkali using bromocresol purple indicator. Results were found to be most satisfactory when standard acid and alkali solutions were approximately the same normality. From the titre before and after sorption and the dry weight of the fibres, the acid sorption per gm. dry sample could be calculated.

In the pH range 0 to 1.5 the above method was modified slightly to give better accuracy. The hydrochloric acid solutions were placed in a carefully calibrated burette (10 mls.) and titrated against a weighed amount of re-crystallized borax in duplicate. Although this represents a change in technique, it is justified as it eliminates errors involved by taking an aliquot, and those introduced by standardizing alkali with standard acid, and then subsequent use of the alkali as a standard. With concentrated solutions, above 0.1 molar, an error of 0.05 mls. may cause a difference of 0.020 to 0.060 millimoles in the sorption value. Previous authors[6,8] have obtained erratic results at concentrations higher than 0.1 molar. Using the above titration procedure, a greater accuracy is obtained.

Sorption measurements in the presence of electrolyte (KCl) have been conducted in such a manner that the final ionic strength of the solution is at a fixed value. A range of ionic strengths 0.005 - 0.10 molar were studied. In dilute acid solutions adjusting the salt concentration is relatively easy, as the final acid concentration is negligible. As the acid concentration increases, however, the total ionic strength becomes dependent on both the acid and the salt

concentration, and adjusting the salt concentration to obtain the correct value at equilibrium is more difficult. An approximate acid sorption for acid-salt mixture was predicted from the standard curve, Fig. 8. From the pH value at this point the final acid concentration was calculated. A hydrochloric acid solution was prepared containing an amount of KCl calculated to bring the ionic strength at equilibrium to a fixed value. Using this solution, a sorption experiment was then conducted. In most cases the experimental sorption values differed considerably from those predicted as above. Experiments were repeated using trial and error methods to obtain the correct amount of KCl. Usually three or four determinations were necessary before the correct value was obtained.

In order to determine whether exposure to the concentrated acid solutions (0.1 to 0.8 molar) produced hydrolysis of amide groups or peptide bonds, an aliquot of solution was reserved for ammonia and soluble nitrogen determinations. For long equilibration periods Steinhardt and Harris[8] found as much as 0.030 millimoles of ammonia at 25°C. Using the pumping method for equilibration, neither ammonia nor soluble protein (after kjeldahl digestion) could be detected by a sensitive colorimetric procedure.[21]

(iii) pH Measurement.

Measurements were made with a Beckman Model C pH meter, using a calomel reference electrode and a small glass electrode. The following buffers were prepared for calibration of the instrument:

	pH value
0.05 m potassium tetroxalate	1.48
0.05 m potassium hydrogen phthalate	4.01
0.025 m potassium dihydrogen phosphate 0.025 m disodium hydrogen phosphate	7.02

Measurements were made at room temperature, using the temperature correcting adjustment on the instrument. The meter was calibrated on an appropriate buffer immediately before every reading.

After the sorption stage an aliquot of acid solution was reserved for pH determination. The electrodes were washed well with distilled water, and then immersed in several lots of acid solution, before the final reading was taken. The overall accuracy of the pH readings using this procedure is approximately $\pm$ 0.05 pH units.

(d). Correction for Water Imbibition of Keratin Fibres.

Before immersion in HCl solution, the conditioned fibres contain 12 to 13% moisture. In aqueous solution, however, the fibres reach a saturation value of approximately 40%. This water is no longer available to the liquid phase and consequently causes an apparent increase in the final acid concentration. This in turn gives rise to lower values for acid sorption. In order to evaluate a correction term, it is necessary to determine the saturation moisture content of the fibres in aqueous hydrochloric acid solutions.

Sookne and Harris[22] have formulated the following correction:

C_1 = initial concentration of HCl by titration

C_2 = final " " " " "

B = gms. of water sorbed by 1 gm. of fibres.

Then, if a conditioned sample contains W gms. water per gram dry fibres, after immersion it will have sorbed (B - W) gms. water/gm. dry fibres. If there are G mls. of acid present and S gms. of dry wool, and v is the volume of (B - W) gms. water (density taken as unity), the corrected acid sorption

$$A = \frac{GC_1}{S} - (G - Sv)\frac{C_2}{S}$$

$$= \frac{G(C_1 - C_2)}{S} + (B - W)\,C_2 \quad \ldots\ldots\ldots\ldots \qquad (2.1)$$

Hence the magnitude of the correction depends on (B - W) and the final concentration C_2, and is only significant for solutions from pH 0 to 1.5.

The evaluation of v presents an intricate problem. Moreover it is not certain whether the water associated with the fibre is held internally or whether part is derived from surface effects, i.e. surface tension, electrical double layer, etc.

Speakman and Stott[6] have estimated the value of v from measurements of diameter swelling; this accounts for the water which has diffused into the fibre causing a lateral displacement of the structure. The procedure devised by Sookne and Harris[22] is more apt for the problem, but unfortunately the experimental results are extremely variable. The concentration change of a standard substance (which has no affinity for the fibre) is measured during the sorption process and from these values the amount of water which has left solution may be determined. Standard substances such as sodium chloride or trehalose are used. The sodium chloride method is based on the evaporation of an aliquot of solution and gravimetric determination. Trehalose is determined polarimetrically. The sodium chloride method was attempted on wool and mohair samples of different initial moisture contents. Although the experiment was carefully performed, the agreement of the results was very poor. This is due to the fact that the accuracy of the method depends on small changes in the concentration of the sodium chloride solutions. The final results are extremely sensitive to small errors. A difference of 0.5 mg. in two weighings is magnified to an error of 20%. Moreover it is doubtful whether the sorption of water remains unaffected by the large concentration of sodium chloride necessary as reference substance.

Work by Preston and Nimkar[23] on the volume swelling of various fibres in aqueous solution provided the basis of the following method. After saturating the fibre with water, the excess is removed by centrifuging, and the moisture content is determined by drying at 105°C. The results, when compared with other methods, are 10 to 15% too high. This is caused by surface moisture held by capillary action between adjacent fibres. By reducing the surface tension with a surface active

agent, a minimum water retention was found. The following experimental procedure was adopted:-

A 1 gm. sample of conditioned wool or mohair was soaked in a hydrochloric acid solution (0.05 to 0.50 m) for 2 hours. After squeezing out the excess liquor, the sample was rinsed quickly in an extremely dilute solution of Invadine JFC (5 drops of 0.1% solution to 200 mls water) and squeezed again. The sample was halved, and each portion placed on a perforated disc in a centrifuge tube. After balancing, the centrifuge was run at 3000 r.p.m. for 10 minutes. The samples were rapidly transferred to weighed 10 ml. flasks, and dried to constant weight at 105°C. The saturation moisture content determined in this manner gave results consistent to 2 to 3%. Average values are listed in Table 1 below.

TABLE 1.

MOISTURE RETAINED BY FIBRES AFTER CENTRIFUGING.

PROCEDURE	% SATURATION WATER	
Wool soaked in water	40.0	39.6
Mohair soaked in water	39.6	38.3
Wool soaked in water rinsed in	36.8	36.0
Mohair " " " Invadine JFC	36.6	37.5
Wool soaked in 0.05 m HCl rinsed in	34.8	34.0
Mohair " " " " Invadine JFC	36.0	35.2
Wool soaked in 0.20 m HCl rinsed in	36.6	36.0
Mohair " " " " Invadine JFC	35.3	35.0
Wool soaked in 0.50 m HCl rinsed in	36.8	36.4
Mohair " " " " Invadine JFC	36.3	36.0

Mean value for fibres soaked in acid and rinsed with Invadine JFC 35.7%

From the previous table it will be seen that the wetting agent has decreased the water retention by 10%. The concentration of acid seems to have little effect at this pH. The results were corrected for the amount of HCl on the dried fibres using the method of Barritt.[24] It is interesting to note that these values for combined acid agreed well with those predicted by the titration curves. This provides proof that the centrifuging process, when used in conjunction with a wetting-agent, removes the excess solution from the fibres, and verifies the accuracy of the method.

This method has the advantage over the technique of Sookne and Harris[22] in that it is a direct measure of the water sorbed, whereas the latter method depends on a small difference in the amount of water present in the aqueous phase before and after sorption. Speakman and Stott[6] have measured the saturation water content from measurements of transverse swelling. However, an error of 0.5 μ in the diameter measurements results in a 10% difference in the volume of the water sorbed. Moreover this refers only to water which has penetrated the fibre, and does not take into account adsorbed water, which is not available to the solution.

Using these values for water imbibition more accurate sorption values in the concentrated region are obtained.

(e) Ash Content.

Unless carefully purified, wool and mohair contain an appreciable amount of inorganic matter consisting of metallic ions and silica. After ashing, these are present as metallic oxides (sulphate is also produced by the oxidation) and SiO_2. The cations are either attached to free carboxyl groups or are incorporated in the structure, e.g. iron and copper in the pigment melanin. Most of the cations can be removed in dilute hydrochloric solution or by electrodialysis. If present on the fibre prior to acid sorption experiments, the cations have a two-fold effect:

(i) They combine with an equivalent amount of hydrochloric acid;

(ii) The salt formed in solution causes a shift towards higher acid sorption.

These effects are particularly noticeable with dilute acid solution and unless the cations are removed, it is impossible to determine the iso-electric point correctly. La Fleur[25] failed to appreciate the significance of the ash content, and rinsed his samples in tap water prior to acid sorption measurement. His results show a step in the titration curve in pH region 4 to 6, and necessitated an empirical and rather unsatisfactory correction. For this reason all samples were subjected to a thorough cleansing scheme described fully earlier.

The ash content of the samples was determined by ashing at 800°C (ignited slowly first with a bunsen burner.) A value of 0.2% was obtained for both the wool and mohair standard samples.

Ash analyses for standard wool and mohair, and samples B1 and H2 are shown in Table 2 to be 0.2 ± 0.05%.

Excluding silica, the residue may be regarded as metallic oxides and a certain amount of sulphate of equivalent weight approximately 100. This will account for a deviation of 0.020 millimoles HCl per gram sample.

A comprehensive cation analysis has been performed to clarify the position.

TABLE 2.

ASH ANALYSIS.

		Standard Wool	Standard Mohair	Mohair B_1	Mohair H_2
% Ash content		0.15	0.20	0.20	0.20
Cations - µgms/gm.dry fibres					
Sodium	Flame	20	20	20	20
Potassium	photometer	..... not detectable			
Calcium		..... not detectable			
Iron	Colorimetric	30	28	30	20
Copper		5	5	5	5
Zinc		10	10	10	10

Analytical Methods described fully by Steyn (26)

The presence of Mn, Mg, Al, Co, Sn and Pb could not be detected in the above samples.[27]

Quantitative cation analysis revealed iron, copper, zinc, magnesium and sodium in trace quantities, but not sufficient to account for 0.2%, not even when calculated either as oxides or sulphates. It may safely be concluded that the ash therefore consists mainly of silica which has no effect on the acid sorption measurements. Several authors have applied empirical corrections for this residual ash, but it was felt that this was unnecessary.

5. EXPERIMENTAL RESULTS.

The results of measurements of acid sorption at a large number of pH values are recorded in Tables 3, 4 and 5. Tables 3a and 3b refer to standard wool and mohair respectively. Table 4 contains the data in the presence of added electrolyte (KCl) and Table 5 is the acid sorption of various mohair samples (A - J). Each result is the mean of three measurements, where only the equilibration period has been altered to verify that equilibrium had been attained. Results on modified fibres are recorded in Tables 6, 7 and 8.

TABLE 3a.

THE SORPTION OF HCl BY STANDARD WOOL AT 25°C.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.00011	0.00004	0.004	0.004	4.96
0.00018	0.00005	0.007	0.007	4.85
0.00049	0.00009	0.023	0.023	4.22
0.00073	0.00014	0.034	0.034	3.96
0.00098	0.00019	0.046	0.046	3.86
0.00147	0.00034	0.065	0.065	3.53
0.00244	0.00071	0.101	0.101	3.31
0.00309	0.00096	0.124	0.124	3.18
0.00412	0.00136	0.161	0.161	2.95
0.00516	0.00181	0.194	0.194	2.82
0.00619	0.00225	0.229	0.229	2.70
0.00722	0.00285	0.254	0.254	2.55
0.00914	0.00390	0.304	0.304	2.43
0.00928	0.00387	0.315	0.315	2.46
0.01117	0.00512	0.352	0.352	2.33
0.01320	0.00617	0.408	0.408	2.20
0.01523	0.00779	0.432	0.432	2.13
0.01776	0.00960	0.474	0.474	2.05
0.03035	0.01920	0.634	0.641	1.77
0.03912	0.02733	0.683	0.692	1.59
0.04010	0.02784	0.697	0.706	1.61
0.04401	0.03203	0.795	0.705	1.55
0.06023	0.04677	0.765	0.778	1.39
0.1066	0.09302	0.780	0.805	1.18
0.1926	0.1793	0.754	0.799	0.97
0.2074	0.1937	0.781	0.829	0.87
0.3059	0.2926	0.760	0.831	0.74
0.4905	0.4774	0.748	0.861	0.50
0.8285	0.8167	0.676	0.867	0.24

TABLE 3b.

THE SORPTION OF HCl BY STANDARD MOHAIR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry moh.	Sorption corrected for water imbibition	pH
0.00011	0.00005	0.003	0.003	4.59
0.00018	0.00008	0.006	0.006	4.37
0.00049	0.00016	0.016	0.019	3.97
0.00073	0.00020	0.030	0.030	3.86
0.00101	0.00033	0.040	0.040	3.66
0.00147	0.00046	0.059	0.059	3.42
0.00244	0.00089	0.091	0.091	3.16
0.00305	0.00106	0.117	0.117	3.07
0.00376	0.00132	0.143	0.143	2.95
0.00451	0.00162	0.168	0.168	2.86
0.00527	0.00200	0.192	0.192	2.75
0.00650	0.00262	0.227	0.227	2.64
0.00770	0.00313	0.267	0.267	2.50
0.01012	0.00446	0.328	0.328	2.40
0.01200	0.00575	0.366	0.366	2.28
0.01401	0.00704	0.405	0.405	2.18
0.01601	0.00832	0.449	0.449	2.10
0.01802	0.00971	0.486	0.486	2.03
0.02113	0.01166	0.546	0.550	2.00
0.02400	0.01448	0.559	0.564	1.89
0.03035	0.01921	0.642	0.648	1.77
0.04010	0.02809	0.693	0.702	1.61
0.05135	0.03847	0.744	0.755	1.49
0.06023	0.04704	0.760	0.773	1.39
0.08055	0.06728	0.766	0.783	1.25
0.1067	0.0930	0.792	0.815	1.20
0.1926	0.1792	0.775	0.817	0.97
0.2074	0.1939	0.780	0.825	0.85
0.3059	0.2927	0.766	0.835	0.75
0.4904	0.4776	0.740	0.847	0.49
0.8285	0.8162	0.703	0.884	0.24

TABLE 4a.

THE SORPTION OF HCl BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.005 MOLAR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.00019	0.00003	0.009	0.009	5.31
0.00039	0.00006	0.019	0.019	5.01
0.00097	0.00018	0.046	0.046	4.41
0.00146	0.00021	0.072	0.072	3.95
0.00194	0.00040	0.090	0.090	3.92
0.00292	0.00053	0.139	0.139	3.45
0.00361	0.00082	0.167	0.167	3.30
0.00486	0.00125	0.210	0.210	2.97
0.00618	0.00310	0.250	0.250	2.79
0.00778	0.00288	0.286	0.286	2.63
0.00972	0.00426	0.317	0.317	2.46

TABLE 4b.

THE SORPTION OF HCl BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.01 MOLAR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.00010	0.00001	0.005	0.005	5.50
0.00020	0.00002	0.010	0.010	5.39
0.00040	0.00005	0.020	0.020	5.20
0.00071	0.00008	0.035	0.035	4.88
0.00098	0.00007	0.053	0.053	4.57
0.00147	0.00017	0.076	0.076	4.30
0.00196	0.00013	0.106	0.106	4.01
0.00243	0.00028	0.125	0.125	3.84
0.00389	0.00064	0.191	0.191	3.35
0.00583	0.00132	0.262	0.262	3.00
0.00681	0.00178	0.292	0.292	2.85
0.00778	0.00231	0.317	0.317	2.71
0.00972	0.00345	0.367	0.367	2.53
0.01458	0.00716	0.443	0.443	2.20

TABLE 4c.

THE SORPTION OF HCl BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.02 MOLAR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.00020	0.00002	0.010	0.010	5.63
0.00040	0.00004	0.022	0.022	5.43
0.00082	0.00005	0.045	0.045	5.00
0.00165	0.00015	0.087	0.087	4.48
0.00258	0.00021	0.178	0.178	4.08
0.00360	0.00037	0.188	0.188	3.78
0.00410	0.00050	0.209	0.209	3.60
0.00515	0.00078	0.255	0.255	3.37
0.00618	0.00104	0.299	0.299	3.18
0.00721	0.00152	0.331	0.331	3.03
0.01030	0.00305	0.422	0.422	2.57
0.01236	0.00437	0.465	0.465	2.48
0.01545	0.00625	0.524	0.524	2.22
0.01854	0.00940	0.532	0.532	2.17
0.02575	0.01550	0.600	0.600	1.91

TABLE 4d.

THE SORPTION OF HCl BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.05 MOLAR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.00021	0.00002	0.001	0.001	6.03
0.00062	0.00004	0.003	0.003	5.74
0.00103	0.00008	0.055	0.055	5.23
0.00154	0.00009	0.085	0.085	4.78
0.00206	0.00017	0.110	0.110	4.57
0.00309	0.00021	0.167	0.167	4.17
0.00412	0.00038	0.218	0.218	3.87
0.00515	0.00044	0.274	0.274	3.57
0.00618	0.00077	0.314	0.314	3.35
0.00721	0.00093	0.365	0.365	3.18
0.00874	0.00154	0.422	0.422	2.89
0.01031	0.00022	0.470	0.470	2.70
0.01339	0.00414	0.538	0.538	2.44
0.01545	0.00545	0.580	0.580	2.38
0.01854	0.00794	0.616	0.616	2.16

TABLE 4e.

THE SORPTION OF HCl BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.10 MOLAR.

Initial conc. of HCl moles/l.	Final conc. of HCl moles/l.	HCl sorption millimoles/ gm. dry wool.	Sorption corrected for water imbibition	pH
0.000195	0.00001	0.011	0.011	5.75
0.000475	0.00028	0.026	0.026	5.50
0.000972	0.00032	0.055	0.055	5.20
0.00227	0.00092	0.136	0.136	4.59
0.00389	0.00021	0.214	0.214	4.15
0.00583	0.00053	0.308	0.308	3.73
0.00778	0.00102	0.392	0.392	3.35
0.00972	0.00164	0.470	0.470	2.98
0.0114	0.00246	0.535	0.535	2.75
0.01470	0.00422	0.602	0.602	2.51
0.02671	0.01452	0.699	0.704	2.10
0.06354	0.05011	0.766	0.789	1.40

TABLE 5.

ACID SORPTION BY MOHAIR SAMPLES A - J.

SIDE SAMPLES.			NECK SAMPLES.		
Sample No.	Corr. HCl sorp. m.moles/g. dry fibres.	pH	Sample	Corr. HCl sorp. m.moles/g. dry fibres.	pH
A_1	0.062	3.60			
	0.293	2.46			
	0.551	2.01			
	0.777	1.38			
	0.790	1.24			
B_1	0.074	3.91	B_2	0.057	3.90
	0.365	2.48		0.322	2.42
	0.575	2.01		0.527	2.00
	0.815	1.40		0.740	1.48
	0.834	1.26		0.800	1.25
C_1	0.052	3.73	C_2	0.050	3.34
	0.347	2.42		0.321	2.38
	0.567	2.01		0.524	1.99
	0.804	1.39		0.760	1.37
	0.815	1.25		0.774	1.26
D_1	0.054	3.46	D_2	0.054	3.52
	0.061	3.58		0.303	2.40
	0.337	2.41		0.505	2.01
	0.548	2.01		0.775	1.25
	0.796	1.25		0.797	1.15
E_1	0.061	3.70	E_2	0.067	3.85
	0.332	2.42		0.387	2.45
	0.558	2.00		0.534	2.02
				0.540	2.00
	0.814	1.27		0.805	1.26

TABLE 5. (contd.)

SIDE SAMPLES.			NECK SAMPLES.		
Sample No.	Corr. HCl sorp. m.moles/g. dry fibres.	pH	Sample	Corr. HCl sorp. m.moles/g. dry fibres.	pH
F_1	0.058	3.64	F_2	0.060	3.51
	0.331	2.54		0.329	2.43
	0.558	2.01		0.553	2.00
	0.813	1.27		0.830	1.26
	0.841	1.15			
G_1	0.060	3.54	G_2	0.054	3.54
	0.316	2.42		0.788	1.26
	0.513	2.05		0.290	2.37
	0.545	2.00		0.529	2.01
	0.820	1.26			
H_1	0.056	3.45	H_2	0.057	3.45
				0.276	2.41
	0.286	2.38		0.469	2.01
	0.523	2.03			
	0.804	1.25		0.774	1.26
J_1	0.060	3.85	J_2	0.051	3.35
	0.309	2.36		0.292	2.35
	0.524	2.01		0.510	1.98
	0.825	1.39		0.812	1.38
	0.840	1.14		0.840	1.16

HCl SORPTION BY MODIFIED FIBRES.

TABLE 6.

TIP PORTIONS OF STANDARD MOHAIR.

HCl SORPTION millimoles/gm.	pH
0.056	3.45
0.142	3.02
0.270	2.50
0.488	2.05
0.770	1.40

TABLE 7.

'LANTHIONINE-RICH' WOOL

HCl SORPTION millimoles/gm.	pH
0.035	4.15
0.362	2.66
0.540	2.04

TABLE 8.

PERACETIC OXIDIZED WOOL.

HCl SORPTION millimoles/gm.	pH
0.040	3.50
0.482	1.91
0.595	1.10

6. DISCUSSION OF RESULTS.

(a) Standard Wool and Mohair.

The titration data for standard wool and mohair is plotted in Fig. 8. Curve (1) shows the acid sorption values for standard mohair without the correction for selective sorption of water. The deviations from the corrected curve are only significant between pH 0 to 1.5; if not applied, however, a completely incorrect maximum value is obtained.

Comparison of the final hydrochloric acid concentration and pH value of this solution reveals that there is a small buffering effect in pH range 3 to 5. (This was also found in the alkaline region 6 to 9). The most probable cause is the solubilisation of small protein fragments or wool gelatin[28].

Experimental results of Steinhardt and Harris [8] for HCl sorption at 25°C are also plotted in Fig. 8. Considering the fact that wool varies considerably in composition, the agreement of the present measurements with the results of these authors is remarkably close. Taking into account the careful preparative and analytical techniques it is felt that the experimental results in the present work are more reliable than previous authors, especially in the strongly acid region, where former results were most erratic.

Titration curves for wool and mohair are very similar in shape, and extend over approximately the same range of pH and acid combination. Small differences occur in pH region 3 to 5 and again at 0 to 1.5, although in the latter case readings are less reliable. The most striking point is that the curves intersect (at approximately pH 2). The reasons for these differences are obviously complicated, and at present are open to much speculation. Probable causes are:

(i) Differences in amino acid composition. The amino acid composition of wool has been shown to vary from one animal to another, Simmonds.[29] If the polypeptide chains are shorter, then there are more terminal amino groups in a fixed weight.

(ii) The arrangement of the amino acids can have a definite influence on the pk values of the ionizing groups e.g. two carboxyl groups in close vicinity, or a polar group (tyrosine hydroxy) although not ionized at

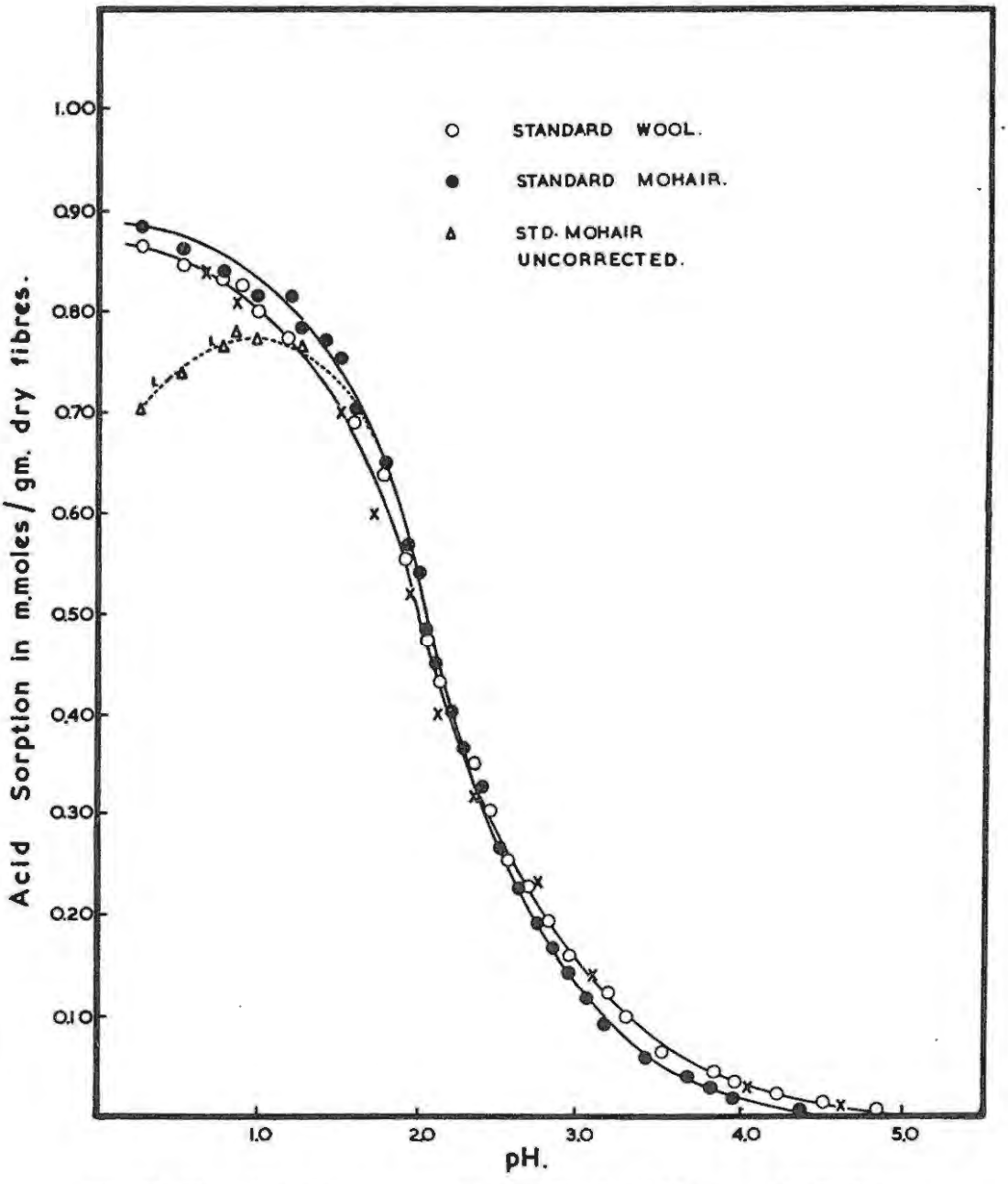


Fig. 8 *The acid titration curve of Standard Wool and Mohair at 25°C. The dotted line is obtained by omitting the correction for water imhibition.*

this pH, may exert an influence by formation of hydrogen bonds near a carboxyl group.

The intersection of the standard wool and mohair curves may be explained by combining these factors.

Amino acid analyses for the dicarboxylic and dibasic (di-amino) acids are shown in Table 9. Column (i) is taken from the chromatographic analyses of merino wool by Simmonds[29], while Columns (ii) and (iii) are merino wool and mohair by Ward, Binkley, and Snell[30] using micro-biological assay. The latter method accounts for only 80% of the nitrogen, and it is felt that they are not accurate enough for this interpretation. However, the differences in Columns (ii) and (iii) indicate that appreciable differences in composition may occur. Unfortunately there are no accurate amino acid analysis on mohair.

TABLE 9.

	COLUMN (i)	(ii)	(iii)
AMINO ACID	Merino 64's Simmonds	Merino 70's Ward, Binkley &	Mohair Snell
		Millimoles/gm.	
(a) Aspartic acid	0.503	0.481	0.550
(b) Glutammic acid	1.020	0.891	0.965
(c) Arginine	0.603	0.525	0.513
(d) Hystidine	0.058	0.062	0.058
(e) Lysine	0.192	0.075	0.210
(f) Terminal amino	0.018		
Amide Nitrogen	0.900	0.78	0.86

Although acid sorption is essentially the back-titration of ionized carboxyl groups, maximum acid binding capacity is determined by the sum of the free amino acids. From Column (i) sum of free amino groups

$$(c+d+e+f) = 0.871 \text{ millimoles}$$

From present experimental data, the maximum acid binding is:

Wool : 0.860 ± 10 millimoles/gm.
Mohair : 0.870 ± 10 millimoles/gm.

These values compare favourably with the value of 0.871 millimoles/gm. obtained by Simmonds for wool.*

Acid titration data in the presence of varying amounts of salt (KCl) is recorded in Table 4, and illustrated graphically in Fig 12.
The salt shift may be analysed into two parts, which are not clearly defined, one passing to the other as the acid concentration increases.

(i) Near the iso-ionic region, small changes in acid sorption occur as salt concentration increases. Nevertheless these are sufficient to change the pH considerably, i.e. pH values are displaced towards a more neutral value.

(ii) As the acid concentration increases, increasing salt concentration increases the acid sorption remarkably, and pH values are less affected. In the region of maximum acid sorption, the curves become superimposed.

* Since the completion of this work, the amino acid composition of mohair has been determined by Simmonds (31). The number of free basic groups calculated from this data is 0.796 m.moles/gm. (using a nitrogen content of 16.5% as found in the present work). Simmonds has analysed only one sample of mohair which may be an extreme case.

Table 10 illustrates the effect of salt addition near the point of half-maximum sorption. (0.01 m. HCl).

TABLE 10.

Total eqlm. ionic strength moles/l.	Acid sorption m.moles/gm.	pH
No salt	0.328	2.40
0.01	0.367	2.53
0.02	0.422	2.57
0.05	0.470	2.70
0.10	0.535	2.75

These results are particularly useful for application of various theories of acid sorption and dyeing.

(b) Mohair, Samples A - J.

Prior to the investigation a series of mohair samples was carefully selected with the co-operation of Grootfontein Agricultural College to investigate the influence of various factors on the acid and alkali titration curves. All samples were removed from animals (Angora goats) which had been maintained under the same conditions, diet etc., and represented an equal period of growth. The following influences were studied:

(i) The effect of mean fibre diameter was studied by choosing samples over a wide range of diameters.

(ii) Samples were taken from animals of three distinct age groups, viz. 6 months (kid), 18 months, and 3 years (adult).

(iii) Mohair samples were removed from the side and neck of each animal, and were labelled 1 and 2 respectively i.e.

B_1 - side sample of animal B

B_2 - neck sample of animal B

Measurements of acid sorption are recorded in Table 5. The titration curves of these samples show distinct deviations from the standard curve.

In Fig. 9a, the largest positive and negative deviations are shown in dotted lines.

Largest +ve deviation - sample B_1

Largest -ve deviation - sample H_2

To investigate whether these differences had resulted from changes in acidic or basic properties of the protein fibre, the iso-ionic point of standard wool and mohair and samples B_1 and H_2 were measured and compared. The following procedure was adopted. Acid sorption was measured as before, in extremely dilute HCl solutions in the presence of 0.1 m KCl. These values were plotted against their respective pH values, and extrapolated to zero acid sorption as shown in Fig. 9b. The intercept with the abscissae gives the iso-ionic point. The deviations from the standard mohair were again observed. These variations can be caused by the following factors:

(i) Presence of acid in the fibre

(ii) Exchangeable cations in the fibre

(iii) Variations in the composition structure of the protein.

The presence of HCl on the fibres after rinsing to the iso-electric region is most unlikely, also the affinity of Cl^- for the fibre is very low. If the samples were not exactly at the iso-electric point before sorption, small deviations could be expected. However, the slope of the titration curve is very small in this region and differences of 0.02 to 0.05 millimoles cannot be explained.

The possibility of exchangeable cations represents a complex problem. Ash analyses are often rather misleading, as these results invariably include substances such as silica. Whether these cations are exchangeable or tightly bound in the structure is uncertain.

Standard samples and mohair samples B_1 and H_2 were electrodialysed for 20 hours using the method of Sookne et al[33]. The excess of HCl was removed by continuous washing till the pH of the extract reached pH 5.0. Measurements of the iso-ionic points of these purified samples were performed as before, and are shown graphically by the dotted lines in Fig. 9b.

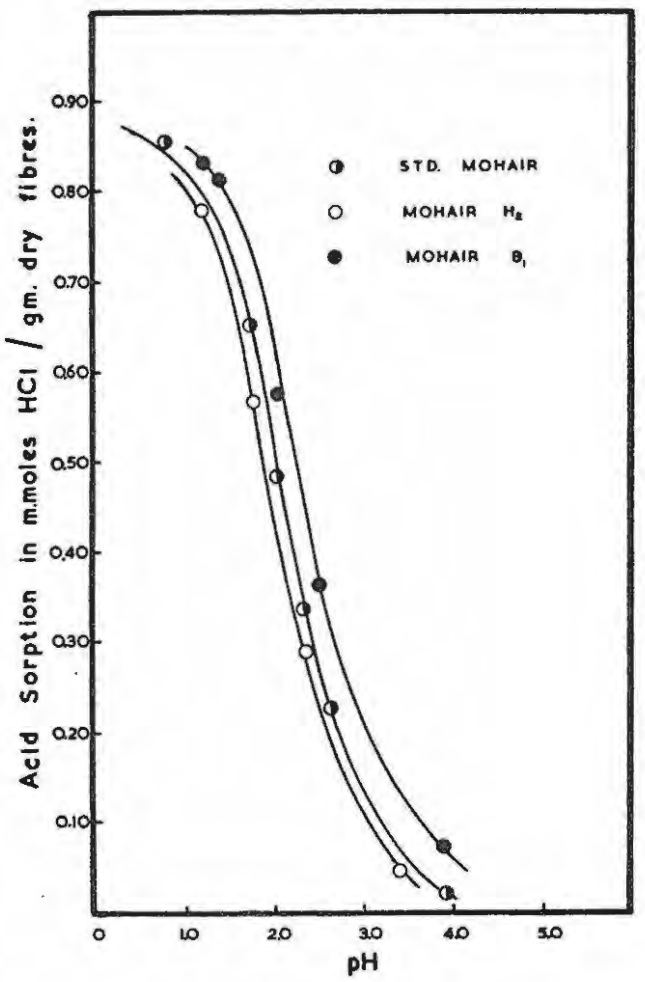


Fig. 9a. *Titration curves of mohair samples which gave the largest deviations from the standard curve.*

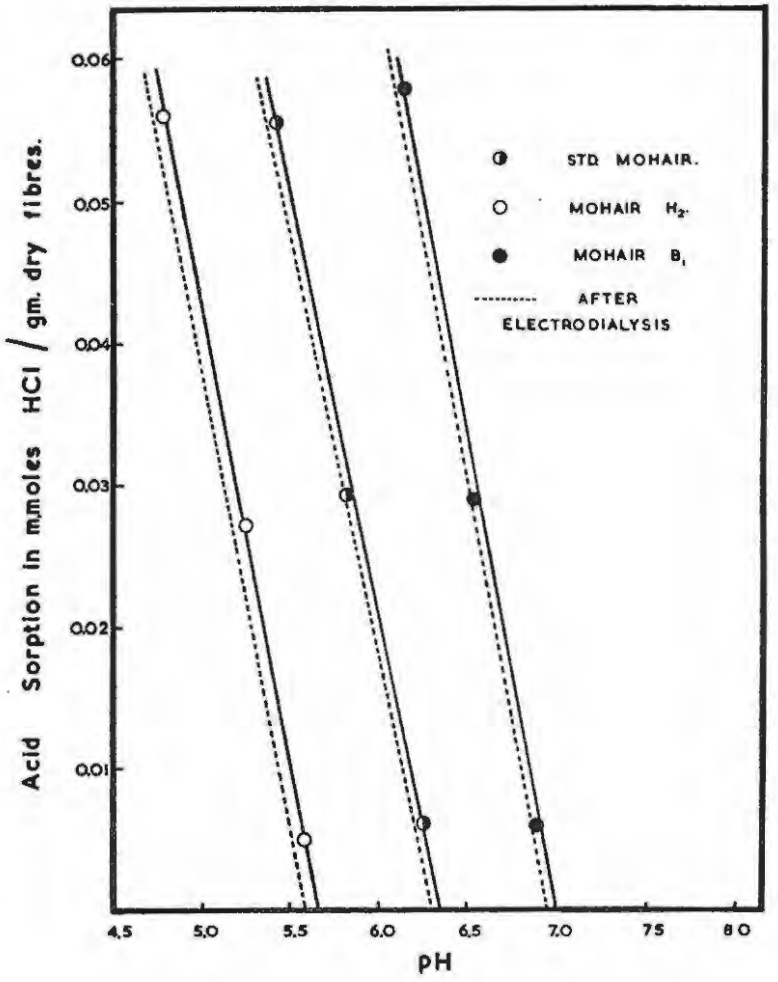


Fig. 9b. *The determination of the isoionic point of wool and mohair keratin by extrapolation to to zero acid combination. (In the presence of KCl).*

Clearly the process of electrodialysis has left the fibres unchanged. Thus it seems more likely that the differences in titration data have arisen from variations in amino acid content, and their arrangement in the protein fibre.

In order that the titration data of mohair and wool samples could be placed on a comparative basis, the acid sorption at pH 2.00 has been selected as an index of acid sorption. The value of the index has been interpolated from Table 5. There are several reasons for choosing this point as a criterion:

(i) It is approximately the pH of half-maximum sorption which will be shown later to be an important characteristic of the titration curves.

(ii) In this region the influence of foreign materials, electrolytes, is less than in the iso-electric region.

(iii) Measurements in this region are more accurate than at any other pH range, since the calculated values of acid sorption are not affected by small errors in titration, and pH values are not readily affected by small traces of impurities, i.e. buffer solution.

Table 11 shows the values of the acid sorption index a, the age A, and mean fibre diameter D of the mohair samples A - J. These results have been statistically analysed to find whether age and diameter influence acid sorption. There is a very strong correlation between age and diameter, which is significant at the 0.01% level (highly significant). Because of this relationship it is necessary to calculate partial correlation coefficients to find the effects of age and diameter on acid sorption.

TABLE 11.

Reference Number	Age of Animal	Mean fibre Diameter μ's	Acid Sorption Index
A_1	0.5	28	0.55
B_1	0.5	29	0.68
B_2	0.5	32	0.52
C_1	0.5	29	0.60
C_2	0.5	34	0.51
D_1	1.5	47	0.51
D_2	1.5	45	0.55
E_1	1.5	36	0.56
E_2	1.5	48	0.54
F_1	1.5	45	0.56
F_2	1.5	48	0.55
G_1	3	47	0.50
G_2	3	53	0.51
H_1	3	54	0.54
H_2	3	49	0.47
J_1	3	41	0.53
J_2	3	46	0.51

Correlation between diameter and acid sorption (age constant)

$$r_{aD.A} = -0.069$$

Degrees of freedom = 12

This is not significant.

Correlation between age and acid sorption (diameter constant)

$r_{aA.D} = 0.31$ (12 degrees of freedom)

This is not significant even at the 10% level, but shows there is a slight trend in this direction.

To investigate whether differences in acid sorption between side (mean value of Index 0.55 millimoles/g.) and neck samples (mean 0.52 millimoles/g.) Student's t has been calculated.

Student's t = 2.10 with 15 degrees of freedom.

This is significant at the 5% level, and it may be concluded that there is a trend towards higher sorption by the side samples.

The mean value for side samples of kid mohair (A_1, B_1 and C_1) is 0.593 m.moles/g. and is significantly different (2% level) from the mean value of the adult side samples (G_1, H_1 and J_1) of 0.516 m.moles/g. There are insufficient samples of kid mohair for a thorough comparison. This relation is not shown in the neck samples, where presumably other factors are involved.

Summing up the statistical analyses, it appears that acid sorption is dependent on more complex factors that have been considered here, i.e. variations in amino acid content, amide nitrogen etc. Consequently although trends appear in the results, they are not statistically significant. A more profitable approach would be to take samples from the same animal at different periods of growth, while the animal was maintained under the same conditions.

The following general observations were made on the results:

(i) The highest values for acid sorption index were obtained for side samples of kid mohair; the neck samples being appreciably lower.

(ii) In the other age groups, the acid sorption index is lower, and the difference between side and neck samples is not pronounced.

It is hoped that complete amino acid analyses will be available in the near future.

(c) Modified Wool and Mohair.

(1) Photochemical Modification.

Tip portions of the fibre staples which were removed during the preparation of standard wool and mohair samples were purified according to the procedure described on page 17 . Acid sorption data for tip mohair is recorded in Table 6. The acid titration curve obtained by plotting this data coincided with the standard mohair curve, which is consistent

with the findings of Speakman and McMahon[33] for wool that photo-chemical damage does not affect the acid titration curve.
Photochemical damage is discussed more fully on page 101.

(2) Chemical Modification.

(i) 'Lanthionine - Rich' Wool.

The method of Zahn and Osterloh[34] was used to convert fraction of the cystine of a sub-sample of standard wool into lanthionine. In this process wool is refluxed at 64°C for 3 hours in 0.05 m. borax dissolved in a water/acetone mixture. After removing the excess solvent, the sample was rinsed in N/1000 HCl till the pH remained at 3. Finally the excess acid was removed by repeated washing till the wash liquor reached a pH of 5.0 to 5.2. After drying at 60°C the sample was conditioned. The wool acquired a slightly yellow coloration, but otherwise suffered no noticeable change in tensile strength or surface properties. Acid sorption data is shown in Table 7 and a plot of these results coincides with the standard for wool. Fig.19 .

(ii) Oxidized Wool.

A sub-sample of standard wool was reacted with 2% peracetic acid, prepared by the method of Greenspan[35], for ½ hour at room temperature (18°C). The excess acid was removed by repeated washing with distilled water till the pH of the extract reached pH 5.0 to 5.2. This treatment resulted in a fibre of slightly yellowish colour, with a considerable loss in resilience and tensile strength. Acid sorption data is shown in Table 8.

Since both treatments (i) and (ii) involve reaction of the disulphide bond, the results are discussed more fully in conjunction with the corresponding values for alkali sorption.

7. THEORIES OF ACID SORPTION BY FIBROUS PROTEINS.

A. Steinhardt and Harris Theory.

The fundamental concept of this theory is that the protein combines with both chloride and hydrogen ions, and the combination of both is subject to the Law of Mass Action or to a Langmuir adsorption law. This implies the formation of partially dissociated linkages such as $RNH_3^+ Cl^-$. The authors make the simplifying assumption that no recognition need be taken of difference in hydrogen ion concentration inside and outside the fibre. 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 variation of activity coefficients in the fibre phase is neglected.

Consider the iso-electric protein $W^{\pm}$ in the presence of acid HA. According to the hypothesis above, the following equilibria occur.

$$WHA \rightleftharpoons WA^- + H^+ \qquad K_{H'} \quad \ldots\ldots\ldots\ldots \qquad (2.2)$$

$$WA^- \rightleftharpoons W^{\pm} + A^- \qquad K_{A'} \quad \ldots\ldots\ldots\ldots \qquad (2.3)$$

$$WH^+ \rightleftharpoons W^{\pm} + H^+ \qquad K_H \quad \ldots\ldots\ldots\ldots \qquad (2.4)$$

$$WHA \rightleftharpoons WH^+ + A^- \qquad K_A \quad \ldots\ldots\ldots\ldots \qquad (2.5)$$

From which it may be shown that

$$\frac{K_H}{K_{H'}} = \frac{K_{A'}}{K_A} \qquad \ldots\ldots\ldots\ldots \qquad (2.6)$$

Where $W^{\pm}$ is assigned to the $NH_3^+ R\ COO^-$ groups

WH^+ " " $NH_3^+ R\ COOH$ "

WA^- " " $ANH_3\ RCOO^-$ "

WHA " " $ANH_3\ RCOOH$ "

Two alternate relationships may be derived from the set of equations (2.2 - 2.6).

(a) If $[WH^+] > [WA^-]$ — low anion affinity.

(b) If $[WA^-] > [WH^+]$ — high anion affinity.

(a) $$\frac{[WHA] + [WH^+]}{[WHA] + [WH^+] + [WA] + [W^\pm]} = \frac{1}{1 + \frac{K_{H'}}{a_A}\left(\frac{a_A + K_{A'}}{a_A + K_A}\right)} \quad \ldots\ldots\ldots \quad (2.7)$$

or (b)

$$\frac{[WHA] + [WA^-]}{[WHA] + [WH^+] + [WA^-] + [W^\pm]} = \frac{1}{1 + \frac{K_A}{a_A}\left(\frac{a_H + K_H}{a_H + K_{H'}}\right)} \quad \ldots\ldots\ldots \quad (2.8)$$

Terms a_A and a_H refer to the activity of anion and hydrogen ion respectively.

Since in this work, the affinity of the anions is low case (a) is used. The fraction of acid sorbed is given by equation

$$\theta = \frac{1}{1 + \frac{K_{H'}}{a_H}\left(\frac{a_A + K_{A'}}{a_H + K_A}\right)} \quad \ldots\ldots\ldots \quad (2.9)$$

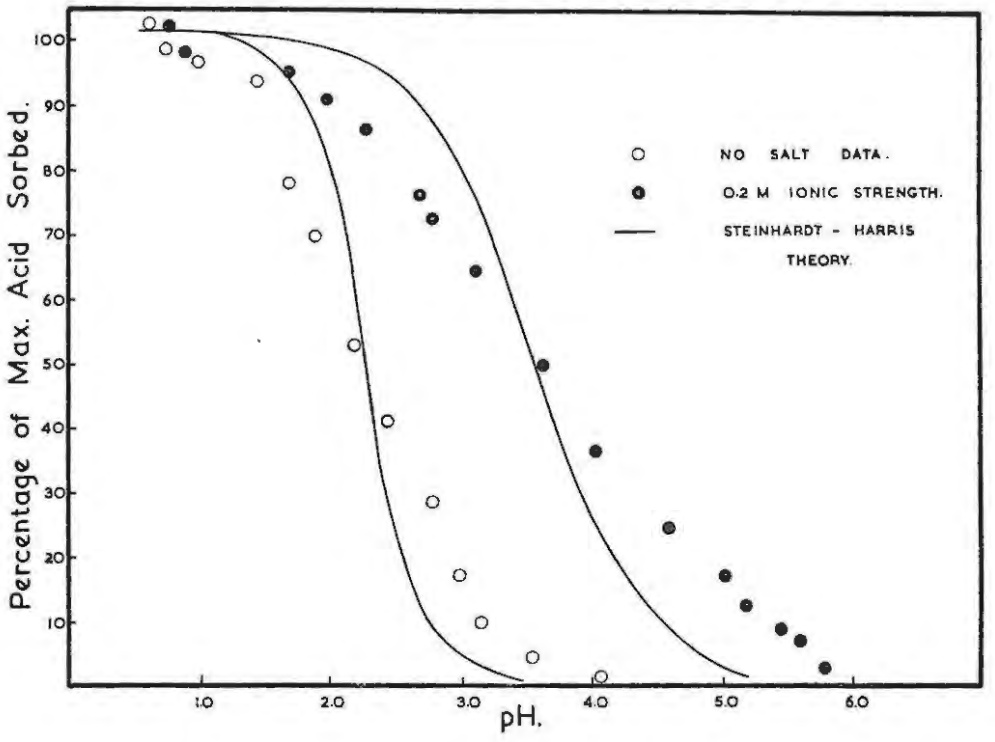
In pure acid $a_A = a_H$, also $K'_A \gg K_A$ thus

equation (2.9) reduces to

$$\theta \approx \frac{1}{1 + \frac{K_{H'} K_{A'}}{a_H^2}} \quad \ldots\ldots\ldots \quad (2.10)$$

From these expressions it follows that the titration curve resembles that of a corresponding mono-basic acid, provided that the dissociating groups have the same intrinsic dissociation constants, and do not interact electrostatically.

$\left(\frac{dpH}{d\theta}\right)_{\theta = 0.5}$ for a simple mono-basic acid is - 0.869 but the equation (2.10) gives a value of - 1.9 when values for K_H etc. are substituted. Although the theory predicts the shape of the titration curve, the theoretical and experimental curves do not coincide. In fact the fit is very poor. Fig. 10a.



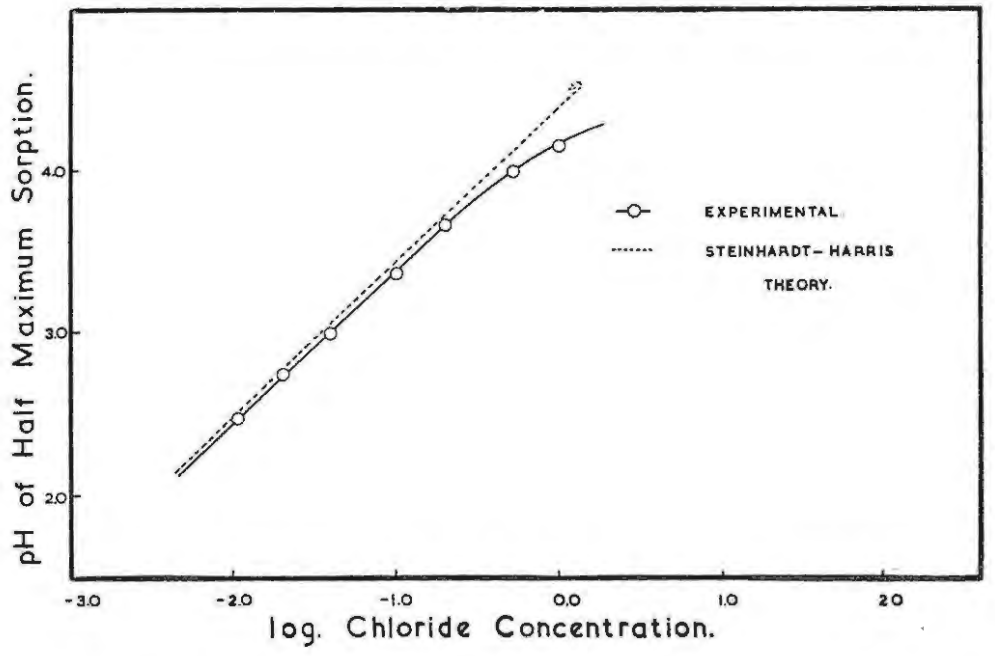


Fig. 10a (above). *Comparison of the experiemental data for acid sorption with the curves calculated from the Steinhardt-Harris theory*[8].

Fig. 10b (below). *The dependence of the pH at half-maximum sorption on the on the chloride ion concentration.*

In the presence of added salt, a_A is large and $K_{A'} \gg K_A$

hence

$$\theta \approx \frac{1}{1 + \frac{K}{a_H}} \quad \ldots\ldots\ldots\ldots \quad (2.11)$$

Where $K = K_{A'}\ K_H$

At half maximum sorption ($pH_{0.5}$ or pk) the slope should be - 1.737 whereas the experimental slope is - 3.9. To account for these differences, Steinhardt and Harris have introduced empirical terms into the equations with little theoretical justification.

The shift of titration curves with varying salt concentration is shown by the dependence of the pH at half-maximum sorption on the anion concentration.

From equation (2.9)

$$pH_{0.5} = pK_H + \log\left(\frac{a_A + K_A}{a_A + K_A}\right)$$

In the presence of salt, this reduces to

$$pH_{0.5} \approx pK_H + \log \frac{a_A}{K_A} \quad \ldots\ldots\ldots\ldots \quad (2.12)$$

The linear relationship between $pH_{0.5}$ and $\log a_A$ (actually $\log Cl^-$) is shown Fig. 10b. The deviations from the straight line are decreased by introducing the activity in place of concentration.

In considering the physical significance of the Steinhardt and Harris theory, there are three particular cases of interest.

(i) Titration with an acid having an anion which has no intrinsic affinity for the protein fibre.

(ii) Titration with an acid having an anion of high affinity.

(iii) Titration with an acid of which the anion has the same intrinsic affinity as the hydrogen ion.

In case (i) $[WA]$ is negligible, and since

$$[WHA] = \frac{[H^+]\ [WA^-]}{K'_H}$$

$[WHA]$ is also negligible. Thus $[WH^+]$ is responsible for the entire sorption, and requires all the anions to be in the adsorbed layer round the fibre. This is only energetically possible if the molecule is of the same order of size as a soluble protein molecule.

The second case is analogous to the first with the anion and hydrion reversed. In the third case all the acid is held in ion pairs, $[WHA]$, functioning as undissociated molecules. It can be shown readily by the Gilbert-Rideal Theory[11] that these cannot be associated on the fibre.

The Steinhardt-Harris treatment was developed in the 1939-40 period, and at that time it was the most advanced and thorough interpretation. It predicts a definite anion affinity for the fibre, and has found success in the explanation of sorption of various complex acids,[36] and the theory of dyeing. It has serious defects, however, mainly in that it is unable to predict the titration curve successfully. Also equation (2.12) relating to pH shift with increasing salt concentrations appears to be an unlimited relationship, whereas it is negligible for salt concentrations above 1.0 molar. These factors probably arise from unjustified assumptions which were made by the authors to simplify the mathematical treatment.

Peters and Speakman[12] have critised this theory on the basis that compounds such as Cl NH_3 R COO^- and Cl NH_3 R COOH are salts and their dissociation is not subject to the Law of Mass Action.

B. <u>The Gilbert-Rideal Theory</u>.[11]

A more successful attempt to explain the titration curves of fibrous proteins was made by Gilbert and Rideal who are credited with the first thermodynamic approach. In accordance with the salt-link theory, the number of positive and negative groups in the fibre are taken to be equal. It is further assumed that an anion is free to occupy any positive site irrespective of whether or not the positive site is adjacent to a carboxyl group that has been neutralized by a hydrogen ion. Only one anion may be sorbed on any site, and are regarded as being sorbed independently, except that their numbers must be equal to the number of protons sorbed, because the electrostatic potential developed by unequal sorption

prevents further selective sorption.

The chemical potential of hydrogen ions in solution at constant temperature and pressure is given by

$$\mu_H = \mu_H^{\circ}(T,P.) + RT \ln a_H \ldots\ldots\ldots\ldots \quad (2.13)$$

$\mu_H^{\circ}(T,P.)$ = partial molar free energy of hydrogen ions at unit activity at constant T and P.

a_H = activity of hydrogen ions in solution.

The relationship between the chemical potential of a charged substance randomly distributed among a limited number of sites, and the fraction of sites occupied, has been obtained by introducing a term F into the Fowler-Guggenheim[37] equation for sorption of uncharged molecules

$$\mu_h = \mu_h^{\circ}(T,P.) + RT \ln \frac{\theta_H}{1-\theta_H} + \psi F \ldots\ldots\ldots \quad (2.14)$$

μ_h = chemical potential of H^+ absorbed on the fibre

$\mu_h^{\theta}(T,P.)$ = " " " " " " " " when $\theta = 0.5$

ψ = electrostatical potential

F = Faraday, R = gas constant, and T temperature in degrees Absolute.

The corresponding equation for anion adsorption

$$\mu_A = \mu_A^{\circ}(T,P.) + RT \ln \frac{\theta_A}{1-\theta_A} - \psi F \ldots\ldots\ldots \quad (2.15)$$

At equilibrium $\mu_H = \mu_h$, and writing $\mu_h^{\circ} - \mu_H^{\circ}$ as $\Delta \mu_H^{\circ}$ equations (2.13 and 2.14) give

$$\ln \frac{a_H(1-\theta_H)}{\theta_H} = \Delta\mu_H^{\circ} + \psi F \ldots\ldots\ldots \quad (2.16)$$

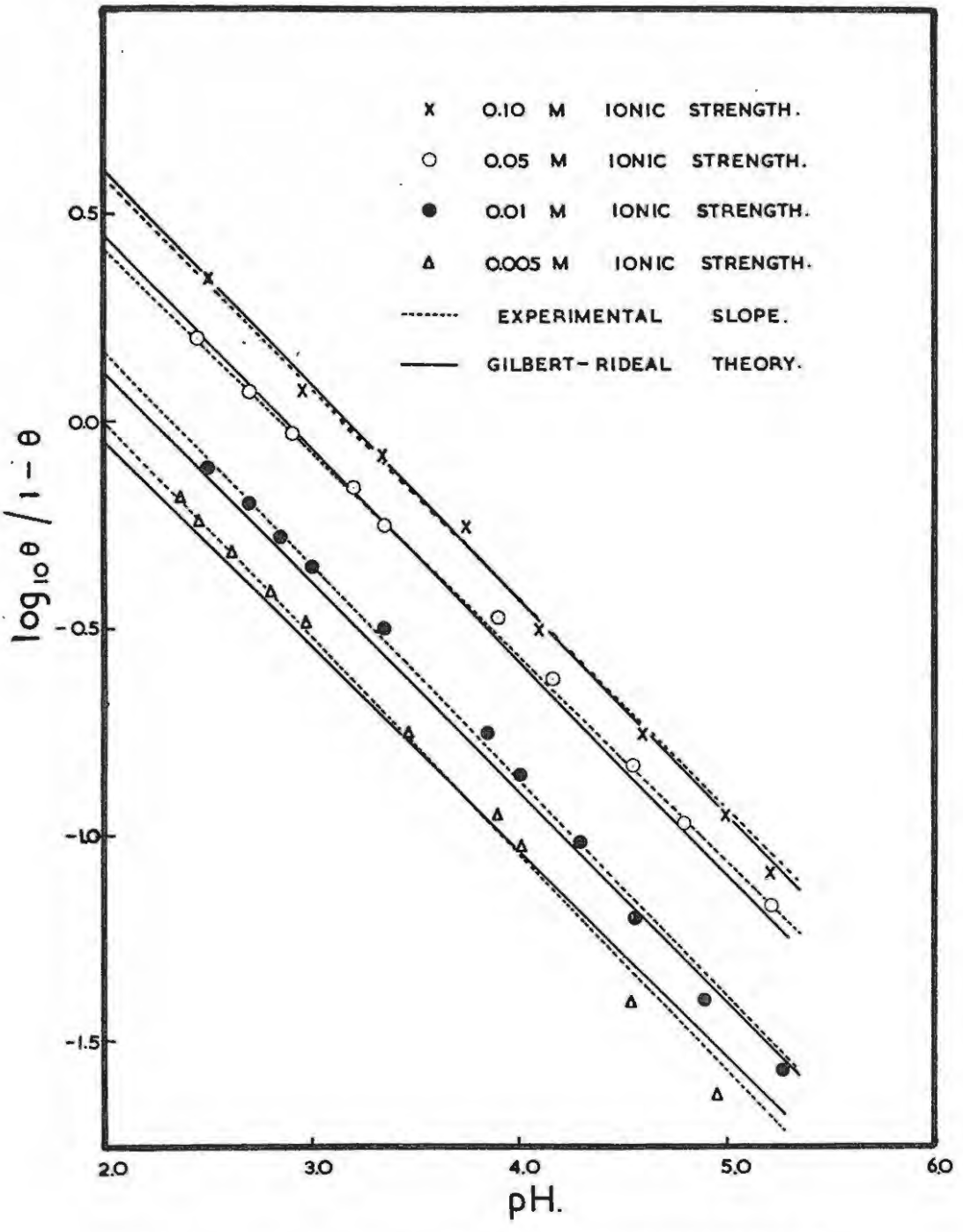


Fig. 11. *The relation between the quantity of acid combined and pH;* $\ominus$ *=fraction of maximum acid bound.*

and for anions

$$\ln \frac{a_A (1 - \theta_A)}{\theta_A} = \Delta \mu_A^o - \not{F} \text{ .....} \quad (2.17)$$

Assuming that there are the same number of acidic and basic sorption sites $\theta_H = \theta_A = \theta$ Equations (2.16) and (2.17) give

$$RT \ln \frac{a_A \; a_H (1 - \theta)^2}{\theta^2} = (\Delta \mu_H^o + \Delta \mu_A^o) \text{ .....} \quad (2.18)$$

taking antilogarithms

$$\sqrt{a_A a_H} \quad \frac{1 - \theta}{\theta} = \sqrt{K} \text{ .....} \quad (2.19)$$

where $RT \ln K = \Delta \mu_H^o + \Delta \mu_A^o$ ..... (2.19a)

The square root of the concentration terms appears in the equation without the arbitrariness of the Steinhardt-Harris theory. For titration in pure hydrochloric acid solution,

$$\log_{10} \frac{\theta}{1 - \theta} = - pH - \frac{\log e}{2 RT}\left(\Delta \mu_H^o + \Delta \mu_A^o\right) \text{ .....} \quad (2.20)$$

A plot of $\log \frac{\theta}{1 - \theta}$ (or $\log \frac{HCl_f}{HCl_s - HCl}$) from experimental values, where HCl_s = saturation sorption value and HCl_f = sorption of acid at any specific pH) should give a straight line of slope - 1 (Fig.11). Also we may calculate the value of $\log_{10} K$ from equation (2.19)

$$pH + \log \frac{\theta}{1 - \theta} = \frac{\log e}{2 RT}(\Delta \mu_H^o - \Delta \mu_{Cl}^o) \text{ .....} \quad (2.21)$$

$$= - \tfrac{1}{2} \log K$$

The mean value for - log K = 4.38

Comparison of equation (2.19a) with the equation for the standard free energy change for a reversible process.

$\Delta F^{\circ} = -RT \ln K$ (K = equilibrium const.) shows that - log K is in fact the pK value for the apparent dissociation (pK_f) of the carboxyl group in the protein fibre. This value is close to that of the dicarboxylic amino acids:-

Aspartic acid	pK_2	= 3.65
Glutamic acid	pK_2	= 4.25
Glycylaspartic acid	pK_2	= 4.45
Wool - COOH	pK	= 4.38

In the presence of added salt MA

$$RT \frac{\theta_H}{1 - \theta_H - \theta_M} = RT \ln a_H + \psi F + \Delta \mu_H^{\circ} \ldots\ldots \quad (2.22)$$

and

$$RT \frac{\theta_M}{1 - \theta_H - \theta_M} = RT \ln a_M + \psi F + \Delta \mu_M^{\circ} \ldots\ldots \quad (2.23)$$

$(\mu_H^{\circ} - \mu_M^{\circ})$ is strongly negative, and hence M may be neglected, giving

$$\log \frac{\theta_H}{1 - \theta_H} = \tfrac{1}{2} pH - \frac{\log e}{2RT} (\Delta \mu_H^{\circ} + \Delta \mu_A^{\circ}) + \tfrac{1}{2} \log a_{Cl} \quad \ldots\ldots \quad (2.24)$$

A plot of $\log \frac{\theta_H}{1-\theta_H}$ ($\log \frac{HCl_f}{HCl_s - HCl_f}$) against pH yields a straight line with slope - $\frac{1}{2}$. The theoretical and experimental values are shown in Fig. 11.

From equation (2.24) we get

$$\frac{1 - \theta}{\theta} = \sqrt{\frac{K}{a_H a_{Cl}}} \quad \ldots\ldots \quad (2.25)$$

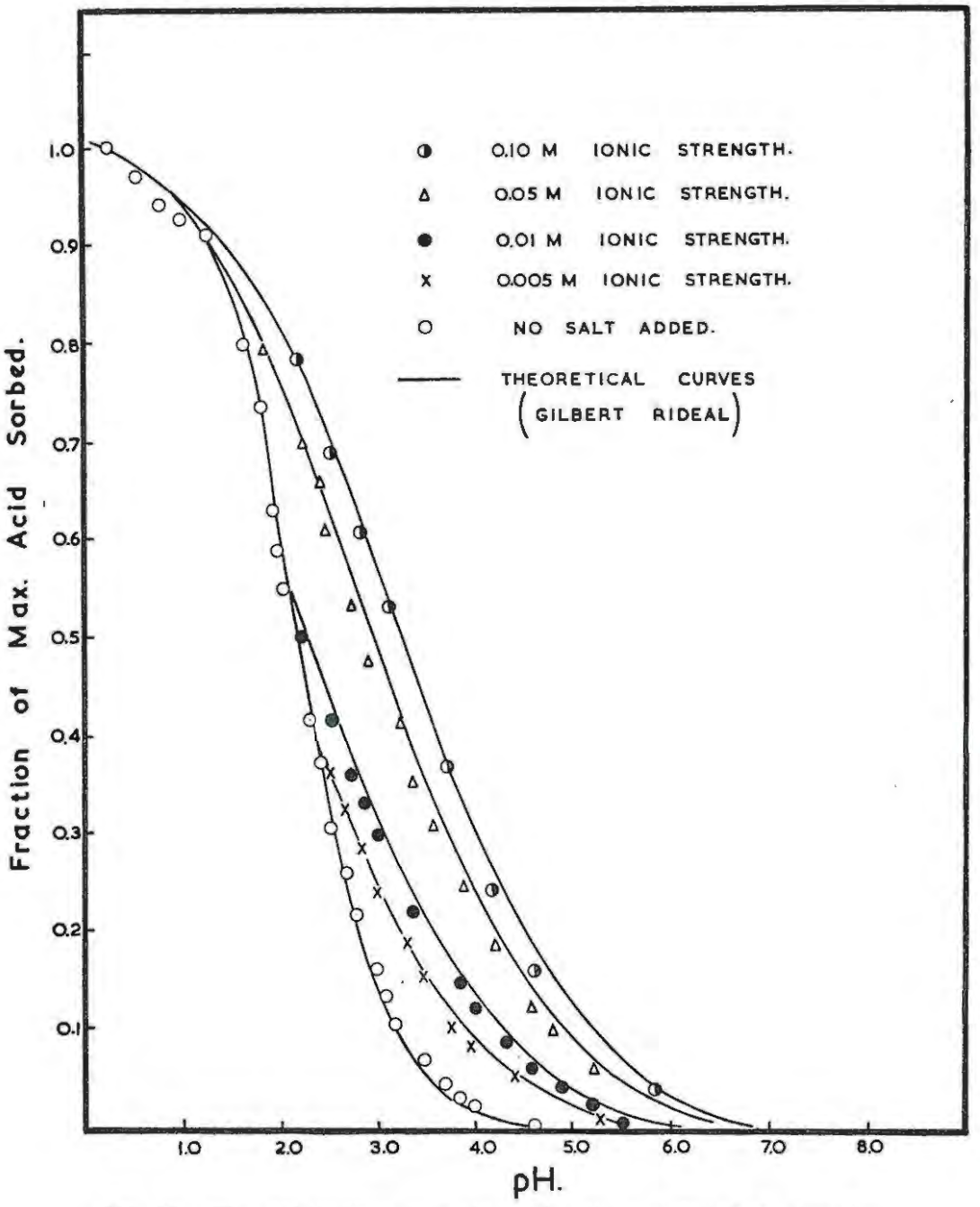


Fig. 12. *The acid titration curve of wool keratin at various ionic strengths. (Solid lines calculated from the Gilbert-Rideal theory).*

Taking the calculated value of K, Θ, pH may be derived. The solid lines in Fig. 12 are those predicted by the Gilbert-Rideal Theory, using equations (2.25).

At the point of half-maximum sorption, $\theta = 0.5$ and equation (2.24) reduces to

$$pH_{0.5} = \log a_{Cl} - \frac{\log e}{RT} (\Delta \mu^{\circ}_{H} + \Delta \mu^{\circ}_{Cl}) \quad \ldots\ldots\ldots\ldots \quad (2.26)$$

This shows the interdependence of the pH at half-maximum sorption and the activity of the chloride ion, and is similar to that deduced by Steinhardt and Harris (see page 48).

The Gilbert-Rideal Theory predicts a definite combination of anions with the positive sites in the fibre, and that a definite affinity for each acid exists (referred to as anion affinity, but strictly speaking it is the mean ion affinity.) Steinhardt et al[36] have determined the titration curve of wool using 16 different strong acids. The titration curves are spread over a wide range of pH, and the displacement of the pH of half-maximum sorption indicates the considerable difference in affinity of the various acids.

The Gilbert-Rideal Theory has been most successfully applied to dye absorption by Lemin and Vickerstaff.[38] These authors have determined the absorption of purified acid dyes under fixed conditions, and have calculated the affinity of the dye acids according to equation

$$-\frac{\Delta \mu^{\circ}_{DH}}{2.303RT} = 2 \log \frac{\theta_D}{1 - \theta_D} + pH - \log a_{HD} \ldots\ldots \quad (2.27)$$

and at half-maximum sorption

$$-\Delta \mu^{\circ}_{DH} = 2.303\ RT\ (pH_{0.5} - \log a_D) \ldots\ldots\ldots \quad (2.28)$$

where DH = dye acid, D = dye anion

The main criticism of this theory is that equation (2.26) suggests that there is no limit to the displacement of $pH_{0.5}$ as the salt concentration increases. Proponents of the Donnan Theory have criticised this approach on the ground that the Fowler-Guggenheim equation for the adsorption of molecules cannot be extended to adsorption of ions merely by the addition of the electrostatic term ψ F.

C. The Donnan Theory.

The combination with acids, and the amount of swelling of gelatin has been successfully explained by Procter and Wilson[39] in terms of the Donnan membrane equilibrium. Subsequently there have been many attempts to extend the treatment to wool and silk, namely Steinhardt et al[8] and Elöd and Silva[40]. These authors made several serious errors, and were very vague as to the phase boundaries, i.e. internal and external. The main difficulties in the use of the Donnan Theory to obtain a quantitative interpretation of acid binding of wool are in assessing the volumes and concentrations within the fibres itself, and in distinguishing between acid bound and acid merely dissolved in the internal solution. The volume of free water is particularly difficult to measure, and no method of pH determination is known.

This obstacle has been overcome finally by Morton and Peters[41] who have devised a method of calculating the internal pH. A simplified derivation of the theory as applied to acid sorption is presented below; a more rigorous treatment is given by Peters and Speakman.[12]

According to the Fowler-Guggenheim equation, the electrochemical potential of hydrogen ion in solution (external phase) is given by

$$\mu_H = \mu^o_H(T.) + RT \ln a_H + P\bar{V}_H \ldots\ldots\ldots\ldots \quad (2.29)$$

In order to simplify the calculation, assume that the pressure P is the same in both phases, and that V_H (partial molar volume of hydrogen ions) also is unchanged.

In the internal phase,

$$\mu_h = \mu^o_h(T,P.) + RT \ln a_h + P\bar{V}_H + \not{\psi} F \ldots\ldots\ldots\ldots \quad (2.30)$$

At equilibrium $\mu_H = \mu_h$, and since the equations differ only in the electrostatic term $\mu^o_H(T.) = \mu^o_h(T)$.

Adding equations (2.29) and (2.30)

$$RT \ln \frac{a_H}{a_h} = \psi F \quad \ldots\ldots\ldots\ldots \qquad (2.31)$$

and similarly for anions

$$RT \ln \frac{a_X}{a_x} = -\psi F \quad \ldots\ldots\ldots\ldots \qquad (2.32)$$

Adding equations (2.31) and (2.32)

$$a_H \; a_X = a_h \; a_x \quad \ldots\ldots\ldots\ldots \qquad (2.33)$$

or $$\frac{a_H}{a_h} = \frac{a_x}{a_X}$$

The conditions of the system are illustrated by the following diagram.

Internal Phase		External Phase	
-COOH	$A\theta$		
-COO^-	$A(1-\theta)$	$[H^+]$ =	H
-NH_3^+	A	$[X^-]$ =	X
$[H^+]$ =	h	Volume =	V
$[X^-]$ =	x		
Volume =	v		

Where A = total number of acidic or basic groups

θ = fraction of carboxyl groups which have combined with a proton

(i) refers to the concentration of the ionic species i. The amount of acid combined is usually measured by subtracting the residual acid from the amount originally present.

$$b = v(A\theta + h) + VH - (V + v)H$$
$$= v(A\theta + h - H) \quad \ldots\ldots\ldots\ldots \quad (2.34)$$

For the condition of electrical neutrality

$$x = A\theta + h = (\frac{b}{v} + H) \quad \ldots\ldots\ldots\ldots \quad (2.35)$$

From equation (2.33)

$$\frac{a_x}{a_X} = \frac{a_H}{a_h} \approx \frac{[x]}{[X]}$$

Taking logarithms

$$pH_{int.} - \log x = pH_{ext.} - \log X \quad \ldots\ldots\ldots\ldots \quad (2.36)$$

$\log x = \log(\frac{b}{v} + H)$ and $H = X$ in the absence of salts.

$$pH_{int.} = 2pH_{ext.} + \log \frac{b}{v} \quad \ldots\ldots\ldots\ldots \quad (2.38)$$

Using this procedure, the 'internal' titration curve was constructed (Fig. 13.) and was shown to be similar to a soluble protein, pK 4.3. In the presence of neutral salts (MX) $[H^+] \neq [X^-]$ equation (2.36) becomes

$$pH_{int.} \approx pH_{ext.} - \log_{10} X + \log_{10} (\frac{b}{v} + H) \ldots \quad (2.39)$$

Using this equation, the experimental results are found to fall in the same internal titration curve as those for pure acid. In addition the pH at half-maximum sorption is shown to be dependent on the salt concentration in the following manner:

$$pH_{0.5} \approx \log_{10} X + \text{const} \quad \ldots\ldots\ldots\ldots \quad (2.40)$$

$pH_{ext.} \approx pH_{int.}$ When $\log (\frac{b}{v} + H) = \log H = \log X$

i.e. when $H \gg b$, as in dilute solutions

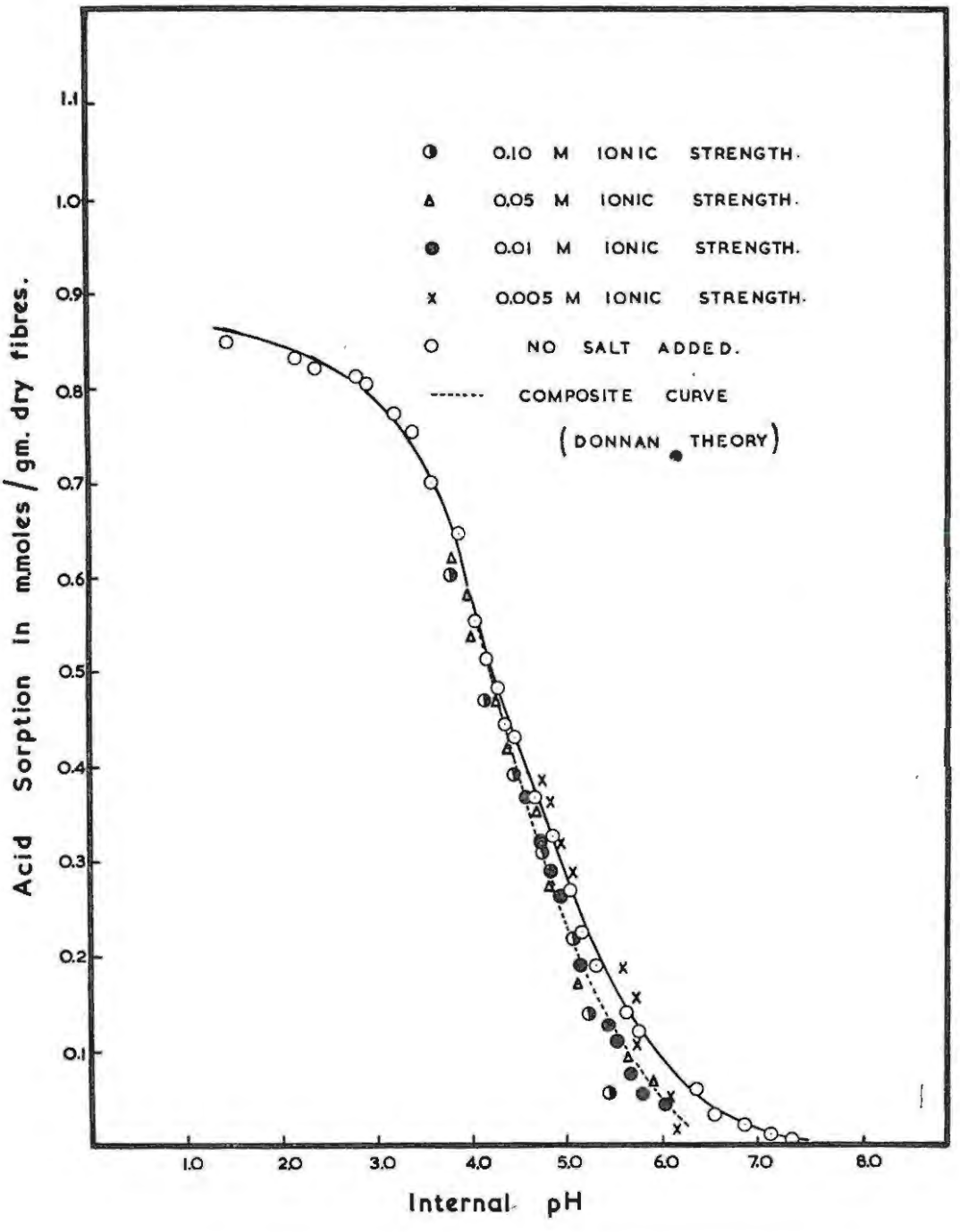


Fig. 13. *The "internal" titration curve of wool keratin calculated from the Donnan membrane theory. The dotted line is the composite curve.*

This membrane treatment stresses the importance of the internal solution, and precludes the possibility of direct anion combination. Although titration data with simple acids is successfully explained, the difficulty of interpreting the effects of an acid with high affinity is the main disadvantage.

D. Alexander and Kitchener Theory[42]

These authors regard the distribution of acid between the solution and fibre phase as governed by the law of mass action:

$$H_s^+ + X_s^- \rightleftharpoons H_f^+ + X_f^- \quad \ldots\ldots\ldots\ldots \qquad (2.41)$$

and $$K \quad \frac{(a_H \cdot a_X)_f}{(a_H \cdot a_X)_s} = \frac{a_f}{a_s} \approx \frac{a_f}{[H^+]_s \; [X^-]_s} \quad \ldots\ldots\ldots\ldots \qquad (2.42)$$

The activity of a Langmuir adsorbate is $\frac{\theta}{1-\theta}$ where θ is the number of sites occupied:

$$\left(\frac{\theta_H}{1-\theta_H}\right)\left(\frac{\theta_X}{1-\theta_X}\right) = a_f = a_s K = K(a_{H^+} \; a_{X^-})$$

$$\log \frac{\theta}{1-\theta} = \tfrac{1}{2} \log_{10} K - \tfrac{1}{2} pH + \tfrac{1}{2} \log a_{X^-} \quad \ldots\ldots\ldots\ldots \qquad (2.43)$$

The authors assume that

$$- \log_{10} a_{HCl} = pH - \log a_{Cl^-} \quad \ldots\ldots\ldots\ldots \qquad (2.44)$$

Although this theory leads to the same conclusion as the Gilbert-Rideal treatment, the authors claim it is a new approach. The only real difference is in the derivation of the equations (Gilbert-Rideal is a more general approach).

La Fleur[25] has made an attempt to interpret the shape and position of the titration curve on the basis of the amino acid composition. Using pK_a values for soluble peptides this author has constructed titration curves for each individual amino acid. Giving each curve its own statistical weight proportional to its % in the protein, a summation

curve was calculated. Comparison with experimental results showed poor agreement. From the arguments presented in Section I pages 9 to 12. it will be seen that La Fleur has made the erroneous assumption that a complex polypeptide such as wool may be regarded as a mixture of its constituent amino acids, simply by choosing pK values from soluble peptides instead of their amino acids. The author has completely forgotten that wool is not a soluble peptide, and as such the shape of the titration curve is not governed entirely by the ionization constants of the constituent amino acids.

Of the theories which have been expounded, the Gilbert-Rideal and the Donnan Theories are superior by far. Accurate measurements of the acid sorption of wool in hydrochloric-sulphuric acid mixtures have been conducted by Olofsson[43] who claims that the Gilbert-Rideal theory is absolutely satisfactory. On the other hand, Peters[44] has analysed the same data according to the Donnan Theory. Consequently a considerable controversy has arisen. Perhaps the mistake these authors have made is to test the veracity of a particular theory by the accuracy with which the theoretical values approach those obtained from experiment. It is not logical to condemn a theory which gives 10% agreement.in favour of one which has, say 7% agreement. On both sides, the authors have chosen the points which their theory confirms, and overlooked to some extent the facts (experimental) which are opposed to it. The ultimate success of any theory of acid or alkali combination depends on whether it can explain all the processes involved, i.e. proton combination and dissociation, swelling, dye absorption, etc.

An interesting case is the treatment of Harrison[45] who considers that the diffusible acid (HCl in this case) competes with the carboxyl groups of the wool (indiffusible acid) in the neutralisation of the basic groups.

$$\begin{array}{l} \vdash NH_2 \\ \quad\quad + HA \longrightarrow \\ \vdash COOH \end{array} \qquad \begin{array}{ll} \vdash NH_3^+ \; A^- & Bz \\ \vdash COOH & \\ \vdash NH_3^+ & \\ \vdash COOH & B(1-z) \end{array}$$

B = Total number of basic groups.

z = fraction of basic groups combined with diffusible acid HA.

1 - z = fraction of basic groups combined with indiffusible acid in the form of salt-linkages.

C_1 = Equilibrium concentration of HA; dissociation constant K_1

C_2 = " " " - COOH " " K_2

From which Harrison obtains

$$z = - \frac{[H^+]\,[A^-] + \sqrt{[H^+]^2\,[A^-]^2 + 4k\,[H^+]\,[A^-]}}{2k} \quad \ldots\ldots\ldots (2.45)$$

The value of k is obtained from the mid point of the titration curve, and on substitution in equation (2.45) gives reasonable agreement between experimental and calculated values of z.

A re-evaluation of this treatment indicates that it has several errors. Consider the concentrations at equilibrium:

$$[HA] = C_1$$

$$[-COOH] = C_2 = Bz$$

$$[A^-] = Bz + C_1K_1$$

$$[-COO^-] = B(1 - z)$$

Harrison has made $[A^-] = Bz$, neglecting the amount which has dissociated from the acid HA at equilibrium. This is particularly serious as HA is a strong acid in the above case.

From the dissociation of the acids we get that

$$\frac{K_1}{K_2} = \frac{[A^-]\,[-COOH]}{[HA]\,[-COO^-]} = \frac{[A^-]}{C_1} \cdot \frac{z}{1-z}$$

but $K_1 C_1 = [H^+][A^-]$

and hence $\frac{[H^+]}{K_2} = \frac{z}{1-z}$

taking logarithms

$$\log \frac{z}{1-z} = -pH + \log K_2 \quad \ldots\ldots\ldots\ldots \qquad (2.46)$$

This is identical in form with equation (2.20) of the Gilbert-Rideal Theory.

Apart from the errors in derivation, the basic assumptions of forward titration of amino groups is not consistent with the Bjerrum concept, although this does not affect the subsequent calculations. Thus it may be seen that although a mathematical expression may coincide with a set of measurements made under specific conditions, it does not necessarily follow that the whole theory is valid.

SECTION III : ALKALI SORPTION.

8. REACTIONS OF THE DISULPHIDE GROUP IN ALKALINE SOLUTION.

Whereas the acid sorption of the keratin group has been investigated fairly thoroughly, measurements on the alkali sorption are relatively few. There are several reasons for this apparent neglect. Firstly, because wool keratin which is stable in acid solution, is extremely sensitive to alkali especially at higher concentrations. Secondly, the accurate measurement of pH in alkaline solutions has in the past presented greater difficulty. With these points in mind it will be seen that measurements of alkali sorption are not as accurate as those conducted in acidic solutions. Authors requiring accurate measurements to verify their hypothetical concepts, have thus investigated acid sorption in preference.

In 1934 Speakman and Stott[6] measured the alkali binding of wool in their classical investigation of salt-linkages. These authors disregarded the degradation of the keratin, and consequently their results are of little interest, except historical.

Harris[46] discovered in 1935 that alkali solutions dilute as 0.1N were capable of destroying as much as 50% of the cystine (disulphide) content in wool. Harris and Rutherford[47] found that alkali was consumed in this process, and realized that alkali sorption measurements should be corrected for this reaction. Using the equation originated by Schöberl,[48] they proposed a correction on the following basis.

$$\rangle CH\text{-}CH_2\text{-}S\text{-}S\text{-}CH_2\text{-}CH \langle \xrightarrow{2\ NaOH} \rangle CH\text{-}CH_2\ SNa + NaOS\text{-}CH_2CH \langle \quad \ldots\ldots\ldots \quad (3.1a)$$

$$\rangle CH\text{-}CH_2\text{-}SONa \longrightarrow \rangle CH\text{-}CHO + NaHS \quad \ldots\ldots\ldots \quad (3.1b)$$

Both the sulphydryl and the sulphenic compounds are acidic, and 2 moles of alkali are removed as the result of the cleavage of 1 mole of cystine. According to equation (3.1b), however, the sulphenic compound decomposes further to give an aldehyde and NaHS. According to this mechanism, the amount of inorganic sulphur is equivalent to the amount of alkali consumed by the reaction. The inorganic sulphur was determined as sulphate by

bromine oxidation. Experimental studies on alkali-treated wools revealed that the loss of cystine sulphur was equivalent to the 'inorganic sulphur' present in the solution. Furthermore, corrections to alkali binding calculated on equations (3.1a) and (3.1b) gave fairly constant results.

At higher pH values appreciable amounts of protein were found to dissolve. Harris and Rutherford corrected for the loss in weight of the wool sample (soluble protein being back-titrated in the determination of alkali sorbed.) This correction assumed that the protein in solution is 'soluble wool', and not fragments of polypeptide chains which have been hydrolysed.

Steinhardt and Harris[8] re-investigated the disulphide correction more thoroughly, and introduced the following modification.

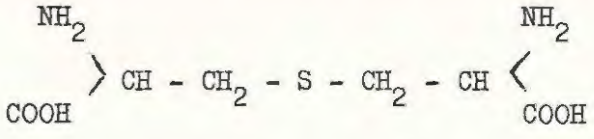
"Comparisons of the ratio of dissolved nitrogen, after kjeldahl digestion, to dissolved inorganic sulphur with the same ratio in the intact protein indicated that a small fraction (usually from 1/10th to 1/5th) of the inorganic sulphide was derived from the wool that had dissolved. Therefore, only the remaining 8/10th - 9/10th of the inorganic sulphur in solution was produced by hydrolysis of the undissolved wool."
This modified correction gives better results especially for long periods of alkaline reaction at higher pH, when the amount of wool dissolving is appreciable. Whether this correction is justifiable is uncertain. Harris, Rutherford and Steinhardt assume that the loss in weight of wool is directly proportional to the amount of protein nitrogen in solution. However this may not necessarily be the case as in acid hydrolysis it has been shown that aspartic acid splits out preferentially.[84]

Elöd and Fröhlich[49] have measured the alkali sorption of wool at low temperatures (0° to $-5^{\circ}C$) in order to suppress the disulphide hydrolysis. These authors have neglected this factor, which is not very serious at such low temperature. However, their value of 1.05 millimoles NaOH/gm. wool is considered to be rather low for the maximum alkali sorption.

There are, however, certain points which cannot be satisfactorily explained in terms of equations (3.1a) and (3.1b). No increase either in -SH or aldehyde groups could be detected in the hydrolysate of alkali-treated wools by Mizell & Harris.[50] The possibility of re-combination between sulphydryl and aldehyde during hydrolysis has been ruled out by Steinhardt et al,[51] who found that few sulphydryl groups exist in alkali-treated wools. Schöberl[52], however, has been able to detect an increase in -SH groups in the alkaline degradation of cystine, and certain cystine derivatives, but these could not be detected by Cuthbertson & Phillips[56] in alkali treated wool. Thus the Rutherford Harris correction, although giving adequate results, is not accurate in its basic concepts.

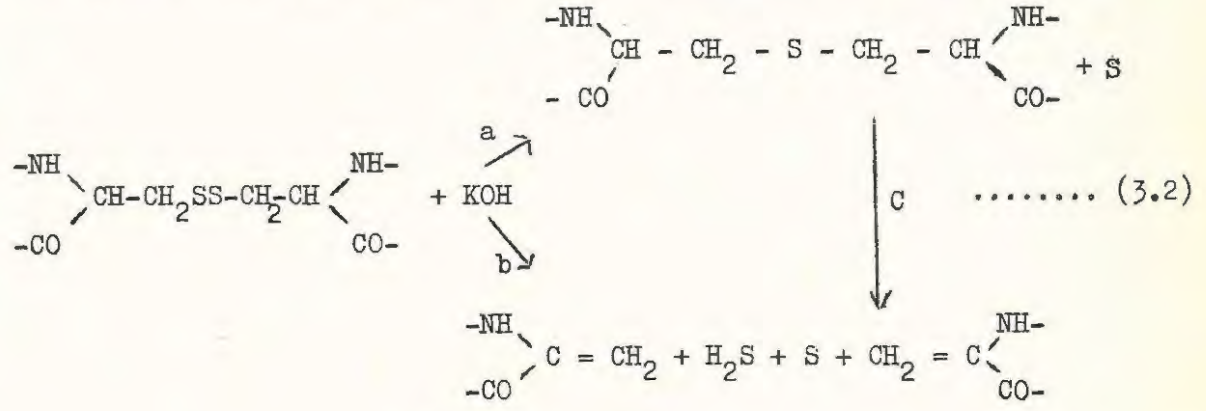
According to reaction (3.1a) and (3.1b) complete rupture of the disulphide bonds occurs during alkaline reaction. The loss of these valuable cross-links should greatly reduce the tensile strength of the keratin fibre, and also greatly increase the solubility in alkaline solutions. This is not the case, as the properties of alkali-treated fibres are such as to suggest that if the disulphide cross-links are destroyed, they are, in a measure, replaced by a new linkage. Speakman[53] suggested that during alkali treatment of wool the -S-S— bonds are converted into stable linkages of the form -C-S-NH-. This cross-linkage and the -CH-N- bond proposed by Phillips[54] are capable of explaining certain aspects of the physical behaviour (permanent set and supercontraction) of the wool fibre. No chemical evidence has been found to substantiate these hypotheses.

In 1941 Horn, Jones and Ringel[55] isolated from the hydrolysate of alkali-treated wool, a new amino-acid, which was named <u>lanthionine</u>:



From the formula for lanthionine it may be seen that the alkali reaction has removed 1 atom of sulphur from the cystine molecule. These stable thioether linkages are responsible for the tensile properties and resistance to solubilisation.

The exact reaction mechanism is complex, and Cuthbertson and Phillips[56] have shown that two reactions occur simultaneously.

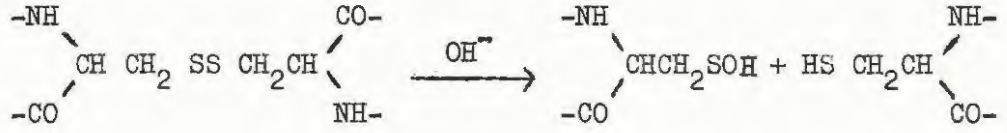


amino acrylic acid
(Dehydro alanine)

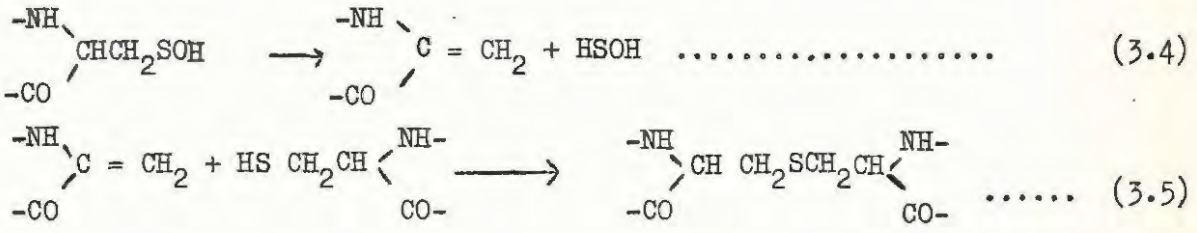
The principal mode of decomposition is given by equation (3.2a). It is unknown as yet whether the dehydroalanine is formed from lanthionine (3.2c) or directly from cystine itself (3.2b). Blackburn and Lee[57] have analysed alkali-treated wool for changes in amino acid composition using quantitative chromatographic procedures. Approximately 1 mole of lanthionine is formed for every mole of cystine destroyed, but the loss of cystine appears to be slightly higher than the amount of lanthionine formed. Whether this is caused by inaccuracies of cystine analyses, or by the influence of reaction (3.2c) is uncertain.

Reaction (3.2b) leads to the formation of α - amino acrylic acid, which on acid hydrolysis gives pyruvic acid. An increase in pyruvic acid content has been observed for alkali-treated wool. On the other hand this may have resulted from the alkaline degradation of the amino acid serine, which is decreased in this process.

Cuthbertson and Phillips[56] have concluded that the most likely reaction mechanism is



...(3.3)



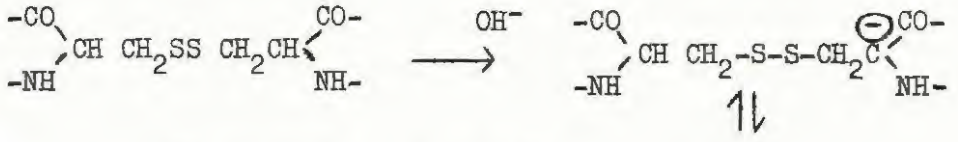
(3.4)

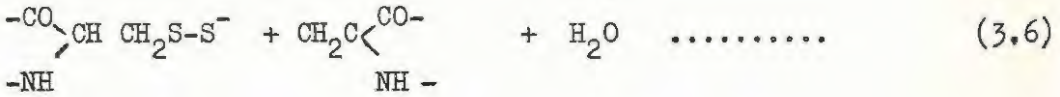
(3.5)

The HSOH (hypothetical dihydrogen sulphoxide) may either give more complex inorganic sulphur compounds or decompose to sulphur and water.

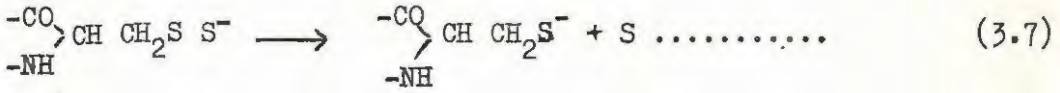
Schöberl and Wagner,[58] using chromatographic techniques, have shown that the alkaline fission of cystine and cysteine yielded alanine and lanthionine, the latter being formed as a result of addition of a cysteine and a dehydroalanine residue as in equation (3.5).

Swan[59] has synthesized cystine-containing peptides and several other cystine derivatives, in order to verify the postulates of Nicolet and Shinn[60] as regards the mechanism of the disulphide reaction.

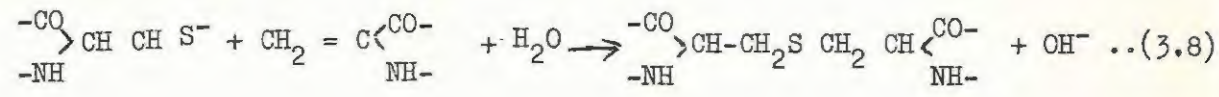




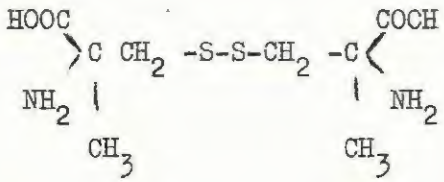
(3.6)



(3.7)



(3.6) is a bimolecular β - elimination reaction, and can only occur when the disulphide in question has an ionizable hydrogen on a carbon β to one or other of the sulphur atoms. To test this hypothesis, Swan[61] employed the synthetic amino acid



α - α' dimethyl cystine.

If direct hydrolysis of the disulphide bond occurs (Schöberl, Cuthbertson and Phillips) then this compound should be as labile to alkali as cystine. However, if the cystine degradation is initiated by ionization of the amino acid hydrogen atom, followed by β - elimination to an unstable disulphide ion, then the new amino acid would be very much more stable to alkali than cystine. An appreciable increase in stability was observed. The amino acid remained unchanged after 3 hrs. boiling in 0.25 m NaOH. Moreover its reactions with disulphide-splitting agents such as $NaHSO_3$, NaCN, and its behaviour at the dropping-mercury electrode were indistinguishable from cystine. Consequently the increase in alkali stability cannot be attributed to steric effects of the methyl group.

This reaction mechanism, equations (3.6 - 3.8) appears to have the widest applicability, and does not have any shortcomings.

Except for postulates of Rutherford and Harris, none of the reaction mechanisms account for the removal of alkali from the solution by the disulphide reaction. Harris and Mizell[50] have investigated the reaction between elemental sulphur and sodium hydroxide, finding that if the sulphur could be dissolved (alcohol added) it reacted mole for mole with the

sodium hydroxide. Since the sulphur eliminated from the cystine molecule is in the atomic form, it is readily soluble in sodium hydroxide solutions. This reaction has been investigated more carefully in section II, p.75.

Apart from the pioneering work of Rutherford and Harris, and by Elöd and Fröhlich[49] the only other measurements of alkali sorption of wool are by Horner[77] in 1954 who has made accurate determinations at 25°C using sodium and potassium hydroxides. This author has adopted the correction procedure of Rutherford and Harris. Thus despite the tremendous advances in the field of disulphide chemistry over the past decade, the corrections applied to alkali sorption measurement, though they may be fortuitously of the right order of magnitude, are calculated on the basis of an incorrect reaction. Hence it is necessary to repeat the work on alkali sorption using more recent and accurate knowledge. The photochemical degradation of wool caused by exposure to sunlight and atmospheric conditions has long been known to result in a decrease in sulphur content of the fibres. von Bergen[62] noted the surface damage caused by exposure of the tip portion of the wool staple, and the subsequent loss in tensile properties and the unlevel dyeing which resulted. Kertesz[63] observed the loss in sulphur and concluded that it was oxidized to sulphuric acid. Speakman[53] however, was first to recognise that the loss of sulphur could be attributed to the photochemical destruction of the disulphide groups, and proposed the following reaction mechanism:

$$\begin{matrix}-CO\\-NH\end{matrix}\rangle CH\ CH_2 : S\text{-}S\text{-}CH_2\text{-}CH\langle\begin{matrix}NH-\\CO-\end{matrix} \xrightarrow[H_2O]{} \begin{matrix}-CO\\-NH\end{matrix}\rangle CH\ CH_2SH + HOS\text{-}CH_2CH\langle\begin{matrix}NH-\\CO-\end{matrix} \quad \ldots(3.9)$$

$$\begin{matrix}-NH\\-CO\end{matrix}\rangle CH\ CH_2\ SOH \longrightarrow \begin{matrix}-NH\\-CO\end{matrix}\rangle CH\ CHO + H_2S \quad \ldots\ldots\ldots \quad (3.10)$$

This is identical to equation (3.1) which was originally proposed for the alkaline degradation of wool. Race et al[64] have provided evidence showing the increase in both aldehyde and thiol groups. The latter analyses

have been confirmed by the findings of Zahn and Traumann[65] that tip wool contains more thiol groups than root wool.

As weathering involves the interaction of sunlight and water, the processes are fundamentally the same as with ultra-violet light under various conditions. Rutherford and Harris[66] have confirmed the evolution of H_2S using U.V. light, but found that the H_2S did not account for the loss of disulphide sulphur. Barritt and King[67] found that by washing exposed wools in dilute alkali, a marked decrease in the total sulphur resulted. This may indicate the formation of an alkali soluble sulphur compound on the fibre.

Recently cysteic acid $\begin{matrix}NH_2\\ \\ CO_2H\end{matrix}\!\!>\!CH - CH_2\ SO_3\ H$ has been identified in the hydrolysate of tip wool[68] which suggests that the process is more complex than postulated by Speakman. However, if new acidic or basic groups are formed as a result of photochemical attack, these will be revealed by shifts in the titration curves of the modified keratin.

9. EXPERIMENTAL PROCEDURE.

A. Materials.

(i) Wool and mohair samplesas purified in Section II.

(ii) Potassium Hydroxide.

A.R. grade potassium hydroxide sticks, washed free of carbonate immediately before use, were used in the preparation of stock solutions, which were stored in polythene containers. All solutions were made up with freshly boiled distilled water (pH 6.0 - 6.5) to eliminate CO_2. Stock solutions were checked regularly for carbonate using barium chloride. Dilute alkali solutions were checked for carbonate by comparison of pH and alkali concentration, taking into account the activity coefficients.

(iii) Potassium Chloride.

A.R. grade potassium chloride was used for varying the ionic strength of solutions. After drying for 6 hours at 105°C, stock KCl solutions were prepared with CO_2-free distilled water.

B. Equilibration Methods.

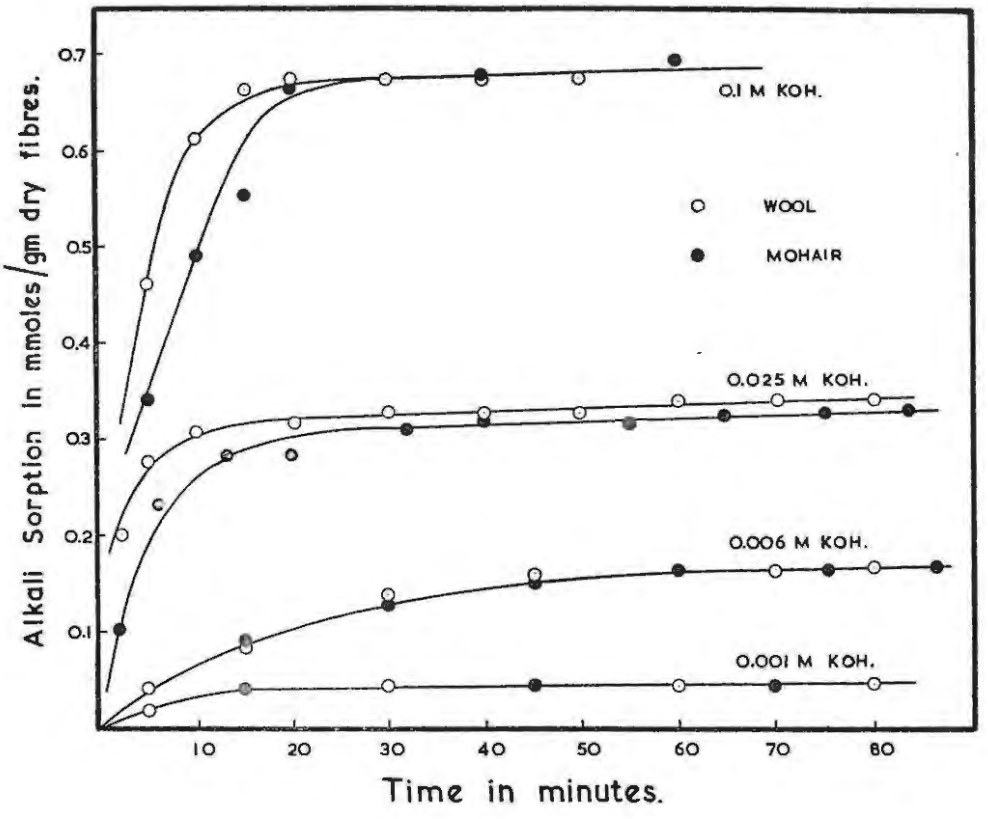
To establish the equilibrium two methods were employed :-

(i) pumping method (as in Section II - high mechanical action rate.) and (ii) soaking (slow diffusion process.)

(i) Pumping Technique.

The apparatus was similar to that employed in Section II but precautions were taken to exclude CO_2 from the pumping chamber. The glass tube was tightly stoppered, and the plunger allowed to travel through a close-fitting brass collar held firmly in the rubber stopper. A stream of purified nitrogen was allowed to bubble into the alkaline solution continuously.

Nitrogen was purified by passing through acid and alkali permanganate, and a U - tube containing 'Carbosorb' to remove CO_2. Thereafter it was saturated in a bubbler immersed in the thermostat-bath. The bubbler contained a potassium hydroxide solution of approximately the same strength as the solution in the pumping vessel.



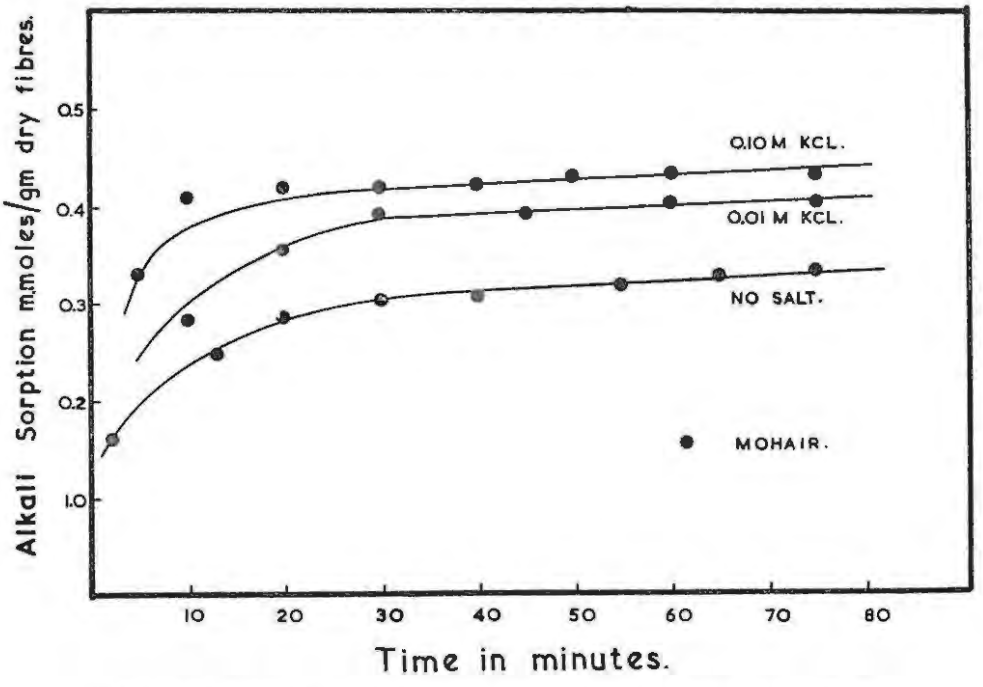


Fig. 14a (above). *The sorption rate of KOH by wool and mohair at 25°C, using the pumping apparatus.*

Fig. 14b (below). *The sorption rate of KOH in the presence of added electrolyte.*

1 gm. of conditioned fibres was weighed and carefully transferred to the ebonite plunger, and lowered into the tube containing 50.0 mls. of solution (without fibres coming into contact with the solution). The tube was then clamped in position in the thermostat-bath and allowed to equilibrate for 15 minutes, with a slow rate of nitrogen bubbling through the system. Pumping was commenced and nitrogen rate increased.

Prior to the determination of alkali sorption, the sorption rate at various alkali concentrations was determined. The method employed is described in Section II, page 20 and is essentially the same. From this data Fig. 14 has been constructed. In order to ensure the attainment of equilibrium all pumping experiments were allowed to proceed for 15 minutes longer than that prescribed by the sorption rate curves.

(ii) Soaking Method.

Unfortunately at high pH (above pH 13) the fibres suffer severe damage, which causes rapid and intense felting of the sample. The fibrous plunger becomes an impervious plug, and equilibrium is not rapidly attained. In this region the method of soaking [6,8] was used, limiting the period of exposure to 2 - 4 hours. 1 g. conditioned fibres was placed in a 100 ml. conical flask containing 50.0 mls. of solution. The flask was stoppered, shaken vigorously and placed in the water bath at 25°C. The flask was periodically shaken to hasten the reaction. When the sorption was complete, the flask was removed, and the contents filtered through a coarse filter (Whatman No. 4) to remove small fragments of fibres.

C. Analytical.

(i) Titration Procedure.

Potassium hydroxide solutions were standardized by titrating a 10 ml. aliquot against standard hydrochloric acid (of approximately the same normality) using bromocresol purple indicator. Hydrochloric acid was in turn standardized with re-crystallized borax as in Section II. HCl standard solutions of less than 0.03 m were prepared by dilution of standard 0.10 m. After the sorption process, a 10 ml. aliquot of

alkali solution was titrated with standard acid. A 10 ml. microburette was used for all titrations.

The amount of alkali removed from solution, β, is given by

$$\beta = 50(C_1 - C_2) \text{ millimoles} \quad \ldots\ldots\ldots\ldots \quad (3.11)$$

where C_1 = initial conc. of KOH (moles/l)

C_2 = final concentration of KOH (moles/l)

In experiments where the final pH was between 7 to 9.0, the apparent base-binding could be calculated from the change in pH's.

(ii) pH Measurement.

Previous authors have avoided investigations of titration curves in concentrated alkali solutions, because of the difficulties involved in accurate pH determination. In regions of high pH (or at high ionic strength) the glass membrane and the solution have a common cation, and sensitivity to the hydroxyl ion is subsequently reduced. Approximate corrections have been devised to allow for these errors, but are not highly satisfactory. These difficulties are partially eliminated by the use of an alkali-resistant glass electrode (Beckmann No. 290-75) in conjunction with a saturated calomel electrode. The assembly was calibrated with 0.05 molar borax buffer, the pH being given by the formula for temperature correction:

$$pH = 9.27 - 0.0085\ (t - 15) \qquad t = {}^{\circ}C$$

Readings weremade with a model D Beckmann pH meter at room temperature. The electrodes were flushed several times with fresh solution before the reading was taken.

Corrections at high pH readings were made by determining the pH values of a series of standard, carbonate-free potassium hydroxide solutions (with varying salt concentration). Observed and calculated pH values could then be compared. Activity coefficient data was obtained from MacInnes,[69] Harned and Cook[70] and where not available, were calculated from the data of Kieland.[71]

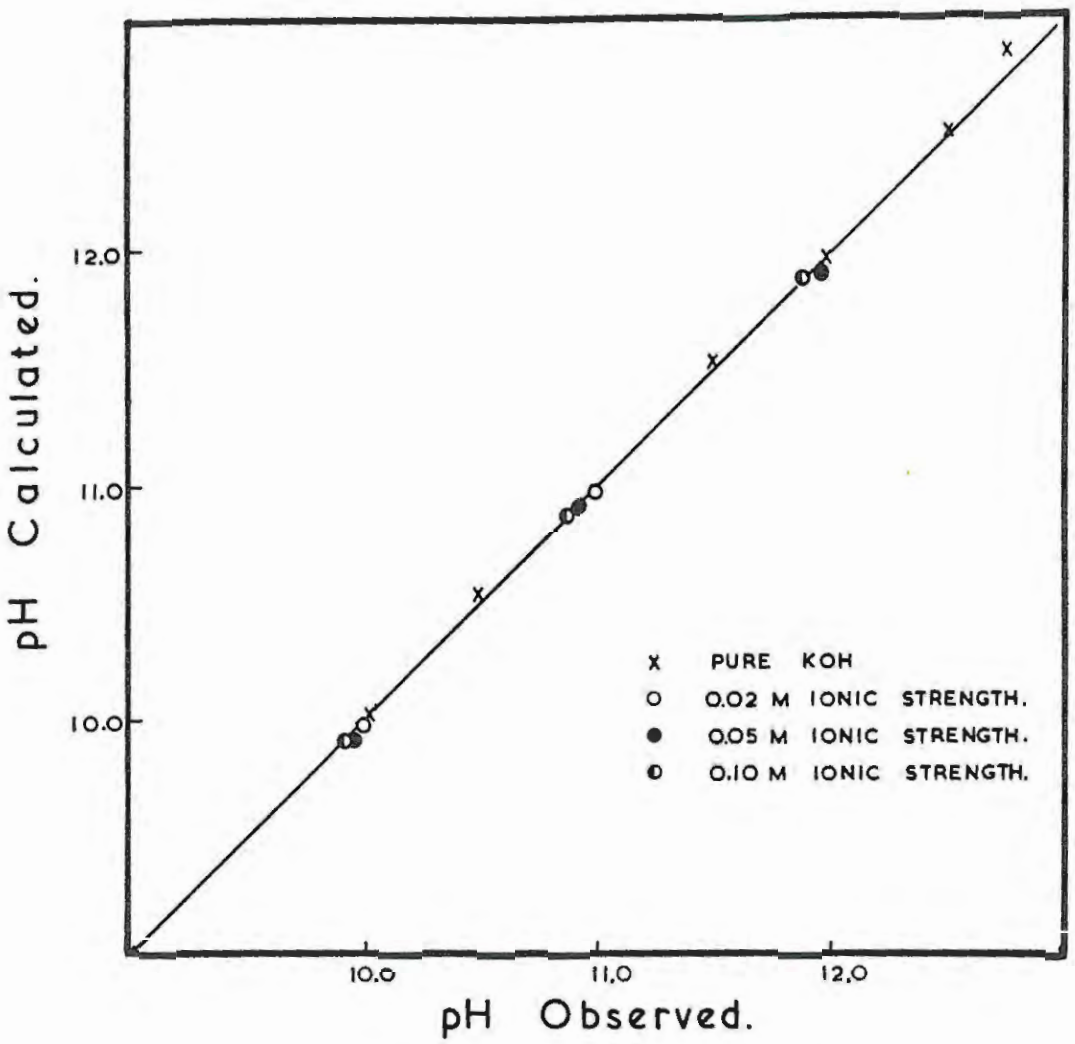


Fig. 15. *Calibration of glass electrode No. 40308.*

Fig. 15 shows the calibration graph of glass electrode (No. 40308) used throughout the experiment. (Not all electrodes behave as well as this, and all tend to become fatigued after a few months use.)

Above pH 12.5 the pH of alkaline solutions was obtained from the concentration and activity coefficients interpolated from the above data.

(iii) Determination of Sulphide and Sulphur in solution.

The analytical method adopted is based on the colorimetric procedure of Budd and Bewick[72] for the determination of microquantities of sulphide and reducible sulphur (sulphur, sulphite and thiosulphate) in alkaline solutions. Hydrogen sulphide evolved on acidification, or by previous reduction and subsequent acidification, is collected in zinc acetate solution and reacted with p-amino dimethyl aniline in the presence of ferric chloride to form methylene blue.

Extreme care is necessary in the preparation of the calibration curve as the sulphide solutions decompose rapidly, and must be standardized iodometrically prior to each determination. Reduction is carried out by heating the alkaline sample with stannous chloride, followed by addition of aluminium strips and hydrochloric acid. Sulphur, sulphite, thiosulphate and polysulphides are reduced, but sulphate is unaffected.

$$Na_2S_2O_3 + Na_2SnO_2 + 2NaOH \longrightarrow Na_2SO_3 + H_2S + Na_2\,SnO_3 + H_2O$$

$$2\,Al + 6\,HCl \longrightarrow 2\,AlCl_3 + 3H_2$$

$$Na_2SO_3 + 2HCl + 3H_2 \longrightarrow \quad H_2S \quad + \quad 2\,NaCl + 3H_2O$$

With this method 2 to 40 µgrams of sulphide may be determined. By choosing suitable aliquots of the test solution, an effective range of 0.01 to 0.16 mg. sulphur may be obtained. For more concentrated solutions, the method was modified as follows:

H_2S evolved was collected in zinc acetate and 10 mls. of 0.025 N iodine added, and acidified with 5 mls. of 0.5 N HC_1. The excess iodine was titrated against standard 0.025 N sodium thiosulphate using starch as indicator.

1 ml. of 0.025 N $Na_2S_2O_3$ consumed = 0.40 mg. sulphur.

Since the colorimetric method was calibrated iodometrically, there can be no objection to this modification. Using a combination of these methods 0.01 - 2.0 mg. sulphur may be determined, and by choosing suitable aliquots of solution the amount of sulphur may be kept within this range.

(iv) Nitrogen Determination.

Protein nitrogen in intact keratin or in solution after the sorption stage was determined by the Kjeldahl procedure of Mackenzie and Wallace [73] using a mercury catalyst. For the determination in the intact fibres, the amount of catalyst was doubled to increase the rate of digestion; this addition had no effect on the final result.

(v) Determination of Cystine and Cysteine.

The procedure of Zahn and Traumann[65] which is a modification of the original Shinohara method was employed. In the case of alkali degraded fibres, more accurate results were obtained by hydrolysis of a 1 gm. sample and dilution to the correct concentration. The accuracy of this method is rather uncertain, especially for chemically modified protein as it has been established that reducing substances interfere in the cysteine determination.

(vi) Water Imbibition.

Wool and mohair fibres swell appreciably in alkaline solution, and the amount increases from approximately 50% at pH 8 to 11 to a maximum value of 150% at pH 13 to 14 [49] where the breakdown of the disulphides allows further penetration into the fibre.

In view of the inaccuracies involved in sorption measurements at pH 12 to 14 the correction for water imbibition was felt to be unjustified.

10. PRELIMINARY INVESTIGATIONS.

Before sorption experiments were commenced, it was felt necessary to investigate the nature of the sulphur liberated in the alkaline reaction more thoroughly. Reaction below pH 12 at 25°C produced little physical change either in the solution or the fibres. Above this pH the swollen fibres became progressively damaged, and the amount of protein dissolved increased rapidly. The alkaline solution acquired a yellow coloration. Acidification of the solution caused the evolution of hydrogen sulphide, and free sulphur was precipitated.(74) Neither sulphite nor sulphate could be detected, but small quantities of thiosulphate(75) were found to be present. The free sulphur and hydrosulphide (sulphydryl) ion are probably combined as polysulphides in the alkaline solution, but little is known about these compounds.

The reaction between sulphur and potassium hydroxide was then studied by dissolving small quantities of sulphur in 50 mls. of ethyl alcohol, and then adding 50 mls. of potassium hydroxide. The results are shown below in Table 12.

TABLE 12.

REACTION OF SULPHUR WITH ALKALI.

M. eqv. of sulphur	M. eqv. of KOH removed	M. eqv. of S^{2-}	M. eqv. of $S_2O_3^{=}$
0.49	0.46	0.040	0.018
0.85	0.80	0.085	0.042

The values for thiosulphate were obtained by strongly acidifying an aliquot with hydrochloric acid and heating for 10 minutes to expel H_2S. After cooling, the solution was diluted and titrated against 0.025 N iodine standard.

These results confirm the findings of Mizell and Harris[50] that sulphur reacts mole for mole with potassium hydroxide, when the former is in solution. The yellowish solutions formed are similar to those produced by the reaction of alkali on wool. Whilst there is very little information available regarding the S + KOH reaction, most authors give the following reactions:

$$4S + 6KOH \longrightarrow K_2S_2O_3 + 2K_2S + 3H_2O \quad \ldots\ldots\ldots\ldots \quad (3.12)$$

$$2K_2S + nS \longrightarrow 2\,K_2S_n + 1 \text{ (polysulphide)}$$

From the evidence above, and the discussion on page 67 it may be concluded that the high sorption of alkali (uncorrected) by wool does not result from the formation of more acidic groups from the decomposition of disulphide groups, but rather by reaction of alkali with the liberated sulphur.

After these analyses had been completed, details of the work of Schöberl and Rambacher[76] were made available, and the finding of elemental sulphur and sulphide sulphur (or polysulphides) in alkaline solution was confirmed.

Using the analytical methods described in 9.C. for the determination of sulphide, reducible sulphur, protein nitrogen, cystine and cysteine, a preliminary sorption experiment was conducted to investigate the relationships between these quantities.

1 gm. sub-sample of conditioned wool and mohair were immersed in 50 ml. of 0.1 m KOH solution at 25°C for various sorption periods, as described on page 71 . At this pH the degradation products are present in sufficient quantities for accurate semi-micro analysis.

After the sorption stage the solution was removed from the fibres by decanting, and filtered aliquots were taken for titration with standard acid, and for the determination of sulphide, reducible sulphur, and protein nitrogen. The keratin sample was washed free of alkali with dilute acid and distilled water. After drying and conditioning, the cysteine and cystine analyses were performed. The moisture content of the unmodified

fibres was approximately 12.3%, but decreased to 11.5 - 11.8% after treatment. This has been taken into account in the calculation of cystine and cysteine content. Analytical results are shown in Table 13 a,b. The cysteine content remained constant throughout, and therefore is not shown. In Fig. 16 the reduction in cystine content is plotted against the total reducible sulphur liberated in solution. The theoretical line is drawn according to the postulate that 1 mole of cystine destroyed = 1 mole of sulphur in solution.

TABLE 13a.

COMPARISON OF CYSTINE ANALYSES WITH THE AMOUNT OF SULPHUR IN SOLUTION.

Wool.

Period of Exposure	Cystine content %	Decrease in Cys. S. mg.	Total Reduc. S. mg.	Sulphide S. mg.	Reducible S. mg.
0 hrs.	8.32				
1	6.98	1.78	1.57	0.28	1.29
3	5.78	3.39	4.43	0.35	4.08
4	5.26	4.08	4.39	0.46	3.93
8	4.03	5.74	4.80	0.56	4.24
10	3.60	6.30	6.17	0.40	5.77
20	3.19	6.84	8.87	0.39	8.48
40	2.86	7.28	9.23	0.74	8.49
60	2.75	7.45	10.0	0.90	9.10
70	2.67	7.54	8.12	0.52	8.30
80	1.84	8.65	9.77	0.41	9.36
100	0.93	10.0	11.63	0.56	11.07

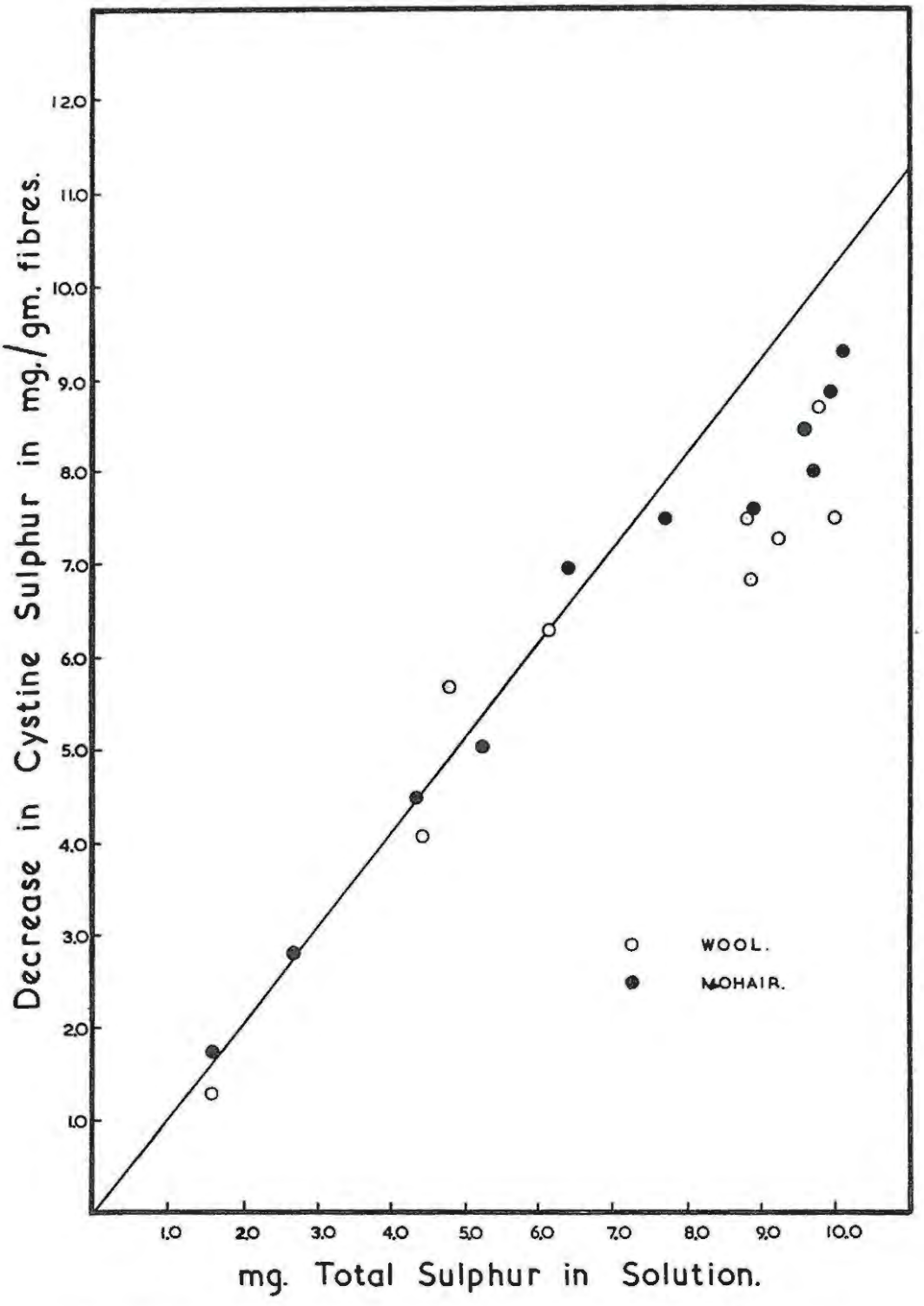


Fig. 16. *The relation between the decrease in cystine content and the total sulphur liberated by alkaline reaction. The solid line is predicted from theory.*

TABLE 13b.

COMPARISON OF CYSTINE ANALYSES WITH THE AMOUNT OF SULPHUR IN SOLUTION.

Mohair.

Period of Exposure	Cystine content %	Decrease in Cys. S. mg.	Total Reduc. S. mg.	Sulphide S. mg.	Reducible S. mg.
0 hrs.	8.84				
1	7.50	1.74	1.57	0.28	1.29
3	5.61	4.31	4.34	0.40	3.94
4	5.04	5.07	5.24	0.46	4.78
8	3.98	6.48	5.43	0.36	5.07
10	3.63	6.95	6.39	0.39	6.00
15	3.25	7.45	7.72	0.72	7.00
20	3.16	7.57	8.90	0.42	8.48
40	3.00	7.79	9.73	0.61	9.12
60	2.63	8.28	9.63	0.80	8.83
70	2.33	8.68	8.97	0.64	8.33
80	1.85	9.32	10.13	0.54	9.59
100	1.91	9.90	11.53	0.50	11.03

The agreement between experiment and theory is close and confirms the earlier findings of Rutherford and Harris.[47] As the reaction proceeds however, deviations occur and there is more sulphur in solution than can be accounted for in terms of cystine decomposed.
Inspection of equation (3.2c) indicates that this sulphur may be formed as a result of lanthionine breakdown. This is consistent with the low yields of lanthionine obtained from alkali-treated wool.

$$\rangle CH\text{-}CH_2\text{-}S\text{-}CH_2\text{-}CH\langle \longrightarrow \rangle CH = CH_2 + S^{2-} + CH_2 = CH\langle \quad .....(3.2c)$$

By subtracting the sulphide ion concentration from the total reducible sulphur in solution, the results follow the theoretical line more closely, Fig. 16. Deviations still persist, however, especially where long exposure to alkali has occurred. Alternate and more satisfactory explanations for these deviations are:

(i) Increase of sulphur in solution as result of soluble protein (especially if this is a cystine-rich fraction). This explanation is quite feasible as the deviations occur only after severe degradation, where appreciable amounts of protein have dissolved;

(ii) The reliability of cystine determinations by the Shinohara method on modified keratin is extremely uncertain, although precautions have been taken to eliminate sampling errors by hydrolysing the whole sample. Thus cystine analyses may be rather high.

The results of apparent alkali sorption measured concurrently with the data in Table 13a,b are shown in Table 14a,b and illustrated graphically in Fig. 17. The correction applied for alkali consumed in the disulphide reaction was calculated on the basis:-

$$\text{m.moles KOH consumed} = \left\{ \begin{array}{l} \text{m.moles total reducible sulphur} \\ - \text{ m.moles sulphide} \end{array} \right\}$$

$$= x$$

The corrected alkali sorption B is given by:

$$B = \text{Apparent Sorption } (\beta) - x$$

$$= 50\,(C_1 - C_2) - x \quad \ldots\ldots\ldots\ldots \qquad (3.13)$$

C_1 = Initial conc. of alkali (moles/l)

C_2 = Final conc. of alkali (moles/l)

x = Millimoles reducible sulphur in solution.

TABLE 14a.

CORRECTIONS FOR DISULPHIDE REACTION.

WOOL.

Time hrs.	Protein Nitrogen in soln. mg.	Alkali m.moles removed from soln.	CORRECTIONS FOR SULPHUR FORMED millimoles (A)	(B)	ALKALI SORPTION CORR. FOR SULPHUR AND N SOLUB. (A) m.moles/g. dry fibres.	(B)
1	3.3	0.645	0.040	0.049	0.620	0.611
3	4.4	0.702	0.128	0.138	0.594	0.583
4	4.8	0.715	0.123	0.137	0.612	0.598
8	6.1	0.701	0.134	0.150	0.592	0.575
10	6.9	0.772	0.180	0.193	0.622	0.608
20	9.0	0.830	0.265	0.277	0.598	0.585
40	9.4	0.846	0.265	0.288	0.621	0.597
60	11.0	0.835	0.284	0.312	0.596	0.566
70	12.5	0.818	0.259	0.276	0.612	0.592
80	12.5	0.850	0.293	0.305	0.609	0.598
100	16.0	0.910	0.346	0.363	0.634	0.615
					Mean 0.610	0.593

A = Modified correction

B = Rutherford - Harris correction.

TABLE 14b.

CORRECTIONS FOR DISULPHIDE REACTION.

MOHAIR.

Time hrs.	Protein Nitrogen in soln. mg.	Alkali m.moles removed from soln. mg.	CORRECTIONS FOR SULPHUR FORMED millimoles (A)	(B)	ALKALI SORPTION CORR. FOR SULPHUR AND PROTEIN DISSOLVED. m.moles/g. dry fibres. (A)	(B)
1	1.5	0.629	0.040	0.049	0.596	0.586
3	3.3	0.705	0.123	0.136	0.596	0.582
4	2.5	0.750	0.149	0.164	0.611	0.596
8	4.0	0.714	0.159	0.170	0.571	0.560
10		0.748	0.188	0.200	0.587	0.563
15	4.1	0.790	0.219	0.241	0.567	0.556
20	5.2	0.835	0.268	0.278	0.587	0.577
40	5.7	0.864	0.285	0.341	0.602	0.544
60	7.7	0.850	0.276	0.309	0.606	0.571
70	6.2	0.846	0.260	0.280	0.612	0.591
80	12.0	0.864	0.300	0.317	0.613	0.595
100	12.0	0.890	0.345	0.360	0.595	0.579
				Mean	0.595	0.575

A = Modified correction.

B = Rutherford - Harris correction.

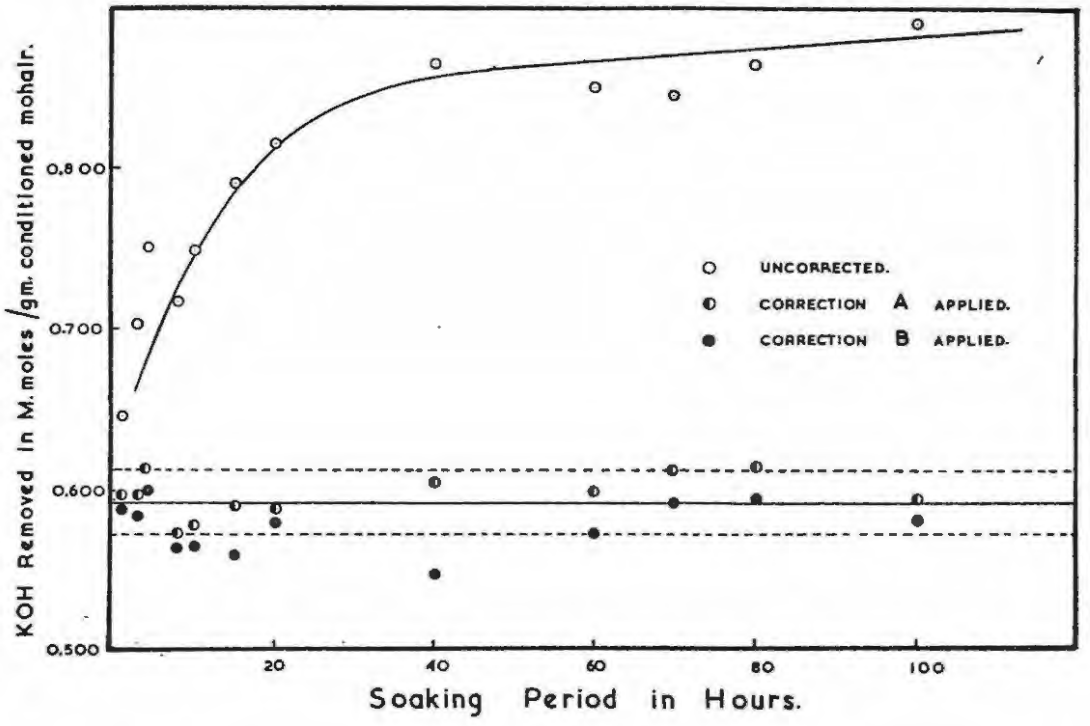


Fig. 17. *The kinetics of the reaction of mohair keratin with KOH (0.10m) at 25°C from the experimental data in Table 14 (b). —O— KOH sorption uncorrected for the disulphide degradation; the shaded circles are for sorption values after application of corrections A or B. The solid line parallel to the abscissae is the mean sorption value after correction A has been applied. The dotted lines represent the accuracy of the titration at this concentration.*

Thus it will be seen that no correction for sulphide was applied, since H_2S is eliminated in the titration of the alkaline solutions with hydrochloric acid. Attempts were made to remove the SH^- ions from solution as insoluble sulphides. In alkaline medium this is only possible with a metal having a soluble hydroxide, and a sulphide which is stable in acid solution pH 3, otherwise a heavy precipitate forms in alkaline solution, and on acidification the sulphide decomposes. Thallium was found to be suitable for this purpose, but at high sulphide concentrations (when sulphur is also present) a brown polysulphide forms which obscures the end point.

Attempts to determine the reducible sulphur by eliminating sulphide before reduction were found unsatisfactory, as the reduction with stannous chloride requires a strongly alkaline solution.

When the Rutherford-Harris correction (B) is applied to the data (i.e. corrected for total sulphur in solution) the final results appear to be overcorrected (Fig. 17) which was noted by Steinhardt and Harris. The correction for soluble protein was that proposed by Rutherford and Harris described in section III page 63.

Thus although the original Rutherford-Harris equations (2.1a,b.) are fortuitously correct for the alkali consumed in the process of disulphide breakdown, their concept of the form of the sulphur was completely incorrect. The actual nature of the reaction which removes alkali from solution is uncertain, but it seems probable that it involves the combination of alkali with the sulphur liberated in solution.

11. EXPERIMENTAL RESULTS.

Alkali sorption measurements over a wide range in pH are recorded in Tables 15 to 21.
Tables 15a and 15b refer to standard wool and mohair respectively. Table 16 records data on these samples at high pH values. Sorption at various ionic strengths is contained in Table 17 while Table 18 refers to mohair A - J.
Tables 19 to 21 refer to various modified fibres.

TABLE 15a.

SORPTION OF KCH BY STANDARD WOOL AT 25°C.

Initial KOH moles/l.	Alkali Sorption m.moles.	S Correction m.moles.	Corrected Sorption m.moles.	Corrected Sorption m.moles/gm. dry wool	pH
0.00001	0.001		0.001	0.001	6.20
	0.001		0.001	0.001	6.25
0.0002	0.007		0.007	0.008	7.20
0.0005	0.010		0.010	0.011	7.35
0.00094	0.041		0.041	0.041	8.32
0.00223	0.082		0.082	0.094	10.39
0.00506	0.130	0.002	0.128	0.146	11.09
	0.127	0.002	0.125	0.143	11.09
0.00702	0.145	0.003	0.142	0.162	11.51
	0.147	0.004	0.143	0.163	11.51
0.01049	0.197	0.003	0.194	0.221	11.75
	0.194	0.003	0.191	0.218	11.73
0.02231	0.308	0.004	0.304	0.347	12.18
	0.310	0.004	0.306	0.349	12.21
0.03025	0.350	0.008	0.342	0.390	12.32
	0.348	0.009	0.339	0.387	12.34
0.04851	0.436	0.015	0.421	0.480	12.43
0.05032	0.446	0.016	0.430	0.491	12.47
0.06373	0.540	0.017	0.523	0.597	12.60
	0.533	0.013	0.520	0.594	12.62
0.07526	0.604	0.072	0.532	0.610	12.80

TABLE 15b.

SORPTION OF KOH BY STANDARD MOHAIR AT 25°C.

Initial KOH moles/l.	Alkali Sorption m.moles.	Sorption correction m.moles.	Corr. Sorption m.moles.	Corr. Sorption m.moles/ dry mohair	pH
0.00001	0.005		0.001	0.001	5.12
0.00020	0.010		0.010	0.012	6.18
0.00050	0.020		0.020	0.023	6.50
0.00094	0.044		0.044	0.051	7.52
0.00101	0.046		0.046	0.053	8.36
0.00150	0.057		0.057	0.065	9.15
0.00202	0.088		0.088	0.102	10.39
0.00506	0.135	0.002	0.133	0.154	11.04
	0.131	0.001	0.130	0.151	11.06
	0.132	0.001	0.131	0.152	11.10
0.00512	0.138	0.002	0.136	0.159	11.25
	0.137	0.001	0.136	0.159	11.22
0.00702	0.157	0.003	0.154	0.177	11.57
	0.160	0.002	0.158	0.182	11.56
0.01003	0.178	0.004	0.174	0.202	11.75
	0.180	0.005	0.175	0.203	11.76
0.01498	0.230	0.005	0.225	0.261	11.95
	0.228	0.003	0.225	0.261	11.92
0.02231	0.283	0.004	0.279	0.324	12.18
	0.285	0.004	0.281	0.326	12.20
0.02693	0.331	0.004	0.327	0.379	12.25
	0.326	0.003	0.323	0.374	12.27
0.03025	0.343	0.006	0.337	0.388	12.31
0.04002	0.401	0.008	0.393	0.452	12.42
	0.405	0.010	0.395	0.455	12.41
0.05031	0.482	0.016	0.466	0.540	12.54
	0.480	0.017	0.463	0.537	12.53
0.06373	0.529	0.010	0.519	0.601	12.65
	0.524	0.009	0.515	0.597	12.66
0.07525	0.600	0.059	0.541	0.625	12.71
	0.607	0.060	0.547	0.635	12.72

TABLE 16a.

SORPTION OF KOH BY WOOL AT 25°C AT HIGH pH VALUES..

hrs.	Initial KOH moles/l.	Final KOH conc. moles/l.	KOH removed m.moles.	Nitrogen soln. mg.	mg. S^{2-}	Total Sulphur mg.	Correction m.moles	Corr. Sorption m.moles.	Sorption m.moles/ gm. dry wool	pH Calc.
1	0.3537	0.3324	1.070	14.5	1.23	8.77	0.275	0.835	0.955	13.39
2	0.3537	0.3290	1.235	18.10	1.10	9.94	0.280	0.955	1.093	13.39
3	0.3537	0.3297	1.200		1.08	9.03	0.250	0.950	1.087	13.39
2.5	0.3537	0.3313	1.120	15.0	1.24	8.39	0.220	0.900	1.030	13.39
2	0.1894	0.1690	1.020	8.1	0.30	5.31	0.160	0.860	0.984	13.12
3		0.1674	1.100	8.8	0.70	6.40	0.178	0.920	1.052	13.11
2.5		0.1693	1.00		0.30	5.40	0.160	0.840	0.961	13.11
2	0.8160	0.7873	1.420	52.0	2.20	17.0	0.460	0.960	1.098	13.77
3		0.7894	1.335	48.0	3.39	13.16	0.305	1.030	1.178	13.77
4		0.7914	1.230	102.0	3.10	14.20	0.350	0.880	1.000	13.77
2	0.5624	0.5358	1.330	48.0	1.00	13.55	0.390	0.940	1.075	12.59
3		0.5365	1.295	55.0	0.86	13.35	0.390	0.905	1.035	12.59
1	0.10175	0.08886	0.645	3.30	0.28	1.57	0.040	0.605	0.695	12.85
4		0.08745	0.715	4.8	0.46	4.39	0.123	0.592	0.677	12.85
6		0.08793	0.691	6.10	0.56	4.80	0.134	0.567	0.649	12.84

TABLE 16b.

SORPTION OF KOH BY MOHAIR AT 25°C AT HIGH pH VALUES.

hrs.	Initial KOH moles/l.	Final KOH conc. moles/l.	KOH removed m.moles.	Nitrogen soln. mg.	mg. S^{2-}	Total Sulphur mg.	Correction m.moles	Corr. Sorption m.moles./gm. dry mohair		pH Calc.
1	0.3537	0.3331	1.030	7.70	1.60	7.93	0.200	0.830	0.957	13.38
2		0.3307	1.150	3.31	1.15	9.90	0.290	0.860	0.992	13.39
3		0.3297	1.200		1.19	9.88	0.275	0.925	1.067	13.39
2.5		0.3304	1.165	8.10	1.40	9.40	0.250	0.915	1.055	13.39
2	0.1894	0.1683	1.060	3.2	0.62	7.03	0.200	0.860	0.992	13.12
4		0.1687	1.035	4.70	0.76	7.07	0.200	0.838	0.966	13.12
6		0.1693	1.000		0.32	5.40	0.168	0.832	0.959	13.11
2	0.8160	0.7886	1.370		2.10	16.11	0.425	0.945	1.090	13.77
4		0.7873	1.435	26.8	3.17	12.97	0.307	1.130	1.303	13.77
2	0.5624	0.5348	1.330	32.0	0.86	14.26	0.419	0.910	1.049	13.59
3		0.5372	1.260	52.1	0.95	14.30	0.417	0.840	0.969	13.59
1	0.10175	0.08896	0.639	1.53	0.28	1.57	0.040	0.599	0.691	12.85
4		0.08678	0.750	2.51	0.46	5.24	0.149	0.601	0.693	12.84
10		0.0875	0.748		0.35	6.39	0.188	0.560	0.646	12.84

TABLE 17a.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.005 MOLAR.

Initial conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry wool.	pH
0.00083	0.00012	0.0050	0.040	7.74
0.00220	0.00029	0.0048	0.105	9.45
0.00346	0.00078	0.0044	0.154	10.81
0.00544	0.00200	0.0030	0.198	11.10

TABLE 17b.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.01 MOLAR.

Initial conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry wool.	pH
0.00083	0.00006	0.0100	0.044	7.35
0.00131	0.00018	0.0100	0.066	8.15
0.00220	0.00020	0.0100	0.105	9.45
0.00346	0.00079	0.0100	0.154	10.45
0.00544	0.00171	0.0081	0.214	10.95
0.01039	0.00542	0.0045	0.260	11.61

TABLE 17c.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.02 MOLAR.

Initial conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry wool.	pH
0.00083	0.00005	0.0200	0.045	7.26
0.00131	0.00016	0.0200	0.067	7.90
0.00220	0.00026	0.0200	0.112	9.00
0.00544	0.00159	0.0182	0.221	10.60
0.00909	0.00464	0.0152	0.291	11.50
0.01039	0.00581	0.0144	0.302	11.55
0.02011	0.01352	0.0650	0.372	12.02

TABLE 17d.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.050 MOLAR.

Initial conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry wool.	pH
0.00083	0.00005	0.0500	0.045	6.73
0.00220	0.00024	0.0500	0.113	8.71
0.00346	0.00056	0.0500	0.167	9.70
0.00544	0.00145	0.0500	0.230	10.62
0.00909	0.00385	0.0455	0.299	11.29
0.01039	0.00502	0.0451	0.306	11.30

TABLE 17e.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.10 MOLAR.

Initial Conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry. wool	pH
0.00083	0.00003	0.100	0.046	6.71
0.00220	0.00022	0.100	0.114	8.50
0.00346	0.00045	0.100	0.174	9.71
0.00544	0.00118	0.100	0.247	10.46
0.00909	0.00288	0.097	0.353	11.20
0.02006	0.01213	0.087	0.447	11.73
0.03016	0.02106	0.079	0.518	11.94
0.05031	0.03913	0.060	0.626	12.42

TABLE 17f.

THE SORPTION OF KOH BY STANDARD WOOL AT TOTAL IONIC STRENGTH 0.50 MOLAR.

Initial conc. of KOH moles/l.	Final conc. of KOH moles/l.	Conc. of KCl moles/l.	Corr. Sorption m.moles/ gm. dry. wool	pH
0.00083	0.00002	0.500	0.047	6.67
0.00131	0.00010	0.500	0.067	7.90
0.00220	0.00020	0.500	0.115	8.00
0.00346	0.00047	0.500	0.171	9.22
0.00544	0.00114	0.500	0.247	9.91
0.02006	0.01093	0.500	0.518	11.80
0.05047	0.03660	0.464	0.739	12.35

TABLE 18.

ALKALI SORPTION BY MOHAIR SAMPLES A - J.

SIDE SAMPLES.			NECK SAMPLES.		
Sample No.	Corr. KOH Sorp. m.moles/g. dry fibres.	pH	Sample No.	Corr. KOH Sorp. m.moles/g. dry fibres.	pH
A_1	0.010	6.51			
	0.087	9.85			
	0.485	12.41			
B_1	0.013	6.80	B_2	0.013	6.90
	0.089	10.10		0.082	10.41
	0.454	12.40		0.450	12.37
C_1	0.015	6.01	C_2	0.008	7.04
	0.092	10.21		0.080	10.52
	0.473	12.40		0.428	12.42
D_1	0.026	5.59	D_2	0.018	6.80
	0.103	9.81		0.082	10.52
	0.460	12.42		0.419	12.45
E_1	0.010	6.96	E_2	0.006	7.70
	0.081	10.30		0.055	10.87
	0.445	12.38		0.406	12.45
F_1	0.018	7.37	F_2	0.025	5.90
	0.089	10.51		0.103	10.21
	0.464	12.39		0.457	12.37

TABLE 18. (contd.)

SIDE SAMPLES.			NECK SAMPLES.		
Sample No.	Corr.KOH Sorp. m.moles/g. dry fibres.	pH	Sample No.	Corr. KOH Sorp. m.moles/g. dry fibres.	pH
G_1	0.008	7.04	G_2	0.021	7.04
	0.076	10.02		0.080	9.92
	0.424	12.37		0.438	12.38
H_1	0.017	6.57	H_2	0.021	6.25
	0.080	10.51		0.077	10.63
	0.470	12.35		0.428	12.42
J_1	0.019	6.50	J_2	0.008	7.09
	0.075	10.51		0.069	10.54
	0.441	12.37		0.425	12.38

TABLE 19.

KOH SORPTION BY STANDARD MOHAIR TIP (PHOTOCHEMICALLY DAMAGED).

Initial KOH concentration moles/litre.	Sulphur Correction m/moles.	Corrected sorp. m/moles gm. fibre.	pH
0.00052	-	0.024	6.90
0.00101	-	0.055	8.09
0.00203	-	0.097	8.85
0.00519	0.004	0.240	10.65
0.0214	0.015	0.425	12.01
0.07525	0.085	0.645	12.80

TABLE 20.

KOH SORPTION BY PERACETIC OXIDIZED WOOL.

Initial KOH concentration moles/litre.	Corr. Sorption KOH m.moles/gm. fibre.	pH
0.0020	0.088	9.05
0.0174	0.342	11.32
0.0203	0.601	11.90
0.07525	1.13	12.62

TABLE 21.

KOH SORPTION BY 'LANTHIONINE' WOOL.

Initial KOH concentration moles/litre.	Corrected Sorption m.moles/gm. fibre.	pH
0.0020	0.075	10.15
0.02027	0.400	12.07
0.07525	0.605	12.68

12. DISCUSSION OF RESULTS.

(i) Accuracy and Reliability of Results.

With dilute alkali (below pH 10) extreme care is necessary to keep solutions carbonate-free, as small quantities of CO_2 produce considerable effects on the pH in this region. (The accuracy of the results is dependent on the extent to which CO_2 is excluded from the system.) Moreover these solutions after the sorption stage, are slightly buffered by small quantities of soluble protein, and it is extremely difficult to establish whether the lowering of pH is caused by CO_2 contamination or by buffer action. By using freshly prepared solutions reproducible results could be obtained. The corrections for disulphide breakdown are negligible at these low alkali concentrations.

The pH measurements from 10 to 12.5 are more reliable; CO_2 or other contaminants do not produce such a pronounced effect. The sorption values vary from 0.100 millimoles at pH 10 to 0.500 millimoles/g. at pH 12.5, and the corrections for disulphide reaction are relatively small (0.001 - 0.010 millimoles/gm.) In this region readings are reproducible to 0.005 millimoles.

Above pH 13 the disulphide correction increases rapidly, reaching a maximum of 400 m.moles (30% of apparent sorption value.) Titrations with concentrated solutions are less accurate even when carefully calibrated glassware is employed. At a concentration of approximately 0.3 molar the keratin is severely degraded, and large quantities of protein are dissolved. Application of the Rutherford-Harris correction for

for soluble protein gives extremely high results, and it seems that either the soluble protein is not back-titrated, or that the wool keratin is modified by the severe treatment. Horner[77] has eliminated the possibility of amide hydrolysis by showing the constancy of amide nitrogen content before and after sorption. Other explanations of these abnormally high values for alkali binding are those of Fröhlich and Elöd,[49] who have postulated the following reaction:-

$$R_1\text{-CH-C(=O)-NH-CH-} \xrightarrow{NaOH} R_1\text{-CH-C(HO)(ONa)-NH-CH-} \quad \text{.....} \quad (3.14)$$
(R$_2$ on the CH)

The addition compound with the keto-group is responsible for the increased alkali sorption.

It seems highly probable that the increase in sorption results from the hydrolysis of peptide linkages. Appreciable quantities of protein are dissolved in the alkaline treatment, and there is no evidence to show that this is 'soluble wool' or a long chained polypeptide, rather than small soluble fragments hydrolysed from the keratin molecule.

Consider the polypeptide chain, split by hydrolysis:

$$\text{Wool - NH - CH(R}_1\text{) - CO - NH - CH(R}_2\text{) - CO - NH - CH(R}_3\text{) - CO - } \xrightarrow{H_2O}$$

$$\text{Wool - NH - CH(R}_1\text{) - COOH} \qquad \text{NH}_2\text{ - CH(R}_2\text{) - CO - Wool}$$

$$\text{NH}_2\text{ - CH(R}_2\text{) - COOH}$$

Soluble

As a result of hydrolysis a fragment has dissolved causing the formation of an additional sorption site. If, however, the peptide is removed from the end of the chain, i.e. included a terminal group, no change would have occurred, unless the R group contained an additional acidic or basic group.

In Table 22 are recorded acid and alkali sorption measurements performed on alkali treated wool. The samples, after the prescribed alkaline treatment, were washed 0.1 m. HCl to remove excess alkali and sulphide, and finally rinsed repeatedly in distilled water till the extract remained constant at pH 5.0. Both acid and alkali sorption are increased by the treatment, showing that more amphoteric sites are available.* Moreover acid and alkali sorption data on standard wool, in which the cystine content had been partially converted to lanthionine[34], coincide with the titration curve of standard wool, indicating that this reaction does not alter the acidic or basic characteristics of keratin.

The fact that both acid and alkali are increased contradicts the hypothesis of Elöd and Fröhlich (equation 3.14).

If the group - C(OH)(ONa) - remains in iso-electric

alkali-treated keratin an increase in subsequent acid sorption may occur, if the sodium ion is displaced in acid solution, but little or no increase in alkali sorption will be produced. If Na^+ is exchanged for hydrions by rinsing to the iso-electric point, no increase in acid sorption would occur, but an increase in alkali binding could be expected.

* Since the completion of this work McPhee[85] has obtained the value of 1.21 millimoles/gm for the maximum alkali sorption of wool by applying a correction for the amount of alkaline hydrolysis. The original 'Rutherford-Harris sulphur correction' has been employed.

TABLE 22.

ACID AND ALKALI SORPTION ON ALKALI-TREATED WOOL.

Description of Pre-treatment of Wool at 25°C.	Acid Sorption 0.02 m HCl millimoles/gm. dry fibres.	Base Sorption 0.02 m KOH millimoles/gm. dry fibres.
Untreated	0.543	0.408
0.01m. KOH, 24 hours Soaking	0.567	0.424
0.02m. KOH, 16 hours "	0.553	
24 hours "	0.583	0.434
40 hours "	0.575	0.430
0.05m. KOH, 3 hours "	0.627	0.463
0.10m. KOH, 3 hours "	0.683	0.549
0.20m. KOH, 3 hours "	0.683	
6 hours "	0.658	
0.50m. KOH, 3 hours "	0.756	0.682

These results confirm the necessity for rapid sorption techniques.

From the data of Simmonds[29] a maximum base binding or hydrogen ion dissociation of 1.2 millimole/gm may be predicted. Owing to the weakly basic nature of the guanidinium group (pk 12.5) complete dissociation does not occur till pH 13.5 to 14.0; Fig. 18 indicates that there is no tendency to reach a maximum sorption value even at pH 13.8. There is little doubt that this is caused by hydrolysis of peptide links with the formation of additional sites for both acid and alkali sorption. Alkali sorption measurements at 25°C using concentrations higher than 0.1 molar cannot be regarded as applicable to unmodified wool. Measurements at low temperature (-5° or -10°C) may provide a more reliable means for determining the maximum base binding capacity.

(ii) Comparison of Standard Wool and Standard Mohair.

Titration data for these samples is plotted in Fig. 18. The curves are similar in shape, and coincide except from pH 6.0 to 11.0 where

small differences (approximately 0.025 millimoles/gm. maximum) are observed. Mohair sorbs more alkali than wool in this region, and combines with less acid in the range 3 to 5.5. Thus wool appears to have more proton-accepting groups (basic groups) between 3 to 11. Differences in content of histidine (pk 6.0) may cause these differences to some extent.

It has been observed that mohair is less soluble in alkali (0.1 molar) at 25°C than wool. This may have resulted from the large difference in mean fibre diameter (mohair 35 μ's, wool 21 μ's) but may also be caused by structural differences.

Sorption measurements at various ionic strengths are recorded in Table 17. Pre-determined amounts of potassium chloride are added to the alkaline solutions prior to sorption, such that the equilibrium solution has a constant ionic strength. These readings are particularly useful in the application of experimental results to the various theories of base sorption. The addition of salt does not influence the disulphide breakdown below pH 11.50. Above this pH, however, the amount of sulphur liberated in solution increases with increasing ionic strength. Peryman[78] has found a similar degradative influence of salts when boiling wool in dilute acid solution.

(iii) Mohair Samples A - J.

Table 18 contains alkali sorption data on mohair samples A - J. Deviations similar to those observed in the corresponding acid sorption data were found. For comparison purpose the alkali sorption at pH 12.4 has been chosen as an index of alkali sorption. At this concentration of alkali the titration is sensitive, pH measurements are reliable, and corrections for the disulphide reaction are relatively small. Values of this index, extrapolated from the data are shown in Table 23 together with acid sorption index, mean fibre diameter, age of animal and weathering damage as determined by the method of Veldsman.[79]

These results have been statistically analysed. To test whether the difference in alkali sorption index for side samples (mean 0.458 millimoles/gm.) and neck samples (mean 0.422 millimoles/gm.) were real, 'Student's t' has been calculated.

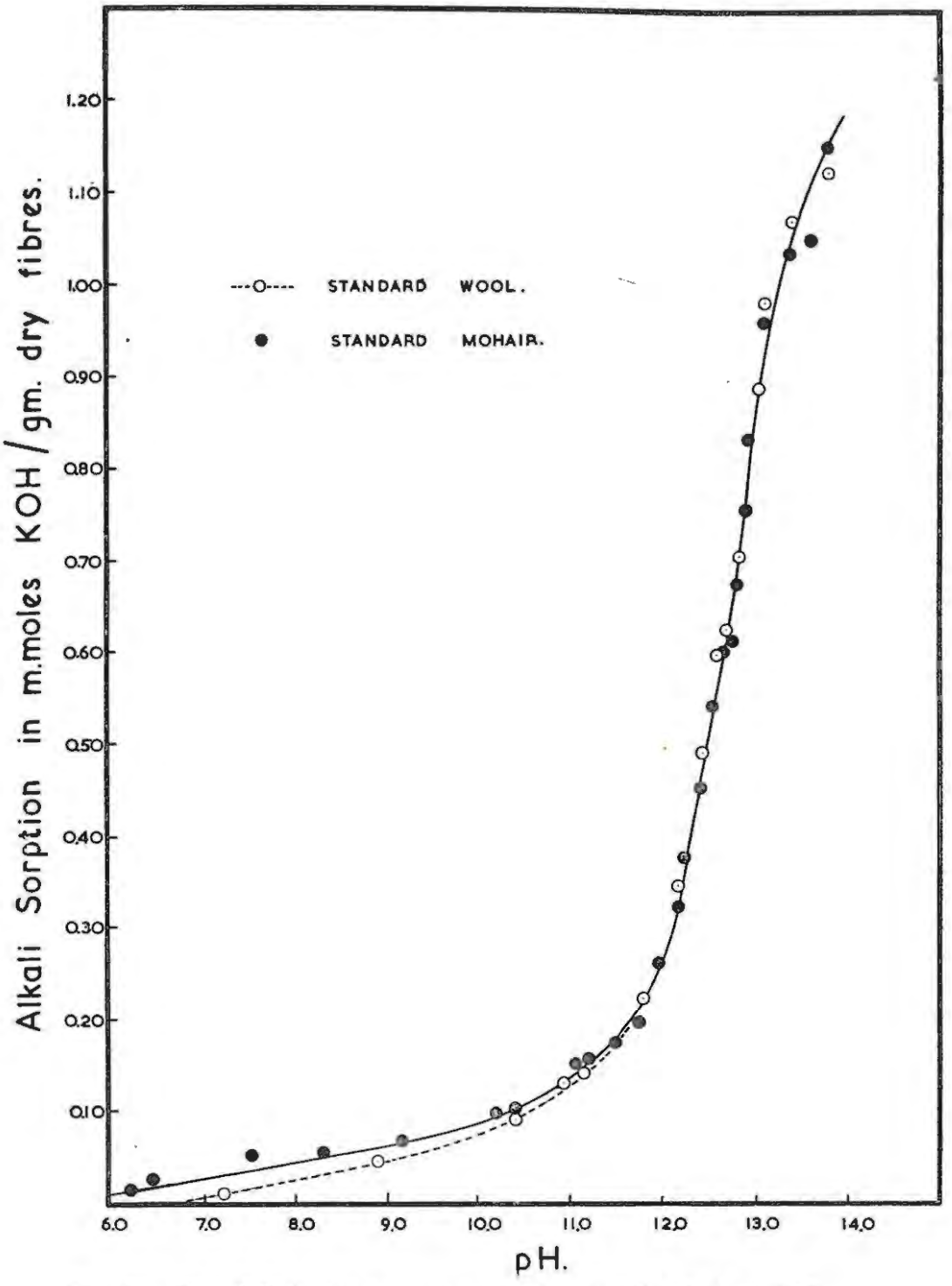


Fig. 18. *The alkali titration curve of standard wool and mohair at 25°C.*

TABLE 23.

MOHAIR SAMPLES A - J.

Mohair Sample No.	Age yrs.	Diameter μ's	Index Acid Sorp. m.moles	Index Base Sorp.	Damage Diameter Index
A1	0.5	28	0.550	0.485	1.00
B1	0.5	29	0.630	0.455	0·95
B2	0.5	32	0.520	0.447	1.14
C1	0.5	29	0.600	0.473	1.45
C2	0.5	34	0.510	0.431	1.55
D1	1.5	47	0.535	0.460	1.09
D2	1.5	45	0.550	0.409	1.15
E1	1.5	36	0.560	0.445	1.03
E2	1.5	48	0.540	0.406	1.45
F1	1.5	45	0.560	0.414	1.02
F2	1.5	48	0.550	0.428	1.26
G1	3	47	0.500	0.449	1.50
G2	3	58	0.510	0.390	1.07
H1	3	54	0.540	0.464	1.18
H2	3	49	0.470	0.438	1.33
J1	3	41	0.525	0.441	1.05
J2	3	46	0.510	0.420	1.03

'Student's t' = 3.2, 15 degrees of freedom

This exceeds the value of t at the 1% level, and thus the difference is statistically significant.

For differences between kid and adult mohair sorption the value of 'Student's t' is significant at the 10% level, indicating a trend towards higher sorption values for kid mohair. This is not a sensitive test as only three samples of kid and three of adult mohair were included in the series A - J.

Partial correlation coefficients between alkali sorption index and mean fibre diameter, and alkali sorption index and age of animal were not significant.

However, calculation of the correlation coefficient between acid and alkali sorption indices gave

r_{AB} = 0.58 with 16 degrees of freedom.

This is a positive correlation significant at the 1% level, indicating a strong relationship between the number of acidic and basic groups in the protein, i.e. an increase in acid sorption sites. This is consistent with the zwitterion concept that equal quantities of positive and negative groups occur at the iso-electric point, coupled in the form of salt linkages.

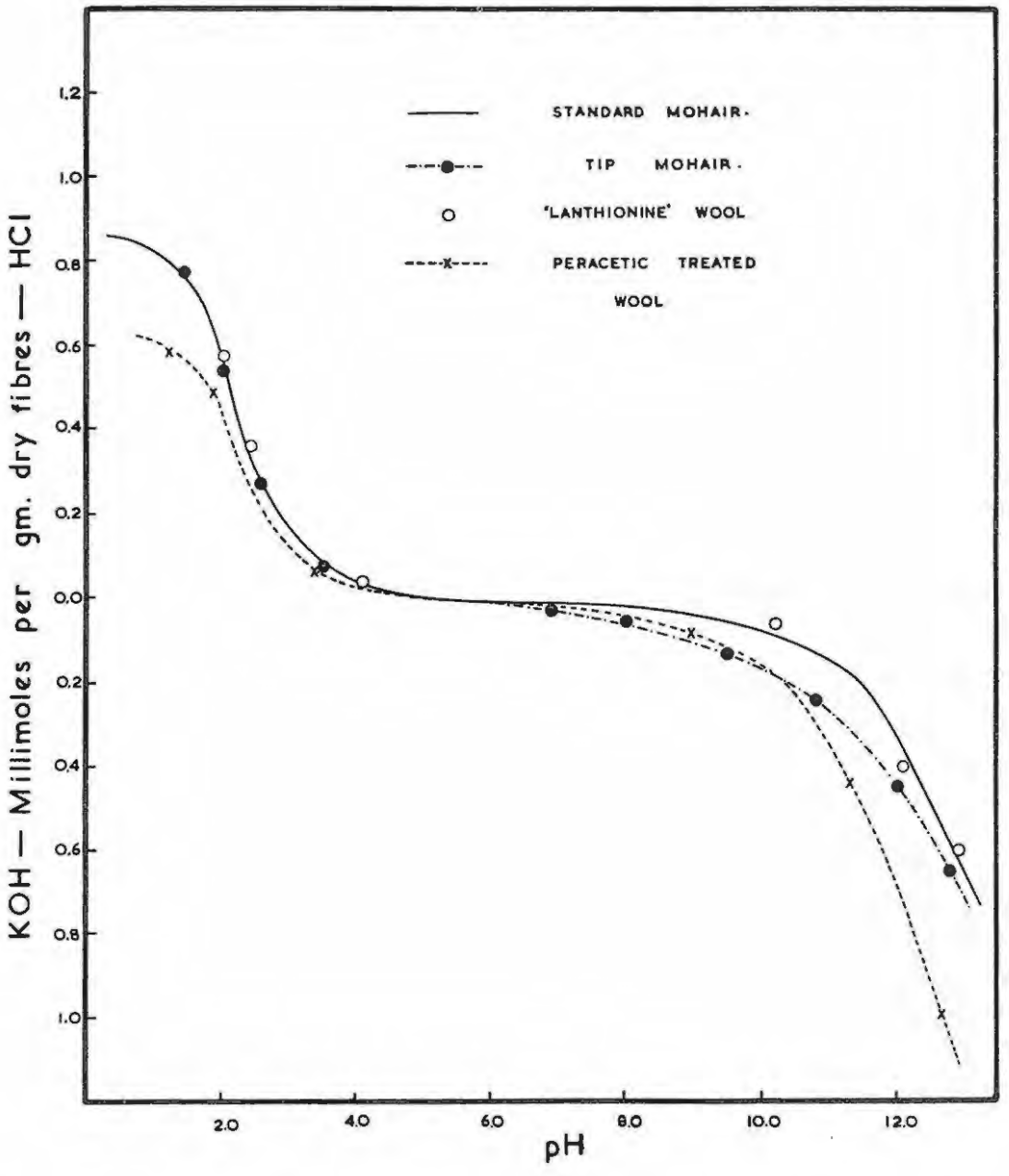


Fig. 19. *Comparison of the titration curves of modified wool keratin with that of the standard.*

(iv) Modified Wool and Mohair.

Photochemical Damage.

Alkali sorption measurements for tip mohair are recorded in Table 19, and together with the data from Table 6 are plotted in Fig. 19. From pH 1 to 6 the curve coincides with the standard mohair indicating that no peptide chain hydrolysis has occurred. In the alkaline region the tip portions sorb more alkali. The effect on the titration curve is more pronounced from pH 7 to 11, where pH varies considerably with small changes in the final concentration. It was also observed that the corrections for disulphide hydrolysis are larger for tip samples which are also more soluble in the alkaline solutions. This can be expected as the decrease in cystine as disulphide bonds allows an increase in swelling, and penetration of the fibre is facilitated.

The actual nature of the groups or side chains in the tip portion which causes the shift in titration curve is unknown at present. There is the possibility that this group is only released after alkaline treatment, and does not exist in the intact protein.

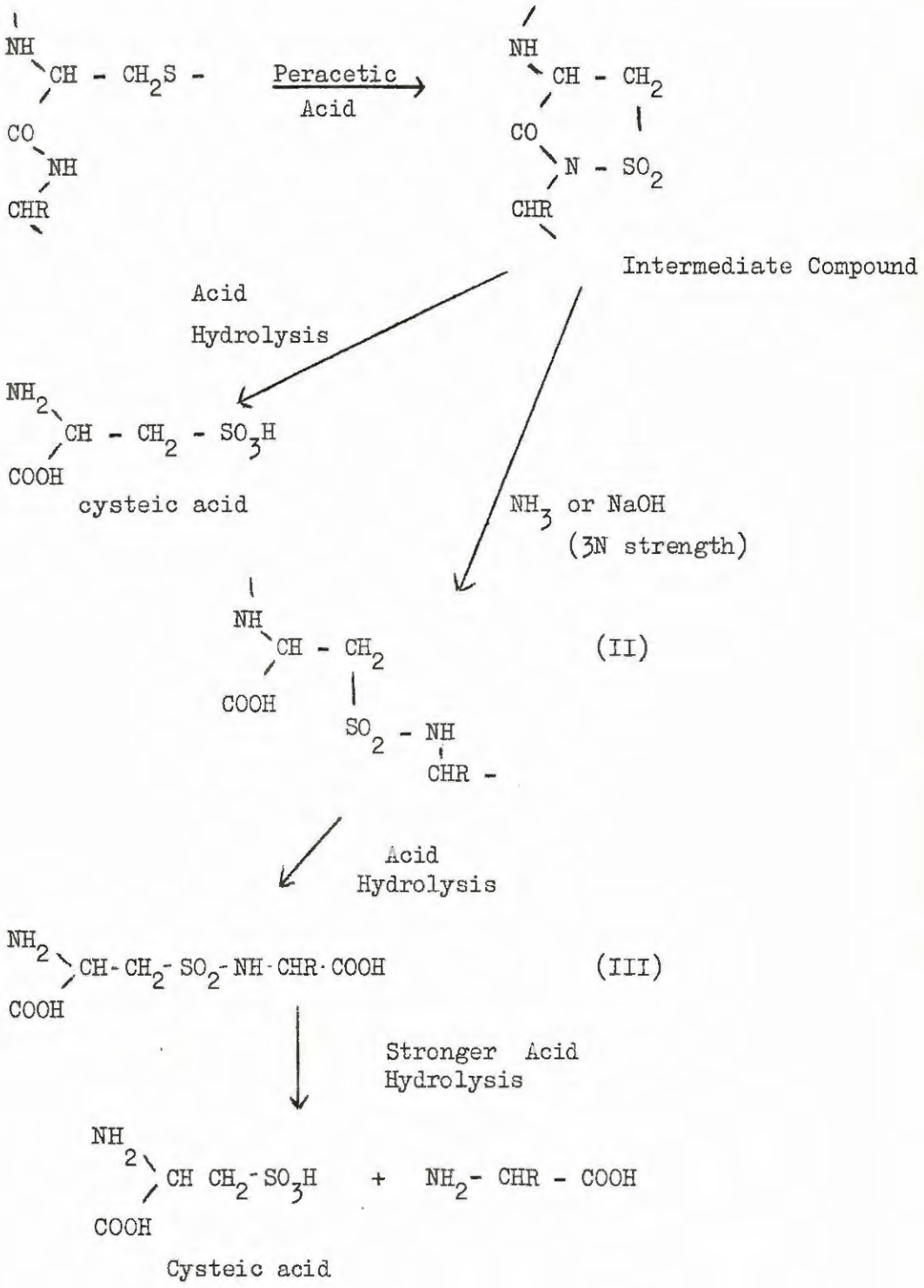
Chemical Modification.

(a) Peracetic oxidation.

The reaction of dilute peracetic acid solutions with wool is reasonably specific and does not oxidize amino acids other than cystine, methionine and tryptophan. If the period of treatment is short no peptide breakdown occurs. Values obtained are:

	% Cystine.	% Cysteine.
Standard Wool	8.32	0.04
Oxidized Wool	4.90	0.03

After acid hydrolysis the oxidized cystine may be determined as cysteic acid[80] but the presence of sulphonic acid groups could not be established prior to hydrolysis. Alexander et al[80] have postulated that cysteic acid is formed only after acid hydrolysis:


Peracetic Acid
Intermediate Compound
Acid Hydrolysis
cysteic acid
NH3 or NaOH (3N strength)
(II)
Acid Hydrolysis
(III)
Stronger Acid Hydrolysis
Cysteic acid

Alexander has shown that treatment of the oxidized wool with alkali (3N ammonia) results in an increase in maximum acid sorption. Compound II has an additional carboxyl group, and is consistent with these findings. However, such a drastic alkali treatment can easily cause extensive peptide hydrolysis, and consequently acid binding may be increased in this manner.

Alkali sorption measurements are shown in Table 20 for the oxidized wool. These values and the data in Table 11 are compared with the Standard Wool curve in Fig. 19. The acid sorption appears to have decreased especially in the region pH 0 to 2. In the alkaline region the curve is displaced towards higher sorption values. Above pH 12.5 the modified protein is extremely soluble, and further investigation was discontinued. The titration curves do not suggest the formation of a strongly acidic sulphonic group, rather it seems as though basic groups have been removed or blocked during the process. These results are not sufficient to support or contradict the ideas of Alexander, but indicate that his interpretations of the titration data are rather suspect.

(b) 'Lanthionine' Wool.

The chemical treatment is described in Section II, page 45. By this method a fraction of the cystine may be converted to lanthionine without further degradation of the protein. Cystine/cysteine analyses are shown below.

	% Cystine	% Cysteine.
Standard Wool	8.32	0.04
'Lanthionine' Wool	4.3	0.04

Alkali sorption data are shown in Table 21, and are compared with the unmodified wool in Fig. 19. These points coincide with the standard curve indicating that the transformation of cystine to lanthionine does not affect the acidic or basic characteristics of the protein.

13. APPLICATION OF THEORIES TO ALKALI SORPTION.

A. Gilbert-Rideal Theory[11]

Consider the alkali MOH under equilibrium conditions with the fibre. In the fibre phase the chemical potential for the M^+ ions is given by:

$$(\mu'_f)_M = (\mu^\circ_f)_M + RT \ln a^f_M + \not{\phi} F \ldots\ldots\ldots\ldots \quad (3.15)$$

and in the solution

$$(\mu_s)_M = (\mu^\circ_s)_M + RT \ln a^s_M \ldots\ldots\ldots\ldots \quad (3.16)$$

Combining equations (3.15) and (3.16)

$$RT \ln a^f_M = (\mu^\circ_s)_M - (\mu^\circ_f)_M - \not{\phi} F + RT \ln a^s_M \ldots\ldots\ldots\ldots \quad (3.17)$$

and writing $\Delta \mu^\circ_M$ for $(\mu^\circ_s)_M - (\mu^\circ_f)_M$ we get

for M^+ and OH^-

$$RT \ln \left(\frac{\theta_M}{1-\theta_M}\right)\left(\frac{\theta_{OH}}{1-\theta}\right)_{OH} = -(\Delta\mu^\circ_M + \Delta\mu^\circ_{OH}) + RT \ln a^s_M \; a^s_{OH} \ldots\ldots\ldots\ldots \quad (3.18)$$

In the presence of pure MOH,

$$\log \frac{\theta}{1-\theta} = -\frac{2.303}{2RT}(\Delta\mu^\circ_M + \Delta\mu^\circ_{OH}) - pOH \ldots\ldots\ldots \quad (3.19)$$

and in the presence of salt

$$\log \frac{\theta}{1-\theta} = \frac{-2.303}{2RT}(\Delta\mu^\circ_M + \Delta\mu^\circ_{OH}) - \tfrac{1}{2} pOH + \tfrac{1}{2} \log a^s_M \ldots(/.20)$$

Equations (3.19) and (3.20) provide the relationship between pH of the solution, the fraction of base sorbed θ, and the affinity $-(\Delta\mu^\circ_M + \Delta\mu^\circ_{OH})$ of the alkali MOH for the fibres.

The essential process in the alkali titration is the dissociation of hydrogen ions from the ionizing groups in the protein. In keratin there is a considerable diversity of groups which may act as proton-donors, i.e. histidine (imidazole group), lysine (amino), terminal amino, and arginine (guanidine group.) In addition the phenoxyl group (hydroxyl group of tyrosine will also dissociate. There is a certain amount of controversy as to whether this group is available for titration. Horner[77] has recently provided evidence for its inclusion as a proton donor. Whether this only occurs after denaturation is unknown (page 14). Although the combination of alkali with protein involves the stripping of hydrogen ions from the charged basic groups, maximum base sorption is in fact governed by the sum of the free carboxyl and tyrosine groups.

Acid sorption is merely the back-titration of ionized carboxyl groups, and it has been shown that the affinity term ($\Delta\mu_H^o + \Delta\mu_A^o$) is equivalent to - RT ln Ka, where Ka is the intrinsic dissociation constant of carboxyl groups in the protein.

Alkali sorption, however, is more complex, because the reaction involves various different groups, each having its characteristic dissociation constant. In the calculation of a theoretical curve it is necessary to take into account the pk values of these groups in the protein and their respective proportions in the fibre. The amino acid analyses of Simmonds have been used for this purpose, and pk values from various sources[81,82] have been collected in Table 24.

TABLE 24.

BASE BINDING CAPACITY OF WOOL PREDICTED FROM AMINO ACID ANALYSIS.[29]

Group.	pk values.	Combining capacity millimoles/gm.
Histidine (imidazole)	6.0	0.058
Terminal amino	7.7	0.018
Tyrosine (hydroxy)	10.4	0.353
Lysine (amino)	10.5	0.192
Cysteine (sulphydryl)	10.8	0.025
Arginine (guanidine)	12.5	0.603
Free carboxyl		0.823
Tyrosine		0.353
Sum of free carboxyl and tyrosine		1.176

Using the equation for the ionization of a weak acid

$$pH = pka + \log \frac{[salt]}{[acid]} \quad \ldots\ldots\ldots \quad (3.21)$$

for the above data, an intrinsic titration curve was constructed, by the summation of the various contributions at each pH value.

Horner[77] has shown that, by assuming that the intrinsic affinity ($\Delta\mu^{\circ}_{M} + \Delta\mu^{\circ}_{OH}$) could be replaced by the term $-RT\ln k_b$, the pOH at which the group was half-saturated could be calculated. This gives the apparent pk value of the group in the fibre, i.e. pk_f.

From equation (3.18) putting $\theta_{OH} = 0.5$, and $\Delta\mu^{\circ}_{M} + \Delta\mu^{\circ}_{OH} = -RT \ln k_b$

$$pH_{\theta = 0.5} = \tfrac{1}{2} \log k_b - \tfrac{1}{2} \log \frac{\theta_M}{1 - \theta_M} = \tfrac{1}{2} \log pk_f \quad \ldots\ldots\ldots \quad (3.22)$$

where

$$\theta_M = \frac{B_M}{B_{M\,max.}}$$

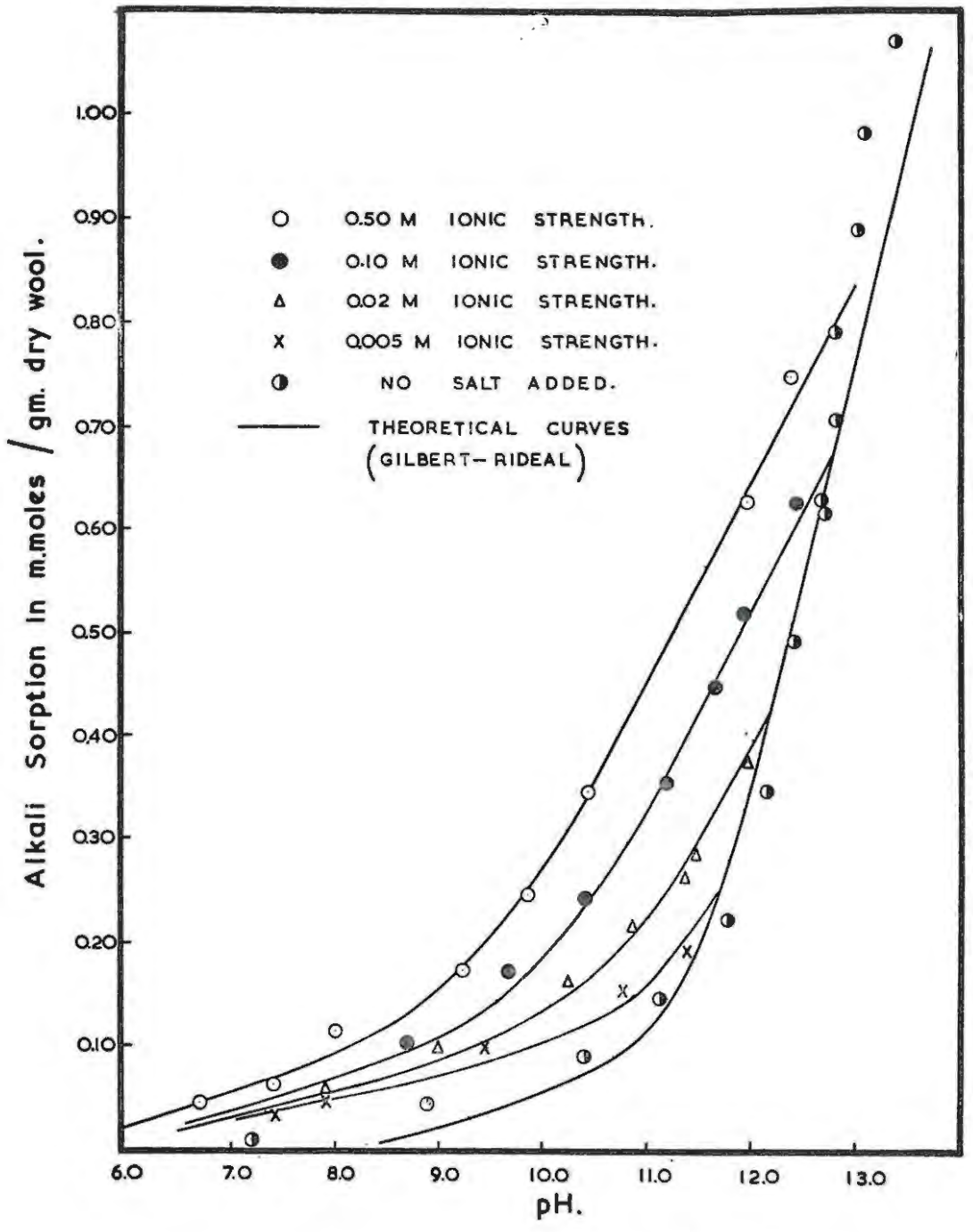


Fig. 20. *The alkali titration curves of wool keratin at various ionic strengths. (Solid lines calculated from the Gilbert-Rideal theory).*

B_M = amount of hydrogen ion dissociated (at $pH_{\theta = 0.5}$) and is obtained from the intrinsic titration curve.

B_M^{max} = sum of free carboxyl and tyrosine groups.

Substituting these values into equation (3.22)

$$RT \log \frac{\theta}{1 - \theta} = \tfrac{1}{2} \log k_f - pOH \ldots\ldots\ldots\ldots \quad (3.23)$$

$$= \tfrac{1}{2} \log k_f - 14 + pH \ldots\ldots\ldots\ldots \quad (3.24)$$

and in the presence of salt

$$RT \log \frac{\theta}{1 - \theta} = \tfrac{1}{2} \log k_f - \tfrac{1}{2} pOH + \tfrac{1}{2} \log a^s_M \ldots\ldots\ldots\ldots \quad (3.25)$$

$$= \tfrac{1}{2} \log k_f - 7 + \tfrac{1}{2} pH + \tfrac{1}{2} \log a^s_M \ldots\ldots\ldots\ldots \quad (3.26)$$

Taking values of pH at intervals of 0.3 units, θ was calculated for each group. Summation of these values gave theoretical curves for alkali-binding; the salt shift was illustrated by using equation (3.26). The theoretical curves are drawn in Fig. 20 together with the experimental data. Bearing in mind the experimental difficulties with dilute alkaline solutions, the agreement between experiment and theory is very close. Deviations occur in pH range 6 to 10.5 where a certain buffering effect occurs, probably as a result of solution of a small quantity of protein, or wool gelatin.

By omitting tyrosine from the calculations a theoretical curve was obtained which is inconsistent with the experimental data (Fig. 21). This provides conclusive evidence that the phenoxyl groups are titrated.

Horner[77] claims that the Gilbert-Rideal theory is unable to predict the shape of the titration curve accurately. However, this was not found to be the case, as theoretical and experimental curves coincide over a considerable range.

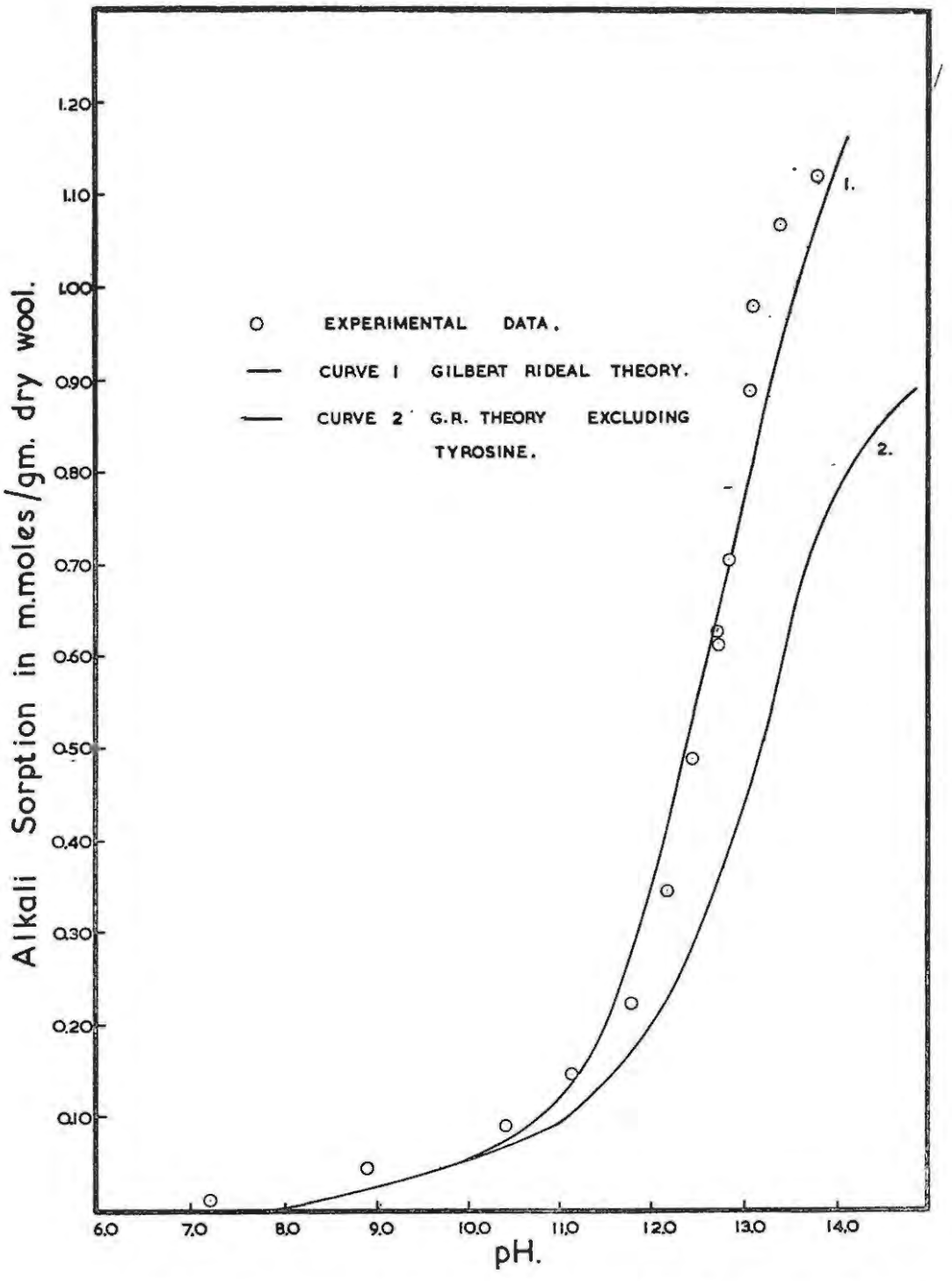


Fig. 21. *Comparison of the experimental data of alkali sorption with the theoretical curves of Gilbert and Rideal. In curve 2, tyrosine has been omitted from the calculations.*

B. The Donnan Theory.

By re-arranging equation (3.17) for the distribution of an electrolyte MOH between fibre and solution, we obtain

$$(\mu^{\circ}_{f})_{M} + RT \ln a^{f}_{M} + \psi F = (\mu^{\circ}_{s})_{M} + RT \ln a^{s}_{M} \quad \ldots\ldots\ldots\ldots \quad (3.27)$$

$$(\mu^{\circ}_{f})_{OH} + RT \ln a^{f}_{OH} - \psi F = (\mu^{\circ}_{s})_{OH} + RT \ln a^{s}_{OH} \quad \ldots\ldots\ldots\ldots \quad (3.28)$$

Adding (3.27) and (3.28) and equating terms to zero.

$$a^{f}_{M} \; a^{f}_{OH} = a^{s}_{M} \; a^{s}_{OH} \quad \ldots\ldots\ldots\ldots \quad (3.29)$$

The conditions of the system are represented in the following diagram, illustrating a swollen fibre containing solvent water.

FIBRE PHASE		SOLUTION PHASE
$-COO^-$	A	$[M^+] = C$
$-NH^+_3$	$A(1-\theta_1)$	$[OH^-] = D$
$-NH_2$	$A\theta_1$	
$-OH$	$T(1-\theta_2)$	
$-O^-$	$T\theta_2$	Volume = V mls.
$[M^+] = c$		
$[OH^-] = d$	Volume = v mls.	

θ_1 = fraction of 'zwitter ion' groups which are occupied

θ_2 = fraction of hydroxy groups which are occupied.

The amount of base combined, as determined experimentally, is given by:-

B = (total original base) - (uncombined base)

This includes base which has entered the fibre, but has not reacted with any group.

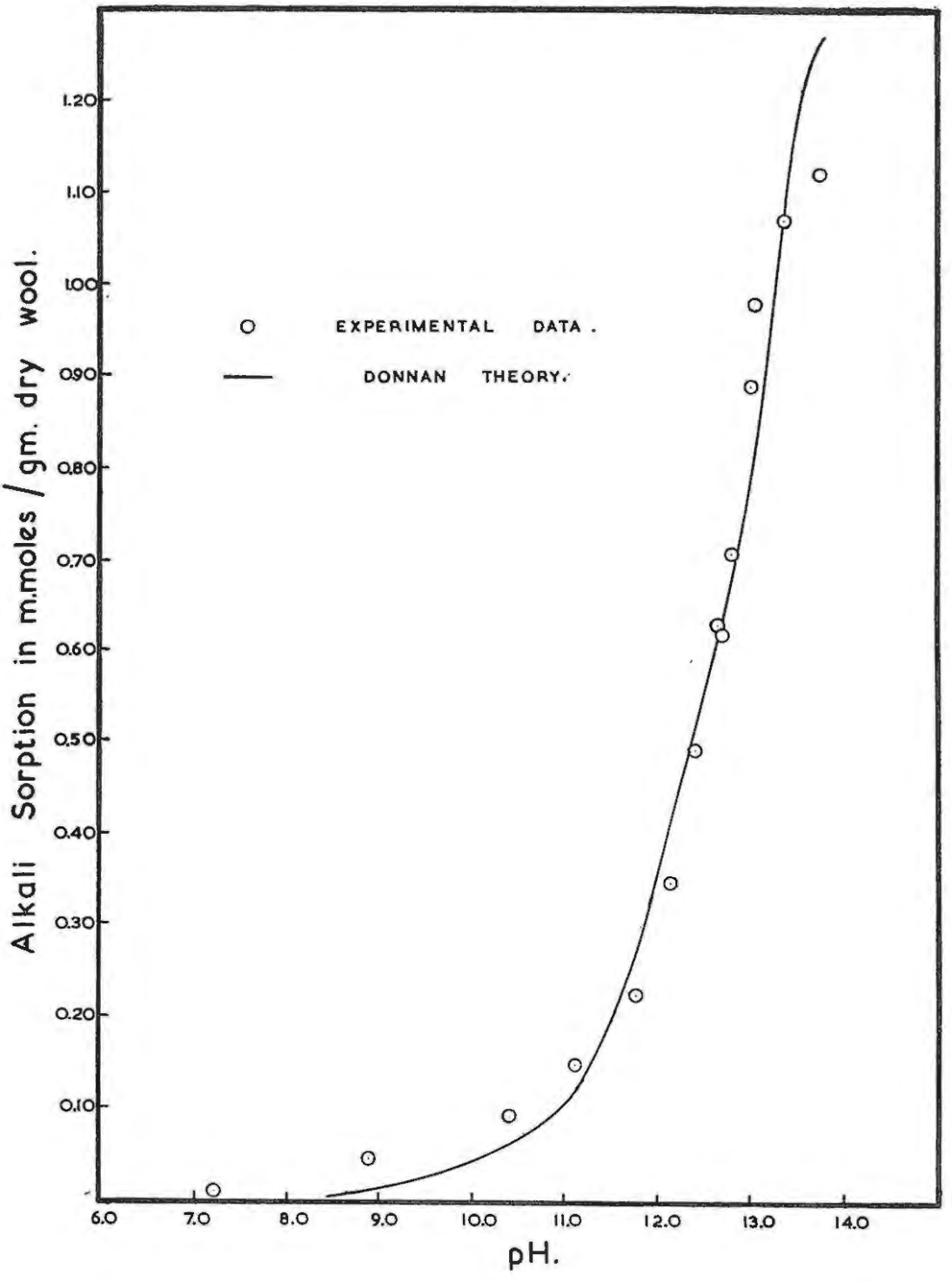


Fig. 22. *Comparison of the experimental data of alkali sorption with the theoretical curve calculated from the Donnan theory.*

$$B = (A\Theta_1 + T\Theta_2 + d) + VD - (V + v)\,D \quad \ldots\ldots\ldots \quad (3.30)$$

$$= (A\Theta_1 + T\Theta_2 + d) - vD \quad \ldots\ldots\ldots\ldots \quad (3.31)$$

B, c and d are in milliequivalents/gm. fibres
C and D are in gm. equivalents/litre.
For conditions of neutrality

$$c = A\Theta_1 + T\Theta_2 + d \quad \ldots\ldots\ldots\ldots \quad (3.32)$$

= total base within the fibre

$$= B + vD \quad \ldots\ldots\ldots\ldots \quad (3.33)$$

From equation (3.29)

$$\log a^f_M + \log a^f_{OH} = \log a^s_M + \log a^s_{OH} \quad \ldots\ldots\ldots\ldots \quad (3.34)$$

and by replacing activities by concentration terms

$$\log a^f_{OH} + \log c \approx \log C + \log a^s_{OH} \quad \ldots\ldots\ldots\ldots \quad (3.35)$$

$$\text{or } pOH_{int} \approx pOH_{ext} - \log C + \log (B/v + D) \quad \ldots\ldots\ldots\ldots \quad (3.36)$$

In the absence of salts C = D

$$pOH_{int} \approx 2\,pOH_{ext} + \log \left(\frac{B}{v}\right) + D \quad \ldots\ldots\ldots\ldots \quad (3.37)$$

The intrinsic titration curve for wool protein as used in the Gilbert-Rideal treatment was transposed to give the corresponding external titration curve for the fibrous protein, using equation (3.37). The theoretical curve is compared with experimental data in Fig. 22.

D is small and may be neglected below pH 11. Since the value of B includes base present in the internal solution, the latter must be added to that which has reacted with the protein to give B. Horner has taken the value of v as 0.285 mls/gm. for the volume of imbibed water in the alkaline reaction. This is the average value over the acid section, but is "chosen in the absence of swelling data". Elöd and Fröhlich[49] have determined the amount of swelling in sodium hydroxide solutions, and this data has been used in the following calculations. Using small values for v, Horner found considerable deviation from the experimental

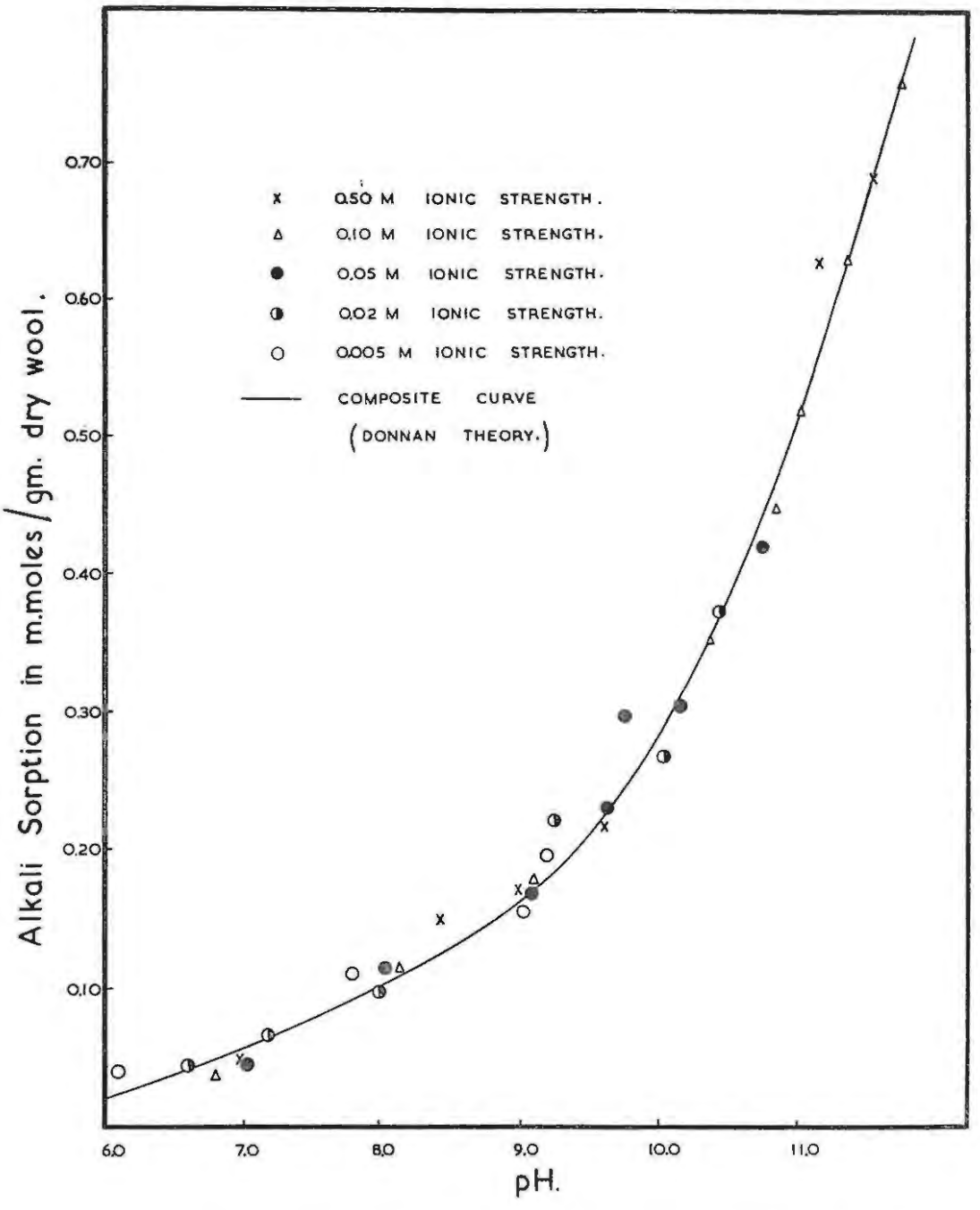


Fig. 23. *The composite curve for wool keratin calculated from the experimental data using the Donnan theory.*

data, but overcame this difficulty by tacitly assuming an excess of 0.050 milli.eqv. of free carboxyl groups. This assumption can hardly be justified. Although the amount of dicarboxylic acids in wool amounts to 1.80 m.eqv./gm., the minimum value of amide nitrogen reported by Leach and Parkhill[83] is approximately 1.0 m.eqv./gm, giving a free carboxyl content of about 0.80 to 0.85 m.eqvs./gm. From Simmonds data the sum of the free basic groups is 0.87 m.eqv./gm. Thus an excess of 0.050 m.eqv./gm. of free carboxyl is most unlikely.

Using the correct values for v, the experimental values both in the absence and presence of salt fall close to a composite curve. Fig. 23.

SUMMARY AND CONCLUSIONS.

1. The main theme of the present investigation has been the establishment of the titration curve of mohair and its comparison with the existing data on wool. However, so many improvements have been made in the methods for the determination of acid and alkali sorbed, that the titration curve of wool has also been determined anew.

   It is obviously more satisfactory if both sets of results are obtained in the same manner. Throughout this study the aim has been to obtain a maximum degree of accuracy.

2. Preparation of all material has been extremely careful. Wool and mohair samples have been purified by low-temperature scouring with non-ionic detergent, soxhlet extraction with ether and alcohol, and careful extraction of ionic impurities with dilute acid. Finally the samples have been rinsed with distilled water to the iso-electric point, pH 5.0 to 5.2. Processes which may cause damage to the fibres, either physical or chemical, have been avoided. This thorough purification programme is essential for the removal of all extraneous matter from the protein, a factor which is essential for accurate determinations of acid or alkali sorption.

3. The rate of sorption of acid or base from aqueous solution is dependent on diffusion both in the fibre and liquid phases, and normally requires an appreciable period before equilibrium is attained. During this period several side reactions occur, such as hydrolysis of peptide and amide bonds in acid solution and protein solubilisation, peptide bond hydrolysis and disulphide bond degradation in alkali solution. These reactions cause complications in the determination of acid or alkali bound. Although corrections for these factors may be applied, it is desirable to keep side reactions to a minimum, not only for the sake of the accuracy of the titration results but because these reactions produce chemical modification of the fibres.

Measurements on the effects of extended exposure to alkaline solutions have shown that considerable peptide hydrolysis occurs even in solutions as dilute as 0.01 m. KOH. A rapid sorption technique has now been developed which dimished the equilibration period from several hours to a few minutes. This method has been successfully applied for the determination of the titration curves between pH 0.5 and 12.5. Using this method, no ammonia or protein nitrogen can be detected in strongly acid solutions at 25°C. In alkaline solution the corrections for disulphide reaction and protein solubilisation are negligible below pH 11.5. Although the rapid sorption method greatly reduces the effects of these side reactions, corrections for several factors still have to be applied.

4. The correction for the disulphide reaction in alkaline solution has been throoughly examined in the light of recent advances in the knowledge of the reaction mcehanism. Previous workers have corrected alkali sorption data on the basis of the reaction

$$\rangle CH\ CH_2\text{-}\ SS\text{-}CH_2\text{-}CH\langle \xrightarrow{KOH} \rangle CH\text{-}CHO + HS^- + KS\ CH_2\ CH\langle$$

i.e. 1 mole KOH removed = 1 mole SH^- formed.

Although the discovery of the formation of lanthionine in alkali-treated wool has disproved this concept, recent authors have continued to make use of it for corrections. This has resulted no doubt from the fact that the lanthionine formation from cystine does not clearly account for the removal of alkali according to the reaction

$$\rangle CH\ CH_2\text{-}SS\text{-}CH_2\text{-}CH\langle \xrightarrow{KOH} \rangle CH\ CH_2S\ -\ CH_2\ CH\langle\ + S$$

OH^- is not consumed in this reaction, nor is there an increase in acidic groups formed in the protein during the process. Analysis of the alkaline liquors after the reaction with wool indicates that the sulphur is not present as sulphide (or hydrosulphide) but in the form

of a polysulphide, equivalent in quantity to the loss of cystine sulphur. On acidification the polysulphide gives the free sulphur and hydrogen sulphide, the free sulphur being readily reducible. Since the sulphide ion is displaced from solution as H_2S in the subsequent titration with standard acid, this has been excluded from the correction for the alkali removed by disulphide reaction. The amount of sulphide formed on acidification varies from 0 to 0.080 m.moles depending on the concentration of alkali, and thus previous data is over-corrected by this amount, which is approximately one-tenth of the total sulphur in solutions. The true correction has therefore been calculated on the basis:

m.moles KOH consumed = m.moles reducible sulphur.

5. Above pH 10 keratin becomes increasingly soluble. A correction for the amount of protein dissolved has been applied as this is subsequently back-titrated. This correction is only effective up to about pH 13.5 where approximatly 10% of the protein nitrogen is in solution.

6. The conditioned fibres contain approximately 15% water but immersion in aqueous solution causes the sorption of a considerable quantity of water, dependent on pH. This results in an apparent increase in the final concentration of sorbate and requires the introduction of a correction term which is negligible only below concentrations of 0.01 m. The alkali sorption values above pH 12.0 are considered to be not sufficiently accurate to warrant this correction, and it has been omitted here. Methods to determine the saturated water content in aqueous acid solution have been carefully examined and a new procedure has been developed. In this method fibres are immersed in aqueous solutions for a few hours and then rinsed in an extremely dilute solution of a non-ionic wetting-agent. The excess moisture is removed by squeezing and subsequent centrifuging for 10 minutes. The samples

are weighed and then dried to constant weight at 105°C. From these weights the saturation moisture content can be calculated. The method gives precise results.

7. The methods and corrections described have been applied to the acid titration curve of wool and the results compared with those obtained by previous workers. This has shown that better accuracy can be obtained by the new techniques.

8. The titration curves of wool and mohair have been established between pH 0.5 and 12.5 at 25°C. The curves are almost identical except from pH 0 to 1.5 and again in the iso-electric range between pH 4 and 9. This close similarity indicates that these proteins are alike in amphoteric properties, and that their content of dicarboxylic and dibasic amino acids may be very close. The differences between the curves in the range pH 4 to 9 suggests that wool may have more histidine than mohair. However, small changes in content of the dibasic amino acids, especially between amino acids of close pk values, will not noticeably effect the shape of the titration curve. Nevertheless, the pronounced difference of behaviour of the two fibres in various industrial processes probably cannot be attributed to a difference in acid-base behaviour.

9. Sorption rate data on wool and mohair have revealed that the latter requires slightly longer equilibration periods, especially in concentrated solutions. This may be caused by structural differences, but can also result from the difference in mean fibre diameter or from experimental difficulties. Mohair was, for example, more difficult to pack into the plunger of the rapid sorption apparatus due to its resilience.

10. Even at the highest concentration of HCl (0.8m.) no amide or peptide hydrolysis at 25°C of wool or mohair could be detected. This seems to refute the popular trade idea that mohair is more sensitive to acid than wool. In alkaline solution wool has been found to be approximately twice as soluble as mohair. This may have valuable application

in processes which are usually performed in alkaline medium, such as scouring and bleaching. Higher temperatures or more concentrated reagents may therefore be employed for mohair before the damage reaches the level experienced with wool.

11. Analysis of a series of mohair samples taken from animals of various ages has shown that the keratin may alter as the animal grows older. The strong correlation between acid and base sorption indicates that these differences probably arise from changes in content of free acidic and basic groups in the fibre, kid samples having more amphoteric sites than the adult samples. Variations have also been found to occur in samples removed from different positions on the same animal. Whether this is caused by differences in the ratio of primary to secondary follicles or by differences in density of fleece is unknown. Amino acid analyses on these samples may provide the solution.

12. The titration curve for tip mohair has been found to follow the same trends shown by photo-chemically damaged wool, i.e. no change in acid titration curve, but an increase in alkali sorption noticeable mainly between pH 8 and 11. This suggests that mohair undergoes photochemical degradation similar to that experienced by wool. The actual processes which occur are still unknown. Corrections for disulphide reaction and protein solubility have been found to be higher for the tip samples, indicating that these portions are more sensitive to alkali than the unexposed portions.

13. Several theories of acid and base sorption have been examined in order to decide whether any particular theory could displace all others. The Donnan Theory and the Gilbert-Rideal Theory are the most satisfactory, and for several years now there has been a considerable controversy as to their respective merits. The data from the present study, which for reasons given above is considerably more accurate than previous data, has been used, together with more recent values for amino acid composition, volume swelling, etc., to test the validity of the two theories. Theoretical titration curves calculated from both

theories with this data are extremely close to the experimental values. This has not been obtained by previous authors who, when using less reliable results, have always found one theory to be more satisfactory than the other. In actual fact, the agreement of the results of these two theories may be expected, as they reach similar conclusions from different viewpoints.

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