THE CONSTITUTION OF THE KERATIN MOLECULE.

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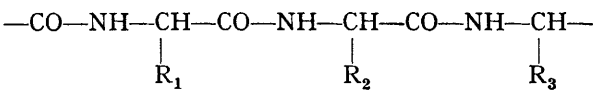
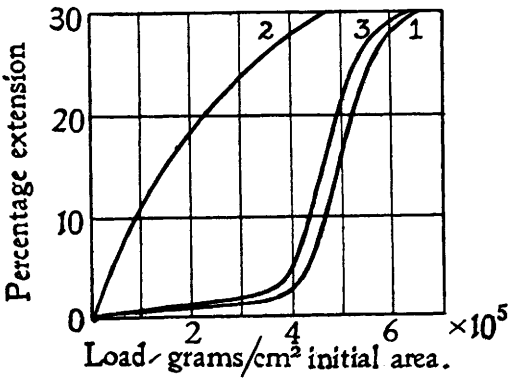
The wool fibre is now known to consist of micelles which are long in comparison with their thickness. They are probably lamellar in shape, their thickness does not exceed 200 Å.U., and they are arranged with their long axes parallel to the length of the fibre.[1] Although the constitution of the micelles is still unknown, X-ray analysis of wool and related animal hairs has led Astbury[2] to suggest a fundamental principle of protein structure, which accounts successfully for the elastic properties of wool and silk and gives a new significance to the preponderance of α-amino acids among the products of protein hydrolysis. Proteins are assumed to consist essentially of long peptide chains having the general formula :—



The specific properties of any particular protein are determined by the configuration of the peptide chain and the constitution of the side chains (R_1, R_2, etc.), whose nature depends on the α-amino acids from which they are derived. In the case of the wool fibre, the peptide chains, which are coiled into a series of pseudo hexagons, are arranged parallel to its length, and cohesion within the micelle is determined either by van der Waals forces between the peptide chains and their side chains, or by chemical combination between the side chains of adjacent peptide chains.[3, 23]

It has recently been shown that acids have a peculiar action on the micelles of the wool fibre.[1] In formic acid, for example, the fibre swells to a striking degree, the Hooke's Law section of the load-extension curve disappears, the fibre acquires a rubber-like elasticity and plasticity is at a minimum. Silk, immersed in formic acid, shows similar properties, but in this case it is also known that the X-ray fibre diagram disappears. By analogy with silk it was argued that the crystal structure of wool must similarly disappear in formic acid and Astbury has since confirmed the deduction. Such observations can be explained only by assuming that the peptide chains within the micelles are separated by the action of acid, *i.e.* a kind of micelle subdivision takes place . The degree of subdivision is unknown, but since the wool fibre is not dissolved by formic acid in the cold, subdivision to simple peptide chains can scarcely have occurred. So great a degree of subdivision is in any event improbable because cystine must form one of the side links between adjacent chains, and subdivision to pairs of chains linked laterally by the cystine molecule is the greatest degree of subdivision to be expected.



1 Water. 2 Formic acid. 3 Water.

FIG. 1.

The most interesting feature of the action of acids on wool, however, is not that they possess this property of subdividing the micelles, but that their action is almost completely reversed by prolonged washing of the fibre in running water. The phenomenon is illustrated by the three load-extension curves of Fig. 1, representing the properties of the same wool fibre first in distilled water, then in 98-100 per cent. formic acid, and finally again in distilled water after prolonged washing in running water.

It is clear that acids are capable of separating the peptide chains of the micelles of the wool fibre against their cohesive forces; and if the peptide chains are linked together chemically through their side chains, such side linkages as are opened by acid must have the ability to reform spontaneously when the acid is removed. Whatever may be the nature of the cohesive forces between the peptide chains, it is evident that a study of the action of acids on wool is capable of giving information as to their character.

Experimental.

(a) Technique.

The above preliminary survey[1] of the action of acids on wool was carried out with purely arbitrary concentrations of different acids selected at random. In the present endeavour to elucidate the nature of the cohesive forces within the micelle, a study was first made of the extent of micelle subdivision as a function of the p_H of the environment, with acids of various strengths. For this purpose, the technique followed in earlier investigations of the structure of the wool fibre was again adopted.[4] Fibres, uniform in diameter along the length, were selected from Cotswold wool which had been purified by successive extraction with alcohol, ether and water. In each case, the cross-sectional area was estimated by making thirty measurements of diameter along the 5-centimetre length used to determine the load-extension curve. Calibration was carried out under desorption conditions in a room maintained at 65 per cent. relative humidity and 22·2° C.

The load-extension curve of each fibre was first determined in distilled water (p_H 5·5) at 22·2° C., using a rate of loading of 1×10^5 g./cm.^{2} per minute, extension being limited to 30 per cent. because it has been shown that wool fibres undergo serious permanent alteration in elastic properties when stretched beyond this point.[4] Each fibre was then immersed in a large volume of acid solution of known p_H, the latter being determined potentiometrically by means of the hydrogen electrode, or the quinhydrone electrode, according to the nature of the acid used. After twenty-four hours, during which time the acid solution was renewed at intervals, the load-extension curve of each fibre was redetermined in the solution with which it had come to equilibrium. The two load-extension curves were then used to evaluate the work (W) needed to perform a 30 per cent. extension in water and in acid solution. The difference between the two values of W gives a measure of the extent of reaction with acid, and it is convenient to express this difference as a percentage of the work needed to stretch the fibre in water at the standard p_H (5·5). Such a method of treating the results has the merit of eliminating possible errors in estimating the cross-sectional area of wool fibres from the comparison of various acid solutions in reaction with wool.

(b) p_H-Stability Curve.[5]

By means of the ultra-centrifugal method developed in his laboratory, Svedberg [6] has measured the stability of a number of soluble proteins as a function of the p_H of the environment. Each of the monodisperse proteins was found to be stable over a fairly wide range of p_H, called the p_H-stability region, which includes the isoelectric point. The technique described above affords a means of extending Svedberg's observations to the insoluble proteins, and since his experiments have not been confirmed by any independent method, an additional interest is associated with the properties of wool keratin. For these reasons, the first experiments to be carried out were not limited to the action of acids on wool but covered a wide range of p_H. The method of experiment was that already described, two determinations of the percentage reduction in the work required to perform a 30 per cent. extension being made at each p_H with different fibres, and the average taken. A summary of the results is given in Table I.

TABLE I.

Reagent.	p_H.	Work Reduction. Per Cent.
Sulphuric acid . . .	$\bar{1}$·96	33·8
,, ,, ...	0·57	33·9
,, ,, ...	1·06	33·2
,, ,, ...	1·85	30·2
,, ,, ...	2·09	24·3
,, ,, ...	2·98	9·4
,, ,, ...	3·55	2·7
,, ,, ...	4·02	1·4
,, ,, ...	4·55	1·5
,, ,, ...	5·36	1·1
Distilled water . . .	5·48	1·2
Borax	7·40	1·1
,, ...	8·02	2·2
Sodium carbonate . . .	8·58	3·0
Borax	9·19	6·0
Sodium carbonate . . .	10·18	5·8
,, ,, . . .	10·62	9·8
,, ,, . . .	11·07	13·5
Caustic soda	11·86	16·3
,, ,, ...	12·82	53·1

From the graph, Fig. 2, it is evident that wool is immune to the action of acid and alkali between p_H 4 and p_H 8 (approx.). The micelles undergo no subdivision within these limits although combination with acid is known to begin at p_H 4·8. Detailed discussion of the p_H-stability curve must be deferred until later in the paper, but the preceding experiments served to show that alkali is unsuitable for precise study of the constitution of wool keratin. The load-extension curves of fibres in alkaline solution indicated quite clearly that attack in such cases is not limited to the side linkages between peptide chains, the latter being themselves attacked as would be expected from their constitution.

(c) The Donnan Equilibrium.

Although sulphuric acid, like formic acid, is capable of facilitating fibre extension by its action in separating the peptide chains of the micelles, the weaker formic acid is twice as effective at p_H 0·0. In order to discover the reason for this peculiarity, and to generalise the observations, attention was next directed to the influence of the strength of the acid on its reaction with wool. A number of acids were chosen, ranging in strength from hydrochloric acid ($K = 10^7$) to acetic acid ($K = 1{\cdot}8 \times 10^{-5}$), and the elastic properties of the wool fibre studied as before in solutions of known p_H. The results are summarised in Table II.

The data for hydrochloric, phosphoric and formic acids are illustrated in Fig. 3, and it is evident that the action of acids in facilitating fibre extension increases smoothly with decreasing ionisation constant. For example, the curve for hydrochloric acid shows a maximum at p_H 1·0, that for sulphuric acid (Fig. 2) is flat below p_H 1·0, whereas the curve for phosphoric acid first tends to become flat and then shows a rapid rise below p_H 0·0. Finally, the curves for chloracetic, formic and acetic acids all show a continuous rise with decreasing p_H, the slope becoming steeper with acids of decreasing ionisation constant. Glycollic acid is exceptional in character, but this may be due to the hydroxyl group, which is known to have a specific action on wool.

TABLE II.

Acid.	p_K.	p_H.	Work Reduction. Per Cent.
Hydrochloric acid .	($K = 10^7$)	$\bar{1}\cdot35$	32·9
		0·08	34·7
		1·02	36·1
		1·68	27·2
		1·98	20·8
		2·63	7·7
		2·95	5·3
		3·90	1·3
Oxalic acid . .	1·42	0·62	37·0
		1·06	34·1
		1·90	24·4
		2·56	11·0
		3·28	4·3
		3·80	2·3
Sulphuric acid . .	1·77	See Table I.	
Phosphoric acid .	2·0	$\bar{1}\cdot14$	53·5
		$\bar{1}\cdot56$	39·5
		0·36	35·9
		1·20	33·3
		1·90	19·7
		2·59	8·8
		3·12	3·6
		3·46	2·2
Mono-chloracetic acid	2·8	$\bar{1}\cdot65$	53·1
		0·98	37·3
		1·65	26·6
		2·14	16·8
		2·55	9·3
		3·02	3·3
Formic acid . .	3·7	0·76	51·7
		1·84	28·9
		2·41	15·8
		2·90	6·6
		3·37	3·6
		4·53	2·4
Glycollic acid . .	3·8	$\bar{1}\cdot56$	44·0
		0·69	37·7
		1·21	34·1
		2·03	18·6
		2·57	8·4
		3·03	3·9
Acetic acid . .	4·7	1·12	46·9
		1·96	25·3
		2·61	8·5
		3·05	3·4
		3·69	1·6

The fact that the reduction in the work required to stretch fibres in hydrochloric acid solution reaches a maximum at p_H 1·0 and then falls, suggests at once that the degree of swelling of the fibre plays some part in determining its resistance to extension; for according to the Procter-Wilson theory,[7] the swelling of proteins in solutions of strong acids must first increase, reach a maximum, and then diminish as the hy-

drogen-ion concentration is increased. The same theory is capable of explaining the greater effectiveness of weak acids in facilitating fibre extension. Since wool and its ions are non-diffusible, the necessary

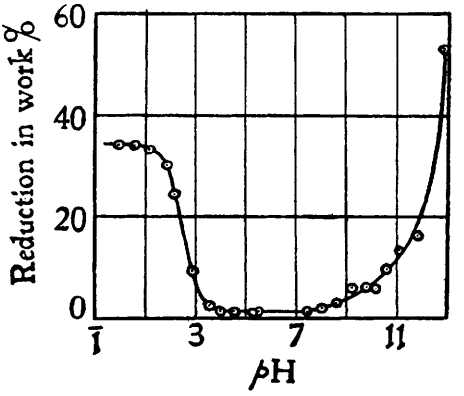


Fig. 2.

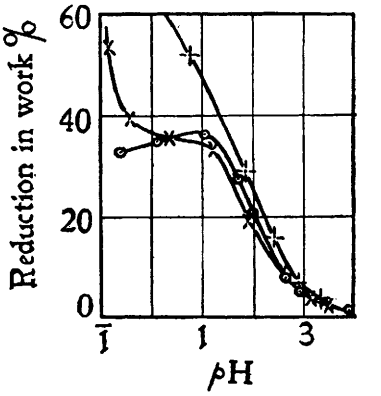


Fig. 3.

conditions for the establishment of a Donnan equilibrium are present, and if the equilibrium concentrations of the several ions are represented by the algebraic symbols in the diagram, then $x^2 = y(y + z)$.

$(z)W^+$	
$(z)Cl^-$	$H^+(x)$
$(y)H^+$	$Cl^-(x)$
$(y)Cl^-$	
(fibre)	(medium)

The excess concentration of diffusible ions inside the fibre determines the extent of swelling and is given by the expression

$$e = 2y + z - 2x = \frac{(x - y)^2}{y}.$$

With weak acids, such as acetic acid, the wool forms highly ionisable salts which depress the ionisation of the free acid inside the fibre, so that y is small and $(x - y)$ is large. The quantity $e = \frac{(x - y)^2}{y}$ is therefore large and swelling is extremely high. It is evident, therefore, that the swelling of wool fibres at the same p_H, but in different acids, will vary inversely as their ionisation constants, and the action of weak acids in reducing the resistance to extension of the wool fibre to a greater degree than strong acids finds a simple explanation on this basis. It need hardly be said that direct observation confirms the preceding deductions—the maximum increase in diameter of wool fibres on transference from water to sulphuric acid is 2 per cent., whereas with formic acid the increase is 50 per cent. or more.

The applicability of the Procter-Wilson theory of swelling to the action of acids on wool does not necessarily imply that strict chemical compounds are formed between wool and acids. Indeed, the evidence so far advanced in this paper has little or nothing to say regarding the mode of combination of acids and wool, and all the preceding phenomena

can be " explained " in terms of swelling without further inquiry. Nevertheless, one striking fact emerges which demands further discussion. Although the swelling of wool fibres in sulphuric acid is so small compared with that in formic acid, the latter is only twice as effective in facilitating fibre extension. This suggests that the first action of all acids on wool is essentially the same and causes a reduction in the resistance to extension. Superimposed on this first effect is that of swelling, which is specific for each acid according to its ionisation constant.

(d) Depression of Swelling.

1. *Neutral salt action.*—The validity of the preceding hypothesis can be examined by studying the action of acids on wool in the absence of swelling. A possible method is to use neutral salts in conjunction with acid, the neutral salt having the effect of depressing swelling without necessarily interfering with the action of acid. Accordingly, the elastic properties of wool fibres were studied in $N/5$ NaCl solutions containing varying amounts of hydrochloric acid. In view of the great interest of the resulting data, the experiment was extended to include alkaline solutions and the complete set of results is summarised in Table III.

TABLE III.

Reagent.	p_H.	Work Reduction. Per Cent.
HCl + $N/5$ NaCl	0·01	32·6
,, ,,	1·00	35·3
,, ,,	1·71	36·5
,, ,,	1·97	31·2
,, ,,	2·72	21·1
,, ,,	3·02	18·6
,, ,,	3·83	9·9
,, ,,	4·95	2·2
Na_2CO_3 + $N/5$ NaCl	7·00	1·5
,, ,,	8·77	3·7
,, ,,	9·97	8·8
,, ,,	10·59	12·2
,, ,,	10·97	15·4
NaOH + $N/5$ NaCl	11·59	22·7
,, ,,	12·00	25·1

The data are graphed in Fig. 4 along with the corresponding results for hydrochloric acid and alkaline reagents in absence of common salt. An examination of the two curves reveals a number of interesting points. In the first place, it has to be remembered that the p_H within the wool fibre is incapable of direct measurement, and all recorded values are those of the media with which the wool was in equilibrium. But in presence of salt, the p_H inside and outside the fibre approximate to one another, so that the curve relating to salt solutions always lies inside the other. Despite the action of salt in minimising differences of p_H and in reducing swelling, the maximum reduction in the resistance to extension of wool fibres in hydrochloric acid solution is precisely the same in presence and absence of salt. Swelling is not entirely eliminated,[8] however, and below p_H 1·6 the curve shows a fall similar to that in absence of salt. Nevertheless the fact that swelling can be reduced without interfering with

the action of acid in facilitating fibre-extension is a confirmation of the view that swelling is not the primary cause of a reduced resistance to extension.

A second important feature of the curve is that the range of the p_H-stability region is reduced in presence of salt and occupies only p_H 5 to 7 instead of p_H 4 to 8. The action of acid begins at p_H 5·0, in good agreement with the value of 4·8 obtained in 1925 [9] for the point at which combination with acid commences. Although no data could be obtained for the alkali side, the isoelectric point of wool was provisionally defined as p_H 4·8. The isoelectric point was subsequently redetermined by Meunier and Rey,[10] who gave the value p_H 3·6 to 3·8, afterwards modified to p_H 4·0 to 4·5. A more recent determination by Elöd and Silva [11]

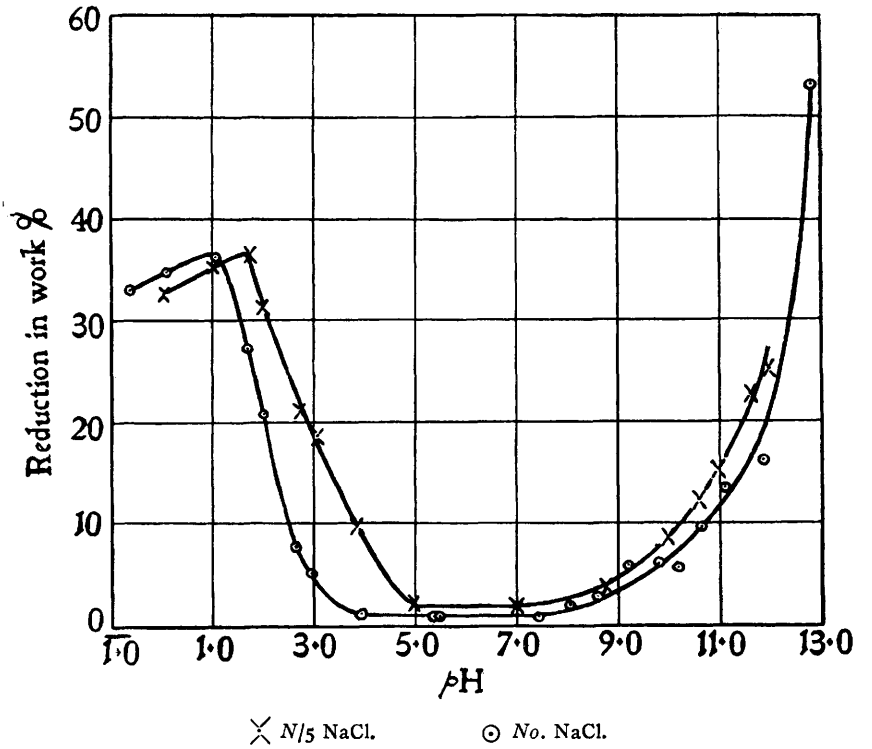


FIG. 4.

agrees precisely with that established in 1925, and it has come to be recognised that the isoelectric point of wool is at p_H 4·8. This view now needs modification. From the curve relating the ease of extension of wool fibres to the p_H of $N/5$ sodium chloride solutions containing varying amounts of hydrochloric acid, it is evident that combination with acid begins at p_H 5·0 or thereabouts. Using the same argument with reference to the alkali side of the curve, it seems probable that combination with alkali commences at p_H 7·0. In other words, *wool does not possess an isoelectric point but an isoelectric region from* p_H 5 *to* p_H 7. Further evidence in support of this view will be given later in the paper.

The diminution in the range of the p_H-stability region in presence of salt is, in part, due simply to its action in making internal and external

p_H identical. It is, however, more than doubtful whether this is the whole explanation because, at p_H 4·8, internal and external p_H are identical even in absence of salt, and both curves should originate in this point. That this is not so seems to indicate that salt does not function simply in the way specified above. Such a deduction is supported by the work of Csapo,[12] who found that considerably more acid is fixed by gelatin in presence of salt than in its absence. Similarly, Gerngross and Loewe [13] found an increased fixation of alkali by hide powder in presence of neutral salts. The action of neutral salts on wool clearly requires further investigation, and there is some probability that it will serve to explain the origin of p_H-stability regions in general.

2. *Diazotised wool.*—Attention was next turned to diazotised wool as a possible means of studying the action of acid in the absence of swelling. The load-extension curves of single wool fibres were first determined in water at p_H 5·5. After being allowed to return to the original length, each fibre was diazotised separately in a mixture of 50 c.c. 1·042 $N/1$ H_2SO_4 and 10 c.c. $N/1$ $NaNO_2$ for sixteen and a half hours. After being washed in running water for twenty-four hours to remove all traces of acid, the fibres were again stretched in water at the standard p_H. Although the two load-extension curves were thus determined at the same p_H, the work required to perform a 30 per cent. extension was 17 per cent. less after diazotisation. Hence nitrous acid has precisely the same action as other acids in facilitating fibre extension, except that its effect on the fibre is not reversed by prolonged washing in running water. Lest it should be supposed that the preceding value of 17 per cent. represents the maximum effect of nitrous acid in facilitating fibre extension, it must be emphasised that complete reaction with nitrous acid is not attained except under well-defined conditions. The concentration of nitrous acid, the p_H of the reaction mixture and the time of reaction all affect the extent of attack. In the preceding experiment, the concentration of nitrous acid is too low to give complete reaction in the time allowed, and the part played by acidity (p_H) is well illustrated by the following experiment in which single wool fibres were diazotised in a mixture of 50 c.c. water, 10 c.c. $N/1$ $NaNO_2$ and 6 c.c. of 98-100 per cent. formic acid for sixteen and a half hours. Although the concentration of formic acid at the moment of mixing was approximately 2·4 N, yet diazotisation caused only 9·3 per cent. reduction in the work needed to perform a 30 per cent. extension in water. The reason for this is, as already indicated, that the p_H given by the relatively high concentration of formic acid is greater than that of $N/1$ sulphuric acid. Such properties are by no means peculiar to wool: they have already been subjected to detailed study by Plimmer [14] in the case of amides and other amino compounds.

Despite the fact that nitrous acid is able to facilitate fibre extension in much the same way as other acids, the swelling of diazotised wool fibres in water at p_H 5·5 is identical with that of untreated fibres, within the limits of microscopic measurement. Actually, the amount of water adsorbed by diazotised wool from atmospheres at different humidities up to saturation is always less than in the case of untreated wool. This would suggest that the swelling of diazotised wool in water is slightly less than that of untreated wool.

Such experiments as the preceding make it certain that acids do not facilitate fibre extension merely by promoting increased swelling. In other words, swelling is a consequence and not a cause of micelle subdivision by acid.

(e) The Adsorption of Acid by Wool.

So far, therefore, it has been shown that nitrous acid is similar to other acids in causing a reduction in the resistance to extension, but its effect on the fibre is incapable of being reversed by prolonged washing in running water. This peculiarity is clearly due to the irreversible conversion of amino groups into hydroxyl groups, and the otherwise similar action of nitrous and other acids on wool suggests that all acids react primarily with the free amino groups present. In the case of strong acids like HCl and H_2SO_4, reference to Figs. 2 and 3 shows that reaction with wool is complete at p_H 1·0 (external) and if such acids react primarily with the free amino groups present, the amount of acid adsorbed at p_H 1·0 should be closely related to the free amino nitrogen content of wool as directly determined. Both sets of determinations were therefore carried out as follows.

1. *The amount of acid adsorbed.*—The wool chosen for experiment was in the form of top and was first freed from adsorbed soap and residual oil by extraction with alcohol and ether in a Soxhlet apparatus. It was then brought to p_H 4·8 by means of a dilute solution of hydrochloric acid. Excess liquid was removed by centrifuging the wool, which was then allowed to dry by exposure to the air. Approximate 2-gram samples of wool were transferred to weighing bottles and their dry weights determined by exposure to phosphorus pentoxide for fourteen days *in vacuo*. Each 2-gram sample was then immersed in 200 c.c. of hydrochloric acid solution of known p_H and allowed at least two days in which to reach equilibrium, the temperature being 22·2° C. At the end of this time, the wool was removed and the p_H of the solution determined electrometrically. With high concentrations of acid, the change in p_H due to adsorption of acid by wool was too small to allow the amount of acid adsorbed to be calculated with any degree of precision. In such cases, direct titration with alkali was used. The results for Cotswold and Leicester wools were obtained in this way and are given in Table IV. The latter also includes data for South Devon wool, obtained in conjunction with Mr. A. E. Battye, using slightly different experimental conditions. The volume of solution used with each 2-gram sample of wool was only 100 c.c. and the temperature 25° C., but since the only significant difference is that of temperature, and as this is small, the results are included for comparison.

TABLE IV.

Cotswold.		Leicester.		South Devon.	
p_H.	C.c. *N*/1 Acid Adsorbed per 100 g. Wool.	p_H.	C.c. *N*/1 Acid Adsorbed per 100 g. Wool.	p_H.	C.c. *N*/1 Acid Adsorbed per 100 g. Wool.
0·75	80·7	0·78	81·9	0·66	80·5
0·89	80·8	1·08	81·8	1·15	78·6
1·28	74·7	1·28	80·0	1·52	70·5
1·54	68·5	1·54	72·1	1·99	47·8
1·80	60·1	1·81	59·0	2·38	28·6
2·33	34·9	2·32	38·8	2·75	16·4
2·70	20·2	2·71	18·5	3·17	6·7
3·18	11·2	3·17	11·5	3·55	4·7

In Fig. 5, the amount of acid adsorbed is shown as a function of the p_H of the equilibrium solution, a mean curve being drawn through the data for the several wools. Much work has been done on the subject of acid adsorption by wool, but this is apparently the first complete titration curve to be obtained. Its form shows quite clearly that combination of wool with acid is complete at p_H 1·0 when 80 c.c. of $N/1$ acid are adsorbed by 100 g. wool. This result is in good agreement with that of Meyer,[15] based on work in which no attempt was made to study adsorption as a function of p_H.

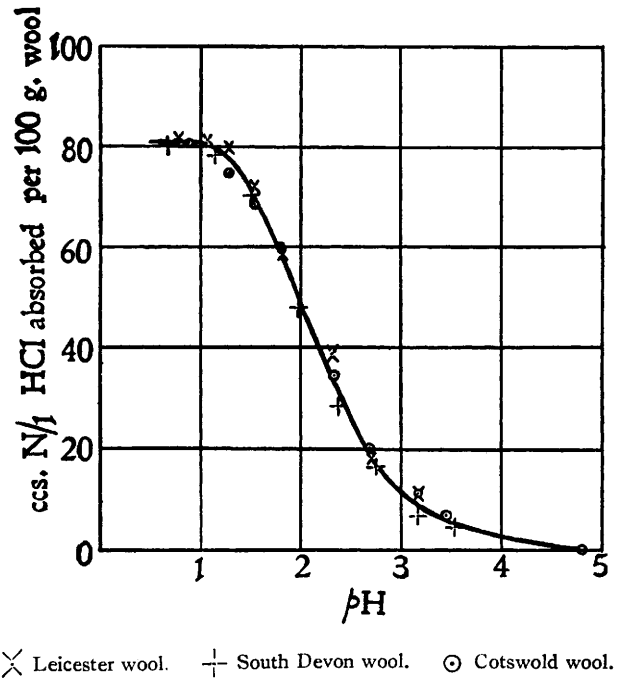


FIG. 5.

If the amount of hydrochloric acid adsorbed by wool from solutions of varying p_H is plotted against the corresponding values for the reduction in the work required to perform a 30 per cent. extension (Table II.), a linear relationship is found to hold between the two, as shown in Fig. 6. Each molecule of hydrochloric acid combined with wool therefore contributes a definite quantum to the total reduction in the resistance to extension observed in strongly acid solutions. A stoichiometric relationship of this kind affords strong evidence in favour of the view that the process of acid adsorption by wool is one of strict chemical combination.

2. *The free amino nitrogen content of wool.*—Van Slyke and Birchard [16] have shown that the free amino nitrogen content of a number of proteins corresponds very closely with half the lysine nitrogen. The conditions of experiment—particularly as regards the time of reaction—were, however, such as to preclude the evolution of nitrogen from arginine. If the time of reaction is increased, arginine gives off increasing quantities of nitrogen and, according to Plimmer,[17] the amount evolved in twenty-four hours corresponds approximately with two reacting nitrogen atoms. In the case of wool, which contains arginine and lysine, the free amino groups from both sources must take part in acid adsorption. For purposes of correlation with the amount of acid adsorbed, estimation of the free amino nitrogen in wool must therefore be carried out under conditions such that arginine, as well as lysine, will react with nitrous acid. In the light of Plimmer's observations, it was decided to estimate free amino

nitrogen in wool by means of the usual van Slyke reagent, allowing the reaction to proceed for twenty-four hours. Using the technique due to Meunier and Rey,[10] the value found for untreated Cotswold wool was 0·94 per cent. Plimmer[14] has also shown that the rate of evolution of nitrogen from compounds such as arginine increases with the acidity of the reaction mixture. Similar experiments have recently been carried out with wool,[18] with similar results, but all the curves showing the amount of nitrogen evolved as a function of the time of reaction, converged to give a limiting amount of free amino nitrogen of 0·92 per cent. The concentration of sodium nitrite was, however, only half that employed by van Slyke, and when the full concentration was used, a limiting amino nitrogen content of 1·19 per cent. was found. In all probability, this value is too high because the form of the curves, showing the rate of evolution of nitrogen, suggested that actual decomposition of the wool had taken place to a small extent. There can, however, be no doubt that the free amino nitrogen content of wool lies between 0·92 per cent. and 1·19 per cent., the lower value being more nearly correct.

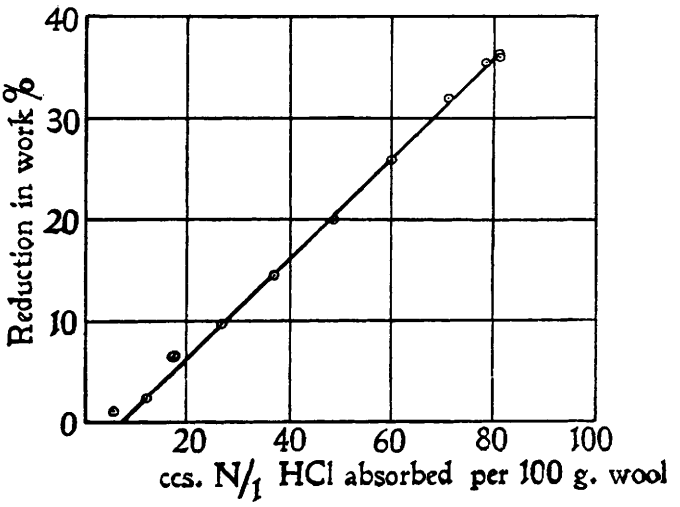


FIG. 6.

If wool contains 0·92 per cent. of free amino nitrogen, the amount of hydrochloric acid adsorbed by wool on this account should be 65·7 c.c. of $N/1$ acid per 100 grams. Adsorption of acid is, however, not limited to combination with free amino groups: according to Vickery and Block,[19] wool contains 0·66 per cent. of histidine, and an additional 4·3 c.c. of $N/1$ acid per 100 grams of wool would be adsorbed on this account. Thus, using the minimum value for the free amino nitrogen content of wool, the calculated amount of hydrochloric acid adsorbed when combination is complete is 70 c.c. of $N/1$ acid per 100 grams. In order to give complete agreement with the experimental value of 80 c.c., the amino nitrogen content of wool would have to be 1·06 per cent., a value lying midway between the two values given above. It is thus possible to account somewhat precisely for the amount of acid adsorbed by wool in terms of its content of histidine and free amino nitrogen.

If Astbury's hypothesis[2] of long-chain protein molecules is correct, the α-amino groups of arginine and lysine must be bound into these molecules, leaving the terminal amino groups free for combination with acid. Hence the amino nitrogen of wool is derived from the guanidine group of arginine and the ε-amino group of lysine. The arginine and lysine content of wool have been determined by various workers, and

there should be close agreement between the amount of free amino nitrogen in wool, calculated from its arginine and lysine content, and the direct determination. The appropriate data are summarised in Table V.

TABLE V.

Observer.	Percentage by Weight of		Calculated Free Amino Nitrogen. Per Cent.
	Arginine.	Lysine.	
Vickery and Block [19] (wool) . .	7·8	2·3	0·85
Marston [20] (wool)	10·2	2·8	1·09
Stewart and Rimington [21] (wool) .	6·0	2·2	0·70
Vickery and Leavenworth [22] (human hair)	8·0	2·5	0·88

Such results suggest that the arginine and lysine content of different wools may be variable, but it is apparent that the free amino nitrogen of wool can be accounted for more or less precisely in terms of its arginine and lysine content. This is especially true since Vickery suspects his determinations to be low, at least in the case of human hair.

There is thus a close relationship between the amount of free amino nitrogen in wool, its arginine and lysine content, and the amount of acid adsorbed when combination is complete.

(f) The Constitution of the Keratin Molecule.[23]

Knowing that the hydrochloric acid adsorbed by wool combines mainly with the terminal amino groups of arginine and lysine, it is neces. sary to inquire how this reaction is able to facilitate fibre-extension. Since the α-amino and carboxyl groups of both these acids (and histidine) are assumed to be embodied in the long peptide chains (*vide supra*), the terminal amino groups are in each case part of a side chain and they may, or may not, be combined with the side chains of adjacent peptide chains. If the amino groups remain uncombined, their dissociation constants will be those of the free amino acids:

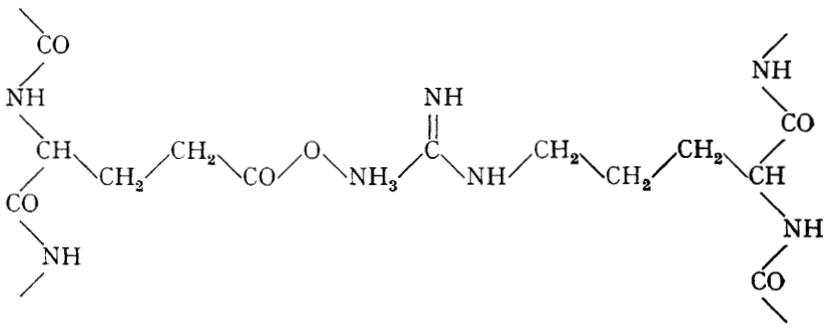
$$\text{arginine} \ldots k_b = 1 \times 10^{-5}$$
$$\text{lysine} \ldots k_b = 3{\cdot}2 \times 10^{-5}$$

and combination of wool with acid should be *complete* at p_H 7. Actually, combination with acid does not *begin* above p_H 5·0.

Conversely, if the terminal amino groups of the side chains are free, the basic dissociation constant of wool should be approximately 1×10^{-5}. The true value can be deduced as follows. It has already been shown that the reduction in the resistance to extension of fibres is strictly proportional to the amount of acid adsorbed at the same p_H (Fig. 6). Hence although the dissociation constant of wool cannot be deduced directly from the acid adsorption curve of Fig. 5, because of the difference between internal and external p_H, it can be deduced from the elastic properties of wool in salt solutions where internal and external p_H are identical. Referring to Fig. 4, it is evident that combination of wool with hydrochloric acid is complete at p_H 1·70, where the reduction in the resistance to extension is 37·0 per cent. When due allowance is made for the action of salt in facilitating extension at p_H 5, it is clear that the wool is half-combined with acid when the reduction in the resistance to extension is 19·25 per cent., *i.e.* at p_H 2·85. The basic dis-

sociation constant of wool is therefore $7{\cdot}1 \times 10^{-12}$, far removed from the values for free amino groups in the side chains. On the other hand, the dissociation constants of the α-amino groups of arginine and lysine are $2{\cdot}2 \times 10^{-12}$ and $1{\cdot}0 \times 10^{-12}$ respectively. Their behaviour as weak bases is due to the *zwitterion* constitution $\overset{+}{N}H_3 - R - \overset{-}{C}OO$, the amino group being already combined, in effect, with the carboxyl group. It thus becomes certain that the terminal amino groups of arginine and lysine are not uncombined in the wool fibre, and since the basic dissociation constant of wool is similar in magnitude to that of the α-amino group of arginine and lysine, it seems probable that they are combined with carboxyl groups. The latter are derived from the side chains of adjacent peptide chains, which are thus linked together by salt linkages of the type: $R_1—NH_3—COO—R_2$. Such carboxyl groups as are needed to combine with the free amino groups of arginine and lysine, as well as with histidine, must be derived from dicarboxylic acids. According to Abderhalden, wool contains 12·9 per cent. of glutamic acid and 2·3 per cent. of aspartic acid, but these values are known to be low. If wool contains 1·06 per cent. of free amino nitrogen, the amount needed to account completely for the acid adsorbed at p_H 1·0, the quantity of glutamic acid required to form salt linkages is 11·1 per cent. To this must be added 0·6 per cent. for combination with histidine, giving a total of 11·7 per cent. There is thus more than enough glutamic acid in wool to form side linkages of the type described above, but it has to be remembered that wool contains 1·2 per cent. of amide nitrogen which is usually assumed to be combined with dicarboxylic acids in the form of an acid amide. The excess of 1·2 per cent. of glutamic acid and 2·3 per cent. of aspartic acid would together combine with 0·35 per cent. of amide nitrogen. Since Abderhalden's determinations of aspartic and glutamic acid in wool are known to be low, it can safely be assumed that enough of these acids will be found present in wool to account quantitatively for the amide nitrogen as well as for what is needed to combine with arginine, lysine and histidine.

It now seems certain that one of the side linkages between the long peptide chains of the wool molecule is a salt of arginine and glutamic acid, qualified by the fact that arginine may be replaced by lysine or histidine and glutamic acid by aspartic acid. The formula of the linkage is given below, using arginine and glutamic acid as typical units:



At the time when a linkage of this type was first suggested,[23] evidence of a striking confirmatory character appeared in the literature. Frankel [24] has been able to prepare salts of arginine with glutamic acid and aspartic

acid of the type described above and they retain their existence in aqueous solution. He goes so far as to suggest that " the discovery of solid molecular compounds between amino acids, and their existence in solution also, compels the fact of intermolecular salt formation to be taken into account in any consideration of the molecular cohesion of solid proteins." In addition, he suggests that salt formation of this type is concerned in the formation of protein micelles, and there can be no doubt that the micelle of the wool fibre affords a striking confirmation of his deductions.

(g) The Properties of the Linkage.

1. *Hydrolysis.*—It is important to note that until recently the possibility of salt linkages playing any significant part in protein structure was rejected because the acid and basic dissociation constants of proteins in general are extremely small, and such linkages would be hydrolysed in water. The misconception has arisen because of the peculiar properties of the *zwitterion*, and it is now recognised that proteins possess fairly strong acid and basic properties.[25] For example, when acid is added to a protein, the ionisation of the acid group of the *zwitterion* is depressed, and what has been regarded as the titration curve of a weak base is, in reality, the back titration curve of a fairly strong acid. In the case of wool, the *zwitterions* are derived from the salt linkage of arginine (lysine or histidine) and glutamic acid (aspartic acid). Taking the case of arginine and glutamic acid, the dissociation constants of the acid and base involved in salt formation are $6{\cdot}3 \times 10^{-5}$ and 1×10^{-5}. The degree of hydrolysis of the resulting salt in water will therefore be

$$y = \sqrt{\frac{K_w}{K_a K_b}} = \sqrt{\frac{10^{-14}}{6{\cdot}3 \times 10^{-10}}} = 0{\cdot}004 \text{ or } 0{\cdot}4 \text{ per cent.}$$

Even in the extreme case of histidine and glutamic acid, the degree of hydrolysis is only 16·7 per cent. The argument that salt formation between amino acids can play no significant part in protein structure because of the hydrolysis which must occur in water, thus loses the whole of its significance.

2. *The p_H stability region.*—Reference to Fig. 4 shows that the slope of the acid side of the p_H-stability curve is much steeper than the alkali side. The reason for this is now evident, for when alkali is added to wool the basic groups of histidine, arginine and lysine are back-titrated. These compounds differ greatly in basicity, histidine being weak and lysine strong. Hence the first effect of alkali is to depress the ionisation of histidine as a base, followed by arginine and lysine. The titration curve, and therefore the p_H-stability curve, is extended over a wide range of p_H in consequence, and remains fairly flat until arginine and lysine begin to enter into the reaction. The latter are such strong bases that complete repression of ionisation is not attained before the peptide linkages begin to be attacked. On the acid side, however, only two acids are concerned, aspartic and glutamic, and their dissociation constants are not widely separated, the values being $1{\cdot}5 \times 10^{-4}$ and $6{\cdot}3 \times 10^{-5}$ respectively. The acid titration curve is therefore more sharply defined, especially as glutamic acid is present in large excess.

The existence of an isoelectric range between p_H 5 and 7 in the case of wool is readily explained on the assumption that the peptide chains are linked across by salts of histidine, arginine and lysine on the one hand, with aspartic and glutamic acids on the other hand. Six types of

salt linkage are possible and each has its isoelectric point. Using the following values for the dissociation constants, the various isoelectric points can be calculated from the formula

$$[H]_{I.Pt.} = \sqrt{\frac{K_a}{K_b} \cdot K_w}.$$

Compound.	K_a.	Compound.	K_b.
Glutamic acid .	$6{\cdot}3 \times 10^{-5}$	Lysine . .	$3{\cdot}2 \times 10^{-5}$
Aspartic acid .	$1{\cdot}5 \times 10^{-4}$	Arginine . .	$1{\cdot}0 \times 10^{-5}$
		Histidine . .	$5{\cdot}7 \times 10^{-9}$

The results are summarised in Table VI.

TABLE VI.

Salt.	p_H of Isoelectric Point.
Histidine—Aspartic acid .	4·79
Histidine—Glutamic acid .	4·98
Arginine—Aspartic acid .	6·41
Arginine—Glutamic acid .	6·60
Lysine—Aspartic acid .	6·66
Lysine—Glutamic acid .	6·85

It is significant that these values define with some degree of precision the range of the p_H-stability region which, in presence of salt, has been shown to extend from p_H 5 to p_H 7.

3. *Elasticity.*—Extension of the wool fibre is accomplished by the uncoiling of the long peptide chains, which are normally folded into a series of pseudo hexagons. Molecular rearrangement of this type is impossible unless the side linkages are capable of free rotation about the long peptide chain, *i.e.* unless the side linkages are opened at the —COO—NH_3— group. The fact that a perfectly dry fibre may be extended as much as 30 per cent. of its length without breaking, seems at first sight to present a difficulty on the present hypothesis. It has, however, to be remembered that the salt linkage is ionised in the dry fibre, giving R_1—$\overset{+}{N}H_3$ and R_2—$C\overset{-}{O}O$ ions. During extension, rotation of each half of the side link is possible, but extension is difficult because it has to be accomplished against the full attraction of the ions. When the wool fibre [1] is immersed in water, however, some water enters the micelles and reduces the attractive force between the oppositely charged —$\overset{+}{N}H_3$ and —$C\overset{-}{O}O$ ions by virtue of its high dielectric constant. For this reason alone, the wool fibre is much more easily stretched in water than in dry air, but it must not be assumed that this represents the entire action of water on wool. There can, however, be no doubt that the ions derived from the salt linkages play a large part in determining the electrical conductivity of wool. Finally, in acid solution, the ionisation of the glutamic and aspartic acids in the side link is depressed, and the attraction between the peptide chains diminished in proportion to the number of acid groups displaced

from combination. Alkali functions in a similar manner and both reagents facilitate fibre extension on this account.

4. *Technology.*—The side linkages identified in this paper have an important part to play in most trade processes. They are clearly of major importance in acid dyeing, not merely because the whole action of acid centres on the linkage, but because the colour acid dyes the fibre by displacing glutamic and aspartic acids from the side link. Indeed, the curve relating the amount of dye adsorbed to the p_H of the dyebath is precisely similar to the acid titration curve (Fig. 5) and the p_H-stability curve (Fig. 4). Where the dyestuff is colloidal, the problem is a little more complicated although fundamentally no different from the simple case of inorganic acids. Similarly, the rupture of the side linkage in acid and alkaline solutions gives a clear and precise interpretation of the action of these reagents in milling. Finally, the ionisation of the link is intimately concerned with the electrical properties of wool, plays a large part in swelling phenomena, and gives a clear interpretation of the action of water in modifying the elastic properties of the fibre.

Conclusion.

Much work remains to be done to perfect knowledge of the micelle structure of the wool fibre. The most urgent need is a complete analysis of wool into its constituent amino acids, and work on these lines has been undertaken in this laboratory. Despite the limitations of our present knowledge, it can be stated with some degree of certainty that the micelles of the wool fibre consist of long peptide chains bridged across by salt linkages on the one hand and cystine on the other, the salt linkages being formed from glutamic or aspartic acid and arginine, lysine or histidine. Other types of linkage must exist, and their discovery will go far to perfect knowledge of the constitution of the keratin molecule.

In conclusion, we wish to express our indebtedness to the Government Grant Committee of the Royal Society and to the Clothworkers' Company of London, for grants in aid of the present investigation.

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