KERATIN DERIVATIVES EXTRACTED FROM WOOL WITH ALKALINE THIOGLYCOLLATE SOLUTIONS

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Summary

In extension of previous work (Gillespie and Lennox 1953), the conditions under which proteins may be extracted from washed Merino wool have been further examined. Approximately 65 per cent. of the wool can be dissolved by a 40-min extraction at 50°C with 0·1M thioglycollate at an initial pH of 12·6. Electrophoresis at pH 11 in thioglycollate-glycine buffer indicated the presence of seven minor and one major component, the latter amounting to 41 per cent. of the wool. The minor components can be completely removed from the wool by five 20-min extractions with 0·1M thioglycollate at an initial pH of 10·5. Extraction of the residue at pH 12·3 yields the major component. This moves as a single peak on electrophoresis between pH 8·0 and 12·0 in the presence of various buffers. It has a mobility of $-7{\cdot}2 \times 10^{-5}$ cm^2 V^{-1} sec^{-1} at a protein concentration of 0·5 per cent. in thioglycollate-glycine buffer of ionic strength 0·22 at pH 11·0. At higher protein concentrations there is anomalous behaviour on the descending boundary and this can be prevented by increasing the ionic strength or replacing thioglycollic acid with mercapto-ethanol. The ascending pattern is unaltered by these changes or by increased protein concentration.

I. INTRODUCTION

A study of the protein constituents of wool is being made in an attempt to assess the contribution to the properties of wool made by individual proteins.

The insolubility of wool in aqueous media constitutes a major impediment in studying its properties. Of the known reagents for converting the proteins of keratinous tissues, such as wool, into soluble derivatives, an alkaline solution of thioglycollic acid is perhaps the most satisfactory. For example, Goddard and Michaelis (1934) found that solutions of thioglycollate extracted greater amounts of soluble protein from wool than did solutions of sulphide or cyanide, and they expressed the view that "it simply reduces disulphide to sulphydryl groups with no other appreciable change." The superior keratin-dispersing action of thioglycollate is likely to be associated with its greater disulphide-bond-splitting activity than equivalent concentrations of cysteine or cyanide (Lennox and Forss 1953).

The partial solution of wool in alkaline thioglycollate solutions has provided a means of investigating the wool dispersions by methods commonly used for characterizing soluble proteins.

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It has previously been reported that the extracts obtained by treating wool with thioglycollate at an initial pH of 12·6 contain at least three components, two of which can be removed by fractional extraction at pH 10·5 before extraction at the higher pH (Gillespie and Lennox 1953). Further details of this work and an extension of the thioglycollate extraction and electrophoretic studies are given in the present paper.

II. Materials and Methods

Merino wool 64's quality from which the tip had been removed was extracted with ethanol, washed repeatedly in distilled water, and dried in warm air. In large-scale preparative experiments wool from the same bale was used in the form of dry combed top. This was extracted six to eight times with light petroleum, twice with ethanol, and repeatedly with distilled water before being dried in warm air.

Except where otherwise stated 30 ml of solution was used to extract each gram of wool and all extractions were made for 20 min at 50°C ± 0·1°. Potassium salts were used throughout to minimize salt errors during pH measurements in the alkaline region. A standard glass electrode assembly was used. The thioglycollic acid was of laboratory reagent grade and solutions were standardized before use by standard iodometric procedures. The wool extracts were stored by freeze-drying and sealing in air-tight containers.

In large-scale experiments the protein solutions were adjusted to the pH of maximum precipitation and the precipitate allowed to settle, the supernatant decanted off, the precipitate quickly dissolved in one-tenth of the original volume of 0·1M thioglycollate at pH 10·5, and this concentrated solution freeze-dried and sealed into air-tight containers.

The amount of wool passing into solution was measured by estimating the total nitrogen in the liquor by the micro-Kjeldahl method and by estimating the loss in weight of the wool. The partly digested wool was repeatedly washed with water to remove soluble degradation products, prior to drying it at 102°C and weighing. Using a factor of 6·25 to convert nitrogen percentage to protein, the results obtained by the two methods agree closely (Fig. 1) for digestion to about the 60 per cent. level but diverge to some extent at higher degrees of digestion.

The pH-solubility relationships of the wool proteins were determined by precipitating the proteins in each extract at a series of pH values in the range 4-11. The final protein concentration and ionic strength were kept constant and the temperature maintained at 2°C. After 18 hr equilibration the precipitates were centrifuged down and dissolved in 0·1M carbonate solution. The wool protein solutions were not optically clear at pH values normally used for measuring concentration—utilizing the absorption near 280 mμ by the aromatic amino acids. An aliquot of this solution was therefore partly hydrolysed by boiling with an equal volume of 5N HCl for 30 min and the absorption at 275 mμ measured. To correct for the absorption at this wavelength by the thioglycollic acid present, the protein in a second aliquot was precipitated by half saturation

with ammonium sulphate, the supernatant from the precipitate hydrolysed with HCl, and the absorption also measured at 275 mμ. The difference between the two values was converted to the equivalent protein concentration using the extinction coefficient of $E^{1\%}_{1\ cm} = 8{\cdot}9$ (air-dry weight).

Electrophoresis of wool proteins was carried out in a Tiselius apparatus at 1°C, using a constant potential gradient in the ⌊⌋ tube of about 2·5 V/cm. The diagonal schlieren method was employed, using as a light source a mercury vapour lamp from which monochromatic light ($\lambda = 546$ mμ) was isolated by means of a filter. The curves were photographed on Ilford 35-mm panchromatic film (FP3). The wool extracts (25 ml) were dialysed in "Visking" sausage casing (24/32 in. size) against 2 l of buffer solution for at least 2 days at 2°C. Protein

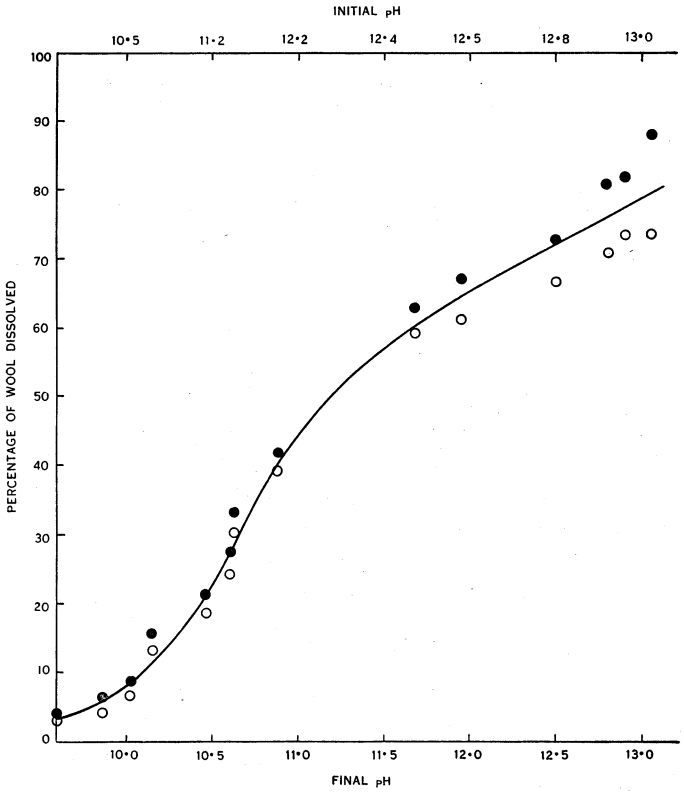

Fig. 1.—Effect of pH on the solution of wool in 0·1M thioglycollate at 50°C for 40 min. O, Estimated from N dissolved. ●, Estimated from weight of undigested wool.

concentrations were determined refractometrically. It was found that a reducing agent was needed in the buffer to prevent oxidation of these sulphydryl proteins. Oxidation was manifested by gelling within 24 hr. Thioglycollic acid or mercaptoethanol at concentrations as low as 0·005M could prevent this oxidation but for routine experiments somewhat higher concentrations were

used. The standard buffer had an ionic strength of 0·22 and consisted of 0·05M thioglycollic acid, 0·1M glycine with KOH added to give pH 11·0.

Mobilities were calculated from measurements made on the descending boundaries at protein concentrations of about 0·5 per cent. The relative concentrations of various components were calculated from the ascending boundaries because of the superior resolution at this boundary and the freedom from anomalous effects.

III. Experimental

It should be noted that all the pH values referred to in this paper are initial pH's; the final values and presumably those prevailing during the major part of the extraction are somewhat lower (see Fig. 1).

Table 1

COMPARISON OF DISPERSING ACTION ON WOOL OF 0·1M THIOGLYCOLLATE WITH 0·5M THIOGLYCOLLATE, AT TWO pH VALUES

Thioglycollate concentration (M)	0·1	0·1	0·5	0·5
Initial pH value	11·6	12·5	11·5	12·5
Final pH value	10·6	12·4	11·2	12·2
Total N in extract (mg N per ml)	1·23	2·59	0·73	1·26
Equiv. protein extracted (%)	23	49	14	24

(*a*) *Preparation of Thioglycollate Extracts*

(i) *Influence of pH.*—The quantity of wool dispersed in 0·1M thioglycollate in 20 min at 50°C increased sharply as the initial pH was increased from 10·5 to 12·3 but thereafter less rapidly (Fig. 1). By dialysing 5-ml portions of the extracts in Visking sausage casing against 5 ml of distilled water for 7 days at 2°C and measuring nitrogen content and volume of both solutions, it was shown that the extracts prepared at pH values below 12·0 contained only traces of dialysable nitrogen, whilst those made at pH 12·6 lost about 3 per cent. of total N and those at pH 13·0 about 50 per cent.

Pretreatment of the wool in alkali at pH 10·6 for 24 hr at 50°C caused a decrease in the amount of protein extracted. The pretreatment was shown to give rise to lanthionine in the wool. However, no lanthionine was present in either the solutions or in the residues produced during thioglycollate extractions.

(ii) *Comparison of Different Cations.*—Thioglycollic acid solutions were adjusted to a final thioglycollate concentration of 0·1M at pH 11·0, using LiOH, NaOH, KOH, NH_4OH, and $Ca(OH)_2$. The solutions containing univalent cations dispersed 15-20 per cent. of the wool under standard conditions whereas the $Ca(OH)_2$ solution dispersed only 8 per cent.

(iii) *Effect of Thioglycollate Concentration.*—Solutions containing potassium thioglycollate varying in concentration from 0·1 to 1·0M, at an initial pH of 11·0, were used to extract protein from wool under the standard condi-

tions of time and temperature. It was found that extent of extraction increased with increasing thioglycollate concentration to a maximum at about 0·7M (Fig. 2). During extraction the pH decreased to values ranging from 9·7 for 0·1M to 10·8 for the 0·5M solution.

The increase in the solubility of the wool protein with increase in pH and decrease with increase in the thioglycollate concentration above a certain maximum value is confirmed by an experiment reported in Table 1. It will be noted that extraction at higher pH values than those reported in Figure 2 lowered the optimum concentration of thioglycollate for extraction to less than 0·5M.

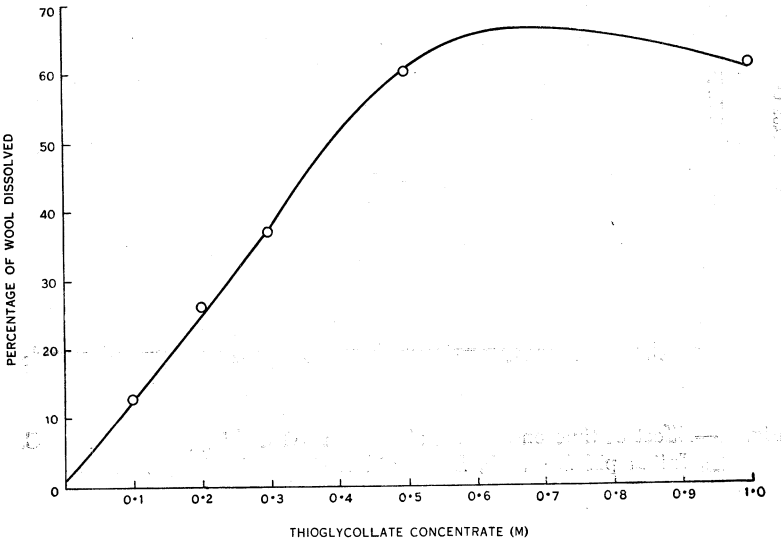

Fig. 2.—Effect of thioglycollate concentration on the solution of wool. Initial pH 11. Heated for 40 min at 50°C.

(iv) *Effect of Time of Extraction.*—Figure 3 shows the relationship between time and extent of digestion at different pH values. At the higher pH values tested, i.e. 12·0 and 12·3, the reaction proceeded in two stages, the first of which was completed in less than an hour with 60-70 per cent. of the wool extracted and the second still incomplete in 40 hr with 90 per cent. of the wool digested.

(v) *Effect of Temperature.*—Increase in temperature of the 0.1M thioglycollate solution at an initial pH of 11·7 had little effect on its dispersing action up to 40°C (Fig. 4) but thereafter increased it sharply up to about 60°C.

Measurement of digestion at intervals during extraction in pH 10·7 thioglycollate at 33·0, 49·2, and 64·0°C (Fig. 5) enabled the curve in Figure 6 to be determined from which the activation energy for the extraction was calculated to be 7·4 kcal per mole.

(vi) *Extraction of Disintegrated Wool and Cortical Cells.*—Wool ground to 40 mesh in a Wiley mill was somewhat more easily extracted by 0·1M thioglycollate at pH 12·5 than was whole wool. At 50°C, 68 per cent. of the ground

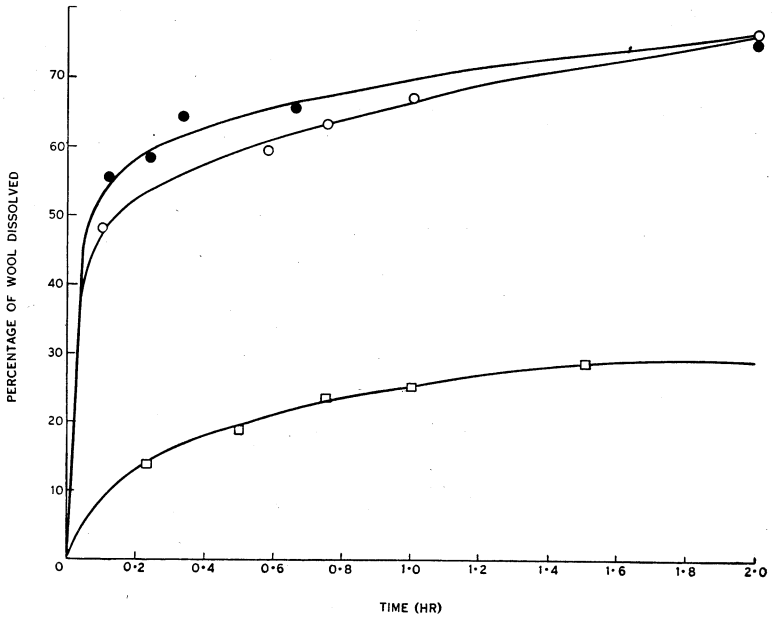

Fig. 3.—Effect of time on solution of wool in 0·1M thioglycollate at 50°C. □, Initial pH 10·5. O, Initial pH 12·0. ●, Initial pH 12·3.

TABLE 2

PERCENTAGE PROTEIN EXTRACTED FROM DISINTEGRATED AND INTACT WOOL DURING 20 MIN AT 50°C IN 0·1 M THIOGLYCOLLATE SOLUTIONS

Experiment No.	Cortical Cells from Trypsin-Digested Wool		Finely Ground Wool*		Intact Wool	
1	pH 10·4 15	pH 11·2 24	pH 10·4 22	pH 11·2 44	pH 10·4 8	pH 11·2 45
2	pH 10·5 19	pH 12·3 27	pH 10·5 24	pH 12·3 53	pH 10·5 13	pH 12·3 62

* The protein digestion was corrected to allow for the presence of 9 per cent. powdered glass in this preparation.

wool dissolved as compared with 62 per cent. of whole wool in a 40-min. extraction. Table 2 shows the results obtained in a similar experiment, in which an extraction was made on cortical cells from trypsin-damaged wool and ground

wool which had been broken down by shaking with glass beads in water for 16 hr.

(vii) *Mechanical Agitation During Extraction.*—When wool was treated with 0·1M thioglycollate at pH 12·6 for 20 min at 50°C, and the reaction mixture disintegrated for 2 min in a Waring Blendor and then heated for a further 20 min at 50°C, 70 per cent. of the wool protein was dispersed. These extracts were very cloudy and they could not be clarified by filtration or centrifugation at 25,000*g*. Electrophoresis revealed a normal pH 12·6 pattern (Gillespie and Lennox 1953).

Agitation of the wool by continuous tumbling in tubes immersed in the thermostat bath for 40 min at 50°C increased the protein extraction in 0·1M thioglycollate at pH 11·6 from 13 per cent. in stationary tubes to 15 per cent. and with 0·1M thioglycollate at pH 12·8 from 57 to 62 per cent.

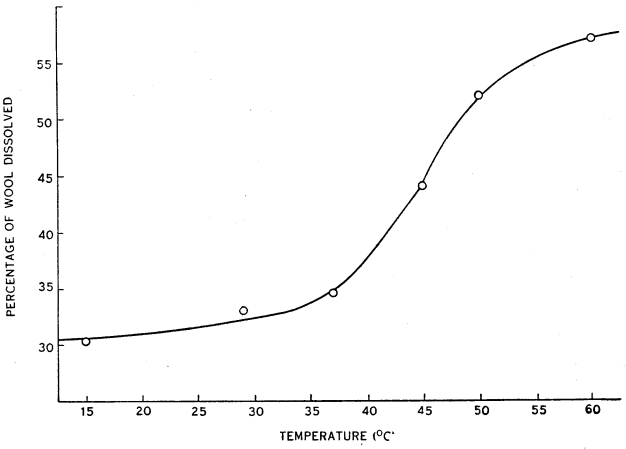

Fig. 4.—Effect of temperature on solution of wool in 0·1M thioglycollate. Initial pH 11·7. Heated for 40 min.

(*b*) *Fractional Extraction of Wool with Thioglycollate*

An extract of wool made with 0·1M thioglycollate at pH 12·6 for 20 min at 50°C contains most of the easily extractable protein (see Fig. 3). Electrophoresis shows that it contains at least four components, the second of which is the major one. Preliminary experiments showed that comparable amounts of protein could be obtained in solution by stepwise extraction at a number of lower pH levels. Furthermore, it was found that the minor components (1, 3, and 4) could be more readily extracted at low pH values than the major one (2), and that by using a series of extractions at an initial pH of 10·5, all these minor components could be removed from the fibre with only partial loss of component 2.

Subsequently the procedure followed was to extract the wool five times for 20 min at 50°C with thioglycollate solutions at an initial pH of 10·5, followed by one extraction with a solution of 12·3. The first extract contained

only 8·5 per cent. of the wool, the final pH being comparatively low, the second contained about 15 per cent. of the wool, the amount dissolved decreasing in subsequent extracts to 3 per cent. of the wool in the fifth extract. The sixth extract made at an initial pH of 12·3 contained almost 20 per cent. of the wool protein and appeared to consist of only one protein. These results are summarized in Figure 7.

In another experiment (Fig. 8) the first five extractions at an initial pH of 10·5 (*A-E*) were followed by five extractions (*F-J*) with 0·1M thioglycollate at pH 12·3, three further extractions with thioglycollate at pH 13·0 (*K-M*), and one with 1·0M KOH (*N*), each extraction being for 20 min at 50°C. The insoluble residue was then dissolved by boiling for 2 min in one-third of the standard volume of 1·0M KOH (*O*).

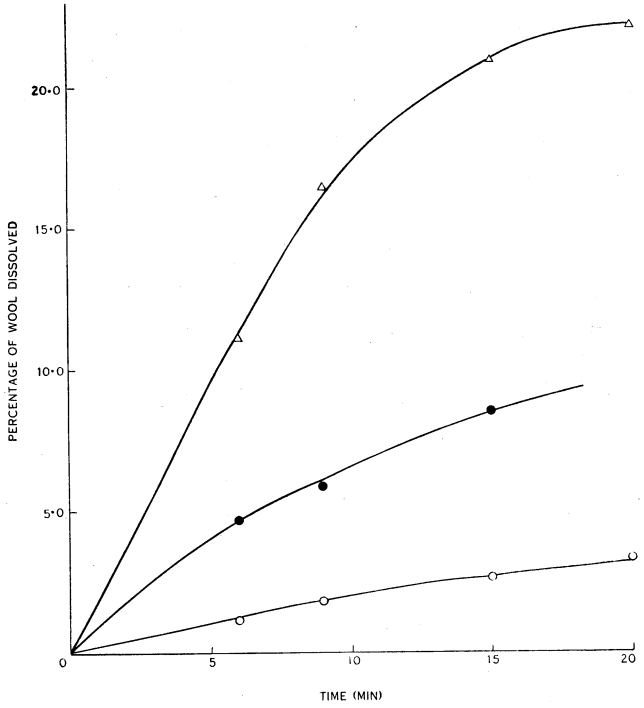

Fig. 5.—Effect of time on solution of wool in 0·1M thioglycollate. Initial pH 10·5. O, At 33°C. ●, At 49·2°C. △, At 64°C.

Dialysis of 5-ml portions of the extracts in "Visking" sausage casing in running tap water with agitation removed less than 10 per cent. of the nitrogen from extracts *A-M*. With extract *N*, however, 24 per cent. dialysed away and with extract *O*, 28 per cent. When 5-ml portions of the extracts were dialysed without agitation for 14 days at 2°C against 5 ml distilled water and the nitrogen content of the solutions was measured on either side of the membrane, making corrections for volume changes, less than 0·1 per cent. of the total *N* was

dialysable from the pH 10·5 extracts (*A-E*) and 2-3 per cent. from the pH 12·3 extracts (*F-J*).

(*c*) *Precipitation Curves of Extracted Wool Proteins*

The thioglycollate wool proteins are readily precipitated by acidification of their solutions, the pH necessary for maximum precipitation varying with the type of material. The pH-solubility relationships of the wool proteins were determined in solutions of equal protein concentration (1 per cent.) containing 0·05M thioglycollate, the pH value having been adjusted with acetic acid. After equilibration for 18 hr at 2°C, the solutions were centrifuged at this temperature and the protein in solution, and in the precipitate, estimated by the spectrophotometric method. The results are shown in Figure 9.

A small proportion of the protein in the first 10·5 extract (approx. 2 per cent.) was not acid-precipitable; however, all subsequent extracts appeared to be completely precipitable. It can be seen (Fig. 9) that the proteins in the pH 12·3 extracts, and those made at pH 13·0, were soluble to a slight extent on the acid side of the point of maximum precipitation. However, the solubility was too low for a satisfactory electrophoresis run to be made at these pH values.

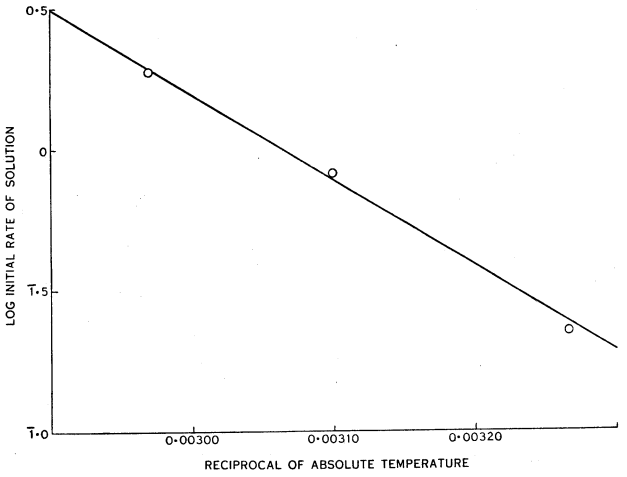

Fig. 6.—Relationship between logarithm of initial rate of extraction and the reciprocal of absolute temperature. Data from Figure 5.

(*d*) *Properties of the Wool Proteins*

The freeze-dried proteins obtained from the thioglycollate extraction of wool are sulphhydryl proteins, colourless, freely soluble in water at pH values above the precipitation range, but readily oxidized to give insoluble materials. By dialysing a solution containing 1 per cent. protein or more against a large volume of water, in order to remove the potassium thioglycollate always present in these preparations, a firm gel was formed and at this stage there were no detectable SH groups present. This gelling was not prevented by "Versene." The

solutions turn yellow on standing at room temperature, presumably owing to the release of sulphur from the cystine residues at the alkaline pH. This reaction is known to proceed more readily with cystine in peptide combination than with the free amino acid (Brand and Sandberg 1926). The exact explanation for the yellowing is not known; however, a strong absorption band appears at 330 mμ which increases in intensity as the reaction proceeds. The alkaline thioglycollate extractant on storage also produced a yellow colour but only in small amount.

The freshly acid-precipitated solids were readily redissolved in thioglycollate at pH 10·5 and these solutions were then freeze-dried for storage. If, however, the solid was packed down by centrifuging, by pressing, or by vigorous stirring, the material became almost insoluble in the thioglycollate and what did dissolve became insoluble after freeze-drying. Compression of the gel probably favoured the formation of relatively stable cross-linkages between the protein micelles. I. J. O'Donnell (personal communication) has observed similar behaviour with some peracetic acid-oxidized wool protein. This material in the acid-precipitated state had to be treated gently and not pressed, otherwise it did not readily dissolve in 0·1N ammonia but merely swelled with some slow dissolution.

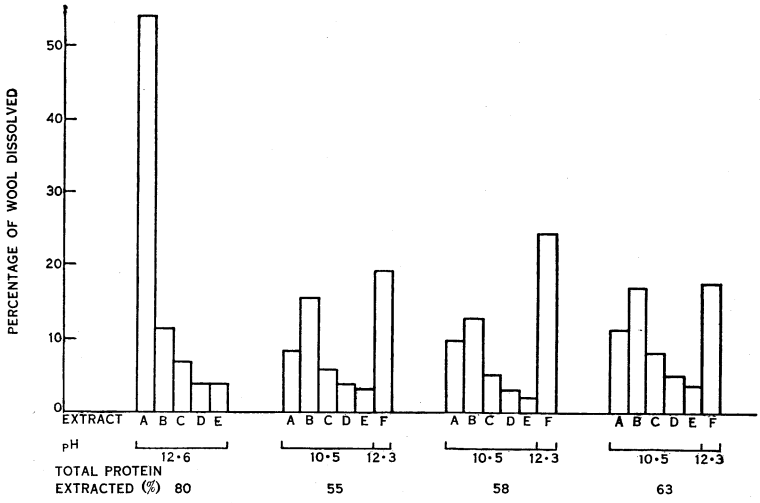

Fig. 7.—Histograms showing the percentage of wool solubilized by successive extractions with 0·1M thioglycollate, each for 20 min. at 50°C.

(*e*) *Electrophoresis of the Wool Proteins*

Electrophoresis patterns obtained with successive thioglycollate extracts, run in the standard buffer at pH 11, are shown in Figure 10. It can be seen that the complexity of the patterns progressively decreased and the sixth extract (*F*) was a relatively pure material. Four conspicuous components appeared in the first 10·5 extract (*A*). Of these the slowest, component 1, was not homo-

geneous and was resolved in this and many other preparations into at least three components. When this extract was precipitated at pH 6, a portion of component 1 remained in the supernatant. Electrophoresis of this supernatant

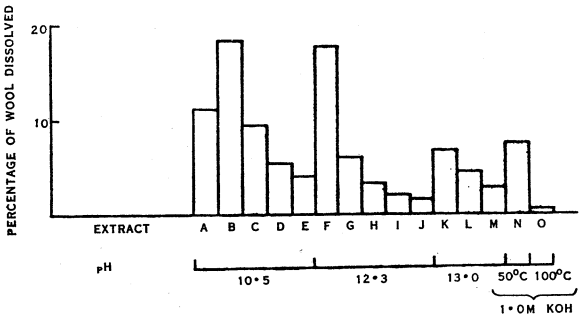

Fig. 8.—Histogram showing the distribution of wool protein in 15 successive extractions, 13 (*A* to *M*) with 0·1M thioglycollate.

fraction showed only a broad low peak after some hours' running and this peak did not appreciably sharpen when the current was passed in the reverse direction for an equal time. Furthermore, the amount of protein detectable on electrophoresis, corresponding to component 1, decreased as the time of dialysis increased. From this evidence and that of Harrap (1955) it seems that the first extract is very heterogeneous and contains some proteins of relatively low molecular weight.

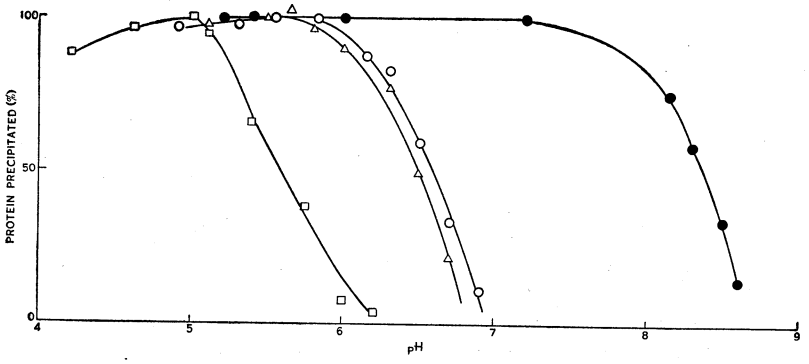

Fig. 9.—pH-solubility curves of wool proteins. ●, First pH 10·5 extract (*A*). ⊙, Second pH 10·5 extract (*B*). △, Subsequent pH 10·5 extracts (*C, D, E*) and pH 12·3 extracts (*F, G, H, I*). □, First and second pH 13 extracts (*K, L*).

The second, third, fourth, and fifth pH 10·5 extracts contained one major component (2) and decreasing amounts of the others (1, 3, 4). The first pH 12·3 extract made on the residue, then yielded a material which on electrophoresis moved as a single peak. This peak was very symmetrical and it showed little spreading and no splitting during a 6-hr electrophoresis run at pH 11 in the standard buffer.

(*f*) *Variation in Extraction Conditions*

Attempts to increase the yield of component 2 by prolonging the first extraction in pH 12·3 thioglycollate (extract *F*) to 18 hr at 50°C or by using thioglycollate at pH values greater than 12·3, or buffered at pH 12·3, for the extraction were unsuccessful. In each case the electrophoresis pattern showed a "spike" component on the leading edge of component 2. A similar pattern was obtained by heating extract *F* at 50°C after removal of the wool residues (Fig. 11).

(*g*) *Location of the Component* 2 *in the First pH* 10·5 *Extract*

The first pH 10·5 extract contains a complicated mixture of proteins and some trouble was experienced in determining the exact location of the pure component in the electrophoretic pattern of this extract. From the values calculated for the mobilities of the pure component and the four main proteins in the mixture it was previously reported that the pure component was located

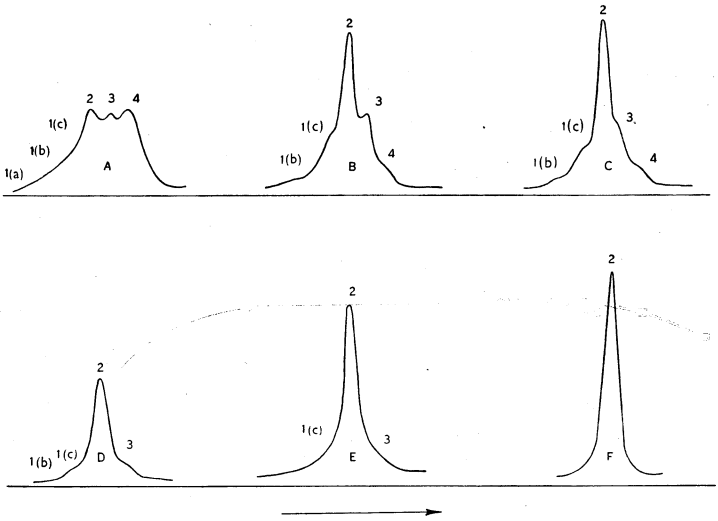

Fig. 10.—Electrophoretic patterns of wool proteins. Ascending boundaries shown. Run at pH 11 in 0·22 Γ/2 buffer. *A-E* are successive pH 10·5 extracts, *F* is a 12·3 extract. The component numbers are shown over the appropriate peaks.

in the first peak (Gillespie and Lennox 1953). However, on adding a preparation of component 2 to the first pH 10·5 extract, the second peak of the electrophoresis pattern was greatly increased in height (Fig. 12), showing this to be identical with the pure component. It is well known that on electrophoresis of a mixture of proteins, only the mobility of the leading component can be calculated with accuracy (Svensson 1946; Armstrong, Budka, and Morrison 1947). This, together with the dependence of mobility on protein concentration, accounts for the difficulties encountered in the present investigations.

(*h*) *Percentage of Electrophoretic Components in Whole Wool*

Having identified the major component, the other components were also identifiable by their relative positions in the electrophoretic pattern of each extract. By measuring the area under each peak, the percentage of the total wool protein in each component was calculated. These results are shown in Table 3. In addition to the three sub-components of component 1, a trace of a faster-moving component, designated 5, was often found.

TABLE 3

PERCENTAGE OF WOOL PROTEIN CORRESPONDING TO EACH COMPONENT

Data from electrophoretic patterns in Figure 10

Component	Proportion of Each Component Present in Each of the Following Extracts (%)						Wool Equivalent to Each Electrophoretic Component (%)
	A	B	C	D	E	F	
1 (*a*)	6	2	—	—	—	—	1·1
1 (*b*)	7	8	8	2	—	—	3·1
1 (*c*)	8	12	14	9	5	—	5·1
2	30	42	56	72	90	100	41·4
3	20	21	14	13	5	—	8·3
4	26	15	8	4	—	—	6·6
5	3	—	—	—	—	—	0·3
Wool equivalent to each extract (%)	11·2	18·3	9·5	5·2	4·0	17·7	Total 65·9 (%)

(*i*) *Studies on the Electrophoresis of the Pure Component*

Electrophoresis of the pure component, i.e. component 2, in the standard buffer at pH 11 showed, on the ascending boundary, only a single symmetrical peak. The descending boundary showed some anomalous effects and experiments were made to explain these.

The electrophoretic mobility of the protein was found to be $-7{\cdot}2 \times 10^{-5}$ $cm^2\,V^{-1}\,sec^{-1}$ at 0·5 per cent. protein concentration in the standard buffer at pH 11, but on increasing the protein concentration to 1 per cent. the mobility decreased to $-6{\cdot}3$, and even lower mobilities were recorded at higher protein concentrations. The descending boundary gave an anomalous multiple peak pattern at protein concentrations greater than 1 per cent., whereas a normal symmetrical peak was obtained at concentrations of about 0·5 per cent. or less.

On electrophoresis of component 2 in thioglycollate solutions containing 1·5 per cent. protein and NaCl to adjust the ionic strength to 0·02, 0·05, 0·10, 0·20, and 0·50, the single component pattern previously observed in the ascending limb was unchanged. The anomalous pattern in the descending limb, how-

ever, was progressively clarified with increase in ionic strength and at $\Gamma/2 = 0{\cdot}5$ only a single peak appeared, similar to that seen in the ascending limb pattern.

Replacement of thioglycollate in the electrophoresis buffer with 0·05M mercaptoethanol or with 0·025M sodium sulphite did not affect the pattern in the ascending limb but, like high ionic strength, eliminated the anomalous components in the descending limb.

Electrophoresis of solutions of component 2 in which the thioglycollate concentration varied from 0·005M to 0·10M, with a constant ionic strength of 0·22, yielded normal single-component patterns in the ascending limb and the usual anomalous pattern in the descending limb.

The patterns were unchanged when glycine was replaced by piperidine-HCl, K_2CO_3, or K_2HPO_4-K_3PO_4 in an electrophoresis buffer of the usual ionic strength and pH.

Electrophoresis at pH values between 8·0 and 12·0 yielded normal patterns except that as the pH fell below 9 the peaks sharpened, perhaps owing to the tendency of the solutions to gel at low pH values. $KHCO_3$ was used in the thioglycollate solutions at pH 8·0, veronal at pH 8·8, and glycine at all higher pH values.

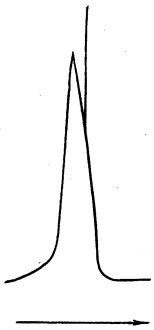

Fig. 11.—Electrophoretic pattern showing modification of component 2 after heating extract *F* at 50°C for 18 hr.

IV. Discussion

The most interesting result of these studies has been the preparation of a protein from wool (component 2) which constitutes over 40 per cent. of the total fibre. It will almost certainly be directly or indirectly responsible for many of the properties of the wool fibre.

This fraction has been run in electrophoretic experiments under as wide a variety of conditions as possible, involving changes in pH and buffer composition. Under all these conditions the material appears to be homogeneous. It has not been possible to run the protein at pH values below its isoelectric point because of its insolubility, nor to run it in the absence of a reducing agent,

consequently it has not been possible to carry out more critical tests for homogeneity.

Electrophoresis of these wool proteins has provided many problems, chief of which has been the anomalous production of new peaks on the descending boundary with increase in protein concentration. The fact that this phenomenon can be repressed or eliminated by increasing the salt concentration or by lowering the protein concentration is consistent with its being an association process.

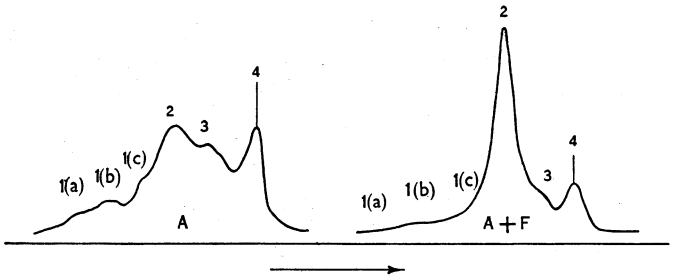

Fig. 12.—Electrophoretic patterns showing the identification of the pure component 2 in the first pH 10·5 extract by mixing extract *F* with *A* to enlarge the component 2 peak.

In some extracts, particularly those prepared with pH values at 13 and higher, the electrophoresis patterns show characteristic spiked peaks in both ascending and descending patterns. The reason for their appearance is unknown although they have been observed previously with other proteins, thus Taylor, Green, and Cori (1948) found a spike in the descending boundary pattern of crystalline aldolase, Hamoir (1953) in the ascending pattern of muscle extracts, attributed to turbidity and high viscosity of the solutions, and Morrison (1954) in both boundaries of heart muscle aconitase. A spike can also be observed in the β anomaly of plasma in the descending boundary (Abramson, Moyer, and Gorin 1942). These phenomena may be unrelated but in certain cases they appear to be caused by association-dissociation processes.

A study of the kinetics of the extraction process indicated an initial rapid solubilization of some 70 per cent. of the wool protein with a subsequent slower extraction of more resistant material. This agrees with the observations of Jones and Mecham (1943) and Booth (1952). The initial rapid reaction corresponds with the appearance of components 1-4 and appears to cease when these have been completely extracted. At this stage microscopical examination showed a grossly swollen tube-like structure with the scale markings still visible and many of the internal cell membranes intact (Fraser and Rogers 1953). This suggests that the high resistance of the residue to thioglycollate extraction as compared with the bulk of the fibre is due to a fundamental difference in its chemical structure.

The first two pH 10·5 thioglycollate extractions caused the S segment to swell and lose birefringence but had little effect on the birefringence of the H segment. This birefringence disappeared during the subsequent pH 10·5 extractions and the fibre swelling increased. It would seem that a number of processes are involved, firstly the diffusion of thioglycollate and OH ions through the membranes into the fibre, then the solubilization of the proteins accompanied by swelling, followed by diffusion of the soluble proteins out. The work of Harrap (1955) suggests that diffusion is a rate-limiting process in the extraction and this is confirmed by the value of 7·4 kcal obtained for the activation energy of extraction using pH 10·7 thioglycollate. This lies within the range of values 4·5-8·0 which typifies the diffusion of ions through a liquid layer. Values within this range have been reported for the diffusion into wool of potassium permanganate (Alexander and Hudson 1949), chlorine (Alexander, Gough, and Hudson 1949), and dyes (Alexander and Hudson 1950). Thus the recovery of proteins from wool with thioglycollate is likely to be diffusion-controlled, the limiting step being either the ingress of the reagent or the egress of protein through a diffusion layer.

A further illustration of the importance of diffusion in the extraction of wool proteins was the repression of the extraction process at high thioglycollate concentrations (Table 1), corresponding with similar observations by Booth (1952). It is also related to the lower depilation and wool-dispersing action of 2·0M Na_2S as compared with 1·0M Na_2S (Gillespie 1951), and to the repressive effect of Na_2SO_3 (Gillespie 1953) on the depilatory action of enzymes from *Aspergillus oryzae* at concentrations exceeding 1·0M. Gillespie observed that the addition of NaCl to sulphide depilatories represses both the swelling of the fibres and the depilatory action. The lesser attack on keratin observed with high concentrations of keratin-dispersing agents could be attributed to slower diffusion caused by repression of swelling.

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