

# INCREASED THIOLYCOLLATE EXTRACTION OF WOOL PROTEINS FOLLOWING SUPERCONTRACTION

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## Summary

Supercontraction of wool or of wool cells by heating in 9M LiCl or 8M phenol facilitates the subsequent extraction of the proteins with alkaline 0.1M thioglycollate solutions during 20 min at 50°C. The amount of protein extracted with 0.1M thioglycollate initially at pH 10.0, for example, approximates to that removed from untreated wool with 0.1M thioglycollate initially at pH 10.5. The electrophoresis patterns of the proteins extracted from supercontracted wool also resemble the patterns of extracts of normal wool obtained at higher pH values. This, together with evidence for the absence of amide bond splitting, suggests that fractional extraction with alkaline thioglycollate yields proteins pre-existing in the wool and not formed during extraction. The electrophoresis patterns would therefore appear to reflect the protein composition of reduced wool.

Extraction of supercontracted wool with alkaline thioglycollate allows the wool to revert rapidly to the original uncontracted state having low thioglycollate extractability. Partial reversal of extractability is likewise achieved by prolonging the period of heating in the LiCl solution and complete reversal by heating the LiCl-supercontracted wool in water.

## I. INTRODUCTION

It has been shown recently that preheating wool in water for 20 min at temperatures above 70°C reduces the amount of protein extractable with alkaline solutions of potassium thioglycollate (Lennox 1956). This effect reaches a maximum at 120°C, the lowest temperature at which hydrothermal supercontraction occurs. In contrast, it has now been observed that wool which has been markedly supercontracted by heating at 96°C in concentrated aqueous solutions of LiCl (Alexander 1951) or phenol (Elöd and Zahn 1949) yields greater amounts of protein to alkaline thioglycollate than does untreated wool. The quantities of protein extracted after supercontracting the fibres under various conditions and the electrophoretic properties of the extracts are described in the present paper.

## II. MATERIALS AND METHODS

### (a) Wool

Merino 64's wool top was repeatedly washed at room temperature with diethyl ether and then with water to remove oil and other impurities, and dried in a current of warm air.

### (b) Wool Cells

The free cells were prepared by aseptic tryptic digestion of the Merino 64's wool followed by thorough washing and drying.

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*(c) Pretreatment*

Supercontraction was effected by heating wool in 9M LiCl or 8M phenol at 96°C in a boiling water-bath for either 20 or 40 min as indicated. In these pretreatments and in the extraction experiments 30 ml of solution were used per 1 g wool treated unless stated otherwise. After supercontraction the samples were rinsed repeatedly in water and squeezed in dry fabric and absorbent paper to remove free water. The weight of the wool was unchanged by supercontraction. The striking reduction in length of a sample of wool top produced by supercontraction is shown in Figure 1.

To heat the wool samples in water at 110°C they were enclosed in a stainless steel pressure-vessel immersed in an oil-bath. About 1–2 ml of the 30 ml water in the vessel were lost as steam when heating it to the temperature of the bath and before closing the valve.

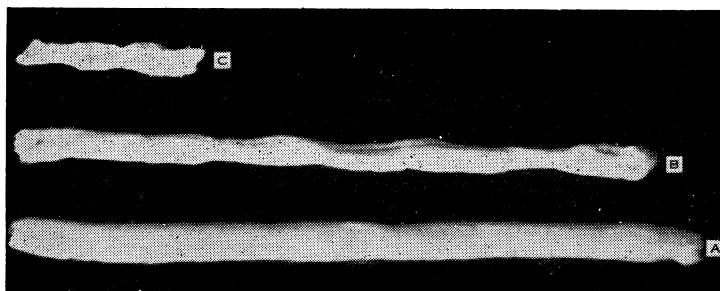


Fig. 1.—Shrinkage of wool top obtained by supercontraction by heating for 40 min at 96°C in 9M LiCl. Individual wool fibres supercontract by only 28 per cent. under these conditions but the bending and twisting of fibres while in the parallel array in wool top cause it to shorten by 75 per cent. A, unheated; B, heated in water; C, heated in 9M LiCl.

*(d) Extraction with Thioglycollate*

The proteins were extracted from wool by heating for 20 min at 50°C in 0.1M thioglycollate adjusted to the desired initial pH value with KOH. The amount of protein extracted was estimated by determining total nitrogen by the micro-Kjeldahl method and applying the conversion factor 6.25.

*(e) Electrophoresis*

Electrophoresis of the wool proteins was carried out as previously described (Gillespie and Lennox 1953, 1955a, 1955b). The experiments were performed using a buffer of ionic strength 0.22 containing 0.05M thioglycollic acid and glycine at 0.1M concentration, the pH being adjusted to 11.0 with KOH. In most experiments a potential gradient of about 2.5 V/cm was applied for 6 hr at 1°C. As in previous electrophoretic studies on wool proteins extracted with thioglycollate (Gillespie and Lennox 1953, 1955a), only the ascending patterns are reported. Anomalous peaks occur in the descending patterns which have been attributed to aggregation (Gillespie and Lennox 1955b).

## III. RESULTS

(a) *Factors Affecting the Extraction of Proteins from Supercontracted Wool*

(i) *Concentration of LiCl.*—The curve relating thioglycollate solubility with concentration of LiCl (Fig. 2) resembles the concentration–supercontraction curve for this salt (Crewther and Dowling, personal communication), both showing maxima at about 8–9M LiCl.

(ii) *Effect of Previous Treatment of Wool.*—Supercontraction by heating to 96°C for 20 min in 9M LiCl at pH 5 (determined with the hydrogen electrode and by comparison with buffer applied to pH indicator paper) allowed less protein to be extracted from carbonized wool containing residual  $\text{H}_2\text{SO}_4$  than from fleece wool

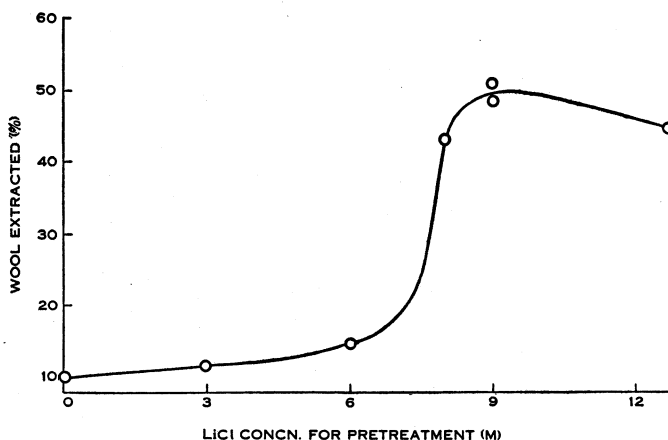


Fig. 2.—Effect of concentration of LiCl used for 40 min pretreatment at 96°C on extraction of wool protein during 20 min at 50°C in 0.1M thioglycollate initially at pH 11.0.

which had been freed of wax and suint by extraction with diethyl ether and water. Fleece wool, in turn, yielded less protein to the thioglycollate than did the slightly alkaline wool top (scoured with soap and soda) used in these investigations.

(iii) *pH of Thioglycollate.*—As with normal wool, the quantity of protein extracted with thioglycollate from wool supercontracted in either 9M LiCl or 8M phenol increased with the initial pH of the extractant (Fig. 3), but the quantity extracted at any particular pH over the range 9.5–12 was appreciably greater for the supercontracted wool than for the normal wool.

(iv) *Time of Heating in LiCl.*—The quantity of protein extractable with thioglycollate initially at pH 10.5 increased with the period of preheating in 9M LiCl up to 20 min but thereafter it decreased (Fig. 4).

(v) *Soaking and Drying Supercontracted Wool before Extraction.*—To ascertain whether drying or additional rinsing after supercontraction for 40 min at 96°C in 9M LiCl affected the extractability with thioglycollate, the standard extraction procedure using 0.1M thioglycollate initially at pH 11.0 was applied to several samples

of supercontracted wool which had been subjected to various additional treatments. The first samples were extracted after washing for only 2 min in running water. The second group was dried in a current of warm air before extraction. The third and fourth groups were soaked for an additional 30 and 180 min respectively after rinsing and before extraction to remove any further traces of LiCl. The amount of protein extracted from all these samples was essentially the same, being in the range 45–49 per cent. In a separate experiment, supercontraction by heating for 20 min in 9M LiCl at 96°C was shown to be irreversible even after soaking in distilled water for several weeks.

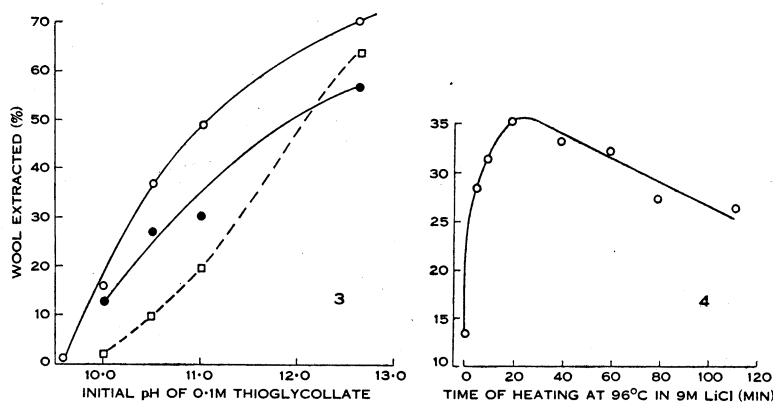


Fig. 3.—Extraction of proteins during 20 min at 50°C with 0.1M thioglycollate at various pH values from wool previously supercontracted by heating for 40 min at 96°C in 9M LiCl(○), or in 8M phenol(●). □ Extraction of untreated wool.

Fig. 4.—Relationship between time of preheating at 96°C in 9M LiCl on subsequent extractability during 20 min at 50°C in 0.1M thioglycollate initially at pH 10.5.

(vi) *Heating in Solutions Containing Both LiCl and Thioglycollate.*—Whereas wool supercontracted in 9M LiCl lost 29 per cent. of its protein during 20 min at 50°C in 0.1M thioglycollate initially at pH 10.5, the addition of 9M LiCl to the thioglycollate solution reduced the extraction to 4 per cent. Heating wool without previous supercontraction in a solution containing 9M LiCl and 0.1M thioglycollate adjusted to pH 10.5 (using indicator paper) extracted no protein, the 9M LiCl apparently repressing the solvent action of the thioglycollate for the wool proteins. In this experiment, wool digestion was measured by washing, drying, and weighing the residue after extraction. The repressive effect of high concentrations of salt on the extraction of proteins with alkaline thioglycollate was confirmed in another experiment in which the concentration of  $K_2CO_3$  in a solution containing 0.1M thioglycollate was increased from 2 to 4M. Although the pH was thereby increased from 10.1 to 10.5 the total protein extracted, as determined by weighing the washed undigested residue, fell from 68 to 17 per cent. In this particular experiment 100 ml of solution per 1 g wool treated were used instead of 30 ml.

(vii) *Reversal of the Effect of Heating in Water or in LiCl Solution.*—In Figure 5 are shown the effects of preheating wool in water and in 9M LiCl on the extractability of its proteins and also the apparent reversal of each of these two effects. The low thioglycollate extractability obtained after heating wool in water at 110°C is seen to be increased to the level of untreated wool by subsequently heating it in 9M LiCl at 96°C. Similarly the enhanced extractability obtained after heating firstly in 9M LiCl is reversed by subsequent heating in water.

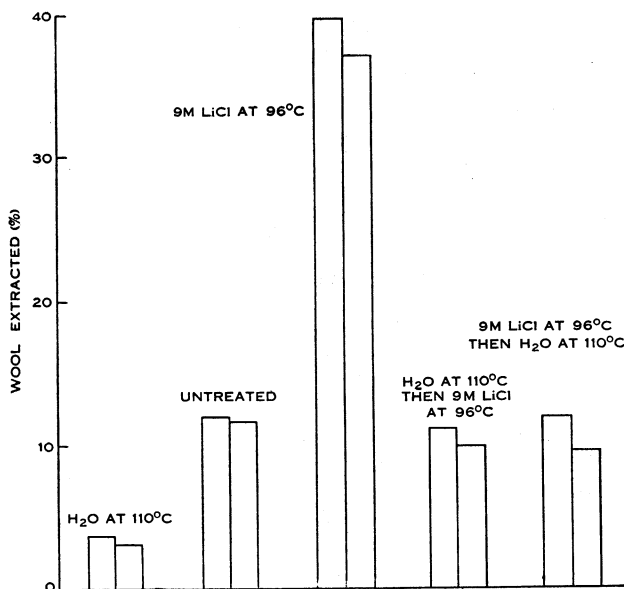


Fig. 5.—Opposite effects of preheating wool for 20 min in water and in 9M LiCl on extraction of protein during 20 min at 50°C in 0.1M thioglycollate (initially at pH 10.5) and the reversal of these effects. Extraction percentages are shown for duplicate experiments.

(viii) *Loss of Supercontraction and of Protein Extractability during Repeated Treatment with Thioglycollate.*—The alkaline thioglycollate fractional extraction method of recovering keratine 2 from wool (Gillespie and Lennox 1953, 1955a), was also applied to normal wool and 9M LiCl-supercontracted wool in the same experiment. This method removed the major portion of the protein from the latter in the first pH 10.5 extraction, in contrast to the normal wool which yielded less than half this amount of protein in the first extraction but a greater amount in the second pH 10.5 extraction (Fig. 6). That the first pH 10.5 extraction of supercontracted wool removed some keratine 2 from the fibres together with other wool proteins is evidenced by the lower content of protein in extract F (Fig. 6), which yields pure keratine 2 in the extraction of normal wool, and also by the presence of the peak corresponding to keratine 2 in the electrophoretic pattern of extract A of supercontracted wool. Extraction of supercontracted wool with alkaline thioglycollate

was complicated by reversal of the supercontracted fibres to their normal length within 3 min of immersion in the 0.1M thioglycollate at pH 10.5. Only the first extraction can therefore be regarded as reflecting the effect of supercontraction. To compensate for the readier extraction of protein from the supercontracted fibres, 0.1M thioglycollate at pH 10.0 was used for the first five extractions in another experiment and the residue was extracted with 0.1M thioglycollate at pH 11.6 (Fig. 6). Although less protein was removed in extract A by 0.1M thioglycollate at pH 10.0 than at pH 10.5, the fibres again reverted to their original length and the protein removed decreased progressively throughout the series.

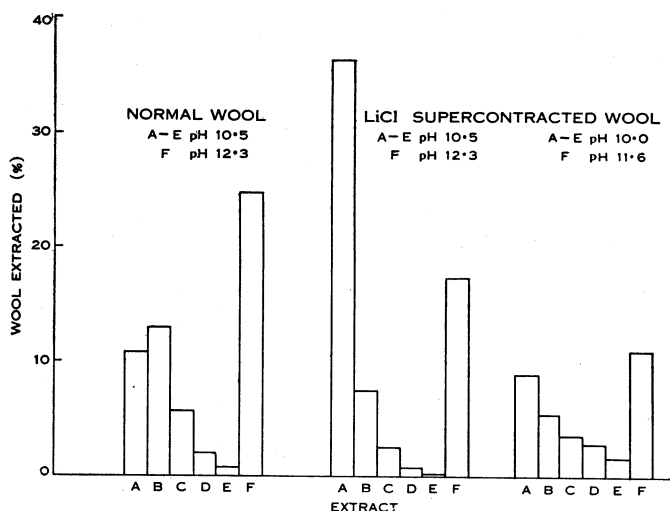


Fig. 6.—Protein extraction of normal and 9M LiCl-supercontracted wool five times with 0.1M thioglycollate initially at pH 10.5 then once with 0.1M thioglycollate initially at pH 12.3, also of supercontracted wool five times with 0.1M thioglycollate at pH 10.0 then once with thioglycollate at pH 11.6.

(ix) *Supercontracted Wool Cells*.—As with the intact fibre, heating wool cells in water at 110°C reduced the extractability of the constituent proteins in alkaline thioglycollate (Lennox 1956) and supercontraction in 9M LiCl at 96°C caused it to increase (Fig. 7). Since more protein is extracted from the untreated wool cells than from intact wool there was less margin for increasing the extraction from the cells by supercontraction than with wool itself.

(b) *Electrophoresis Patterns of Extracts of Normal and Supercontracted Wool*

(i) *Wool Supercontracted in LiCl*.—The electrophoresis patterns of the extracts of normal and LiCl-supercontracted wool, reported in Figure 3 and obtained with 0.1M thioglycollate at the initial pH's 10.0, 10.5, 11.0, and 12.6, are shown in Plate 1. The pH 10.0 and 10.5 extracts were freeze-dried to allow the solutions to be adjusted where possible to 1.5 per cent. protein concentration for electrophoresis; the pH 10.0 extract of normal wool yielded only sufficient protein for a 1.1 per cent. solution.

In the patterns corresponding to the pH 10.0, 10.5, and 11.0 extracts of normal wool, peaks can be seen corresponding to the four components designated keratines 1, 2, 3, and 4 (from left to right in order of increasing mobility). The main effect of supercontraction appears to have been to allow as much wool protein to be extracted at the low pH values as was recovered from normal wool at a higher pH, and also to yield extracts at the lower pH values having similar electrophoretic patterns to those obtained from normal wool at higher pH. Thus the pattern of the pH 10.5 extract of supercontracted wool resembles more closely the pH 11.0 pattern of normal wool than the pH 10.5 pattern.

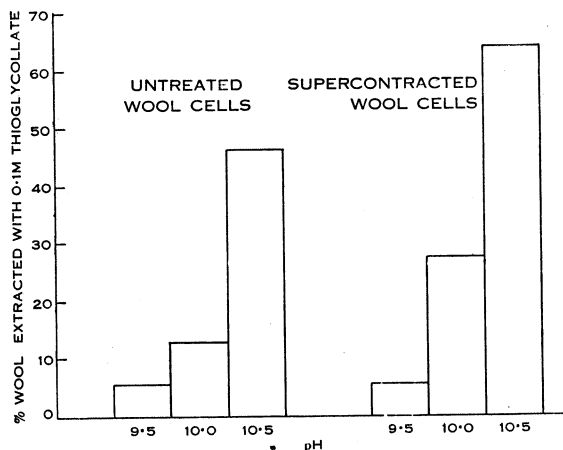


Fig. 7.—Extraction of protein from cortical cells during 20 min at 50°C with 0.1M thioglycollate at various pH values before and after supercontraction by heating for 40 min at 50°C in 9M LiCl. Each extraction was performed on a separate sample of cells.

Although keratines 1–4 can be readily distinguished in the electrophoresis patterns of extracts of normal wool prepared at pH values 10.0, 10.5, and 11.0 (Plate 1), in the pH 11.0 extract the movement of keratine 4 was retarded, and in the pH 12.6 extract its apparent mobility was reduced to that of keratine 3. The peak corresponding to keratine 3 in extracts prepared at pH 12.6 therefore carries a spike. Just as the pH 10.5 extract of supercontracted wool closely resembles the pH 11.0 extract of normal wool so also does the pH 11.0 extract of supercontracted wool resemble the pH 12.6 extract of normal wool, the movement of the keratine 4 again being retarded and its spiked peak therefore coinciding in position with the peak of keratine 3. Retardation of the leading component in the ascending limb with increase in concentration of the other proteins in the mixture is a well-known phenomenon in the electrophoresis of proteins. A similar retardation of keratine 4 appears to have occurred in the pH 10.0 extract of supercontracted wool. This has been confirmed in two other experiments.

(ii) *Wool Supercontracted in Phenol*.—The patterns of the pH 10.5 and 11.0 0.1M thioglycollate extracts of wool supercontracted in 8M phenol resemble those

of the thioglycollate extracts of the LiCl-supercontracted samples. They exhibit a greater ratio of keratine 2 to the other components than do the patterns of extracts of untreated wool obtained with thioglycollate at the same pH values (Plate 2).

#### IV. DISCUSSION

In a previous paper (Lennox 1956) the loss in thioglycollate extractability of wool proteins, produced by heating wool in water, was attributed to slight changes in the molecular configuration of the major proteins, enabling the formation of additional hydrogen bonds which oppose solution. In an analogous manner the enhanced extractability of the proteins in supercontracted wool may be ascribed to the elimination of some of the intermolecular forces such as hydrogen bonds and van der Waals forces by the far more extensive changes in molecular configuration which accompany supercontraction. This may explain the extraction of a greater proportion of the reduced wool at a particular alkaline pH value than from normal wool, fewer cross-links being present to restrain the mutual repulsion of the negatively charged centres in the reduced proteins. Mutual repulsion of negatively charged centres with consequent greater lability of hydrogen bonds was also suggested by Crewther and Dowling (1956) instead of direct splitting of hydrogen bonds (Alexander 1951) to explain supercontraction in LiCl and in some other salt solutions. It was held that if adsorption of  $\text{Cl}^-$  anions occurs in preference to the highly hydrated  $\text{Li}^+$  cations this would increase the net negative charge on the wool proteins thereby favouring structural rearrangements culminating in supercontraction. Reversal of supercontraction by heating to  $50^\circ\text{C}$  in alkaline thioglycollate suggests that the disulphide bonds in wool must not be split if the fibre is to remain supercontracted. If they are ruptured the wool proteins revert to their original length.

Increasing the concentration of LiCl beyond 9M may have failed to enhance further the extractability of the proteins by thioglycollate (Fig. 2) through withholding water from them. Water is essential to supercontraction just as it was previously shown to be essential to thermal changes leading to decreased thioglycollate extraction (Lennox 1955). Presumably it facilitates movement of the chemical groups and peptide chains in the wool proteins into new positions.

Non-reversal of supercontraction after heating in an acidic solution of LiBr and subsequently soaking in water was attributed by Alexander (1951) to acid hydrolysis of peptide bonds in the wool proteins. Pretreatment with a boiling solution of 0.12 per cent.  $\text{H}_2\text{SO}_4$  has also been shown to increase the alkali solubility of wool (Dusenbury, Mercer, and Wakelin 1954). The enhanced extractability with alkaline thioglycollate following supercontraction in 9M LiCl at pH 5, however, arose from the supercontraction itself and not from the accompanying hydrolysis of some peptide bonds due to the low pH of the LiCl solution. This latter possibility is excluded by the observation that heating wool for 20 min at  $96^\circ\text{C}$  in buffer at pH 4, or in LiCl solutions of the same pH as the 9M LiCl but of lower concentration, failed to cause either supercontraction or increased extractability in the thioglycollate.

Electron microscopy of the fibrils from wool supercontracted in  $\text{NaHSO}_3$  (Jeffrey, Sikorski, and Woods 1955) showed that the protofibrils of the cortical cells, the structures which embody the bulk of the wool protein, themselves undergo



contraction. The enhanced extractability of wool following supercontraction with LiCl is also probably attributable to changes in the extracted fibrillar proteins and not primarily to changes in the membranes or intercellular proteins. This view finds support also in the increased thioglycollate solubility accompanying the supercontraction of the wool cells.

Reversal of the LiCl-enhanced thioglycollate extractability, by heating the supercontracted wool in water (Fig. 5), confirms the fall in extractability after the initial 20 min heating in 9M LiCl (Fig. 4). Supercontraction appears to proceed more rapidly than the hydrothermal changes which lower the extractability or it commences earlier during the heating process. The hydrothermal changes supervene only after the initial 20 min in 9M LiCl.

It is significant that the electrophoretic patterns of the proteins obtained by extraction of the supercontracted wool with thioglycollate are identical in their main features with those obtained from normal wool at higher pH values. This observation suggests that the change in pattern of a thioglycollate extract of normal wool, accompanying an increase in pH of the thioglycollate, for example from 10.5 to 11.0, is not due to alkaline hydrolysis of components appearing in the pH 10.5 extract but reflects an increased extraction of protein components pre-existing in the wool. In this respect it supports earlier observations indicating the absence of hydrolytic damage to the wool proteins under the conditions used in the alkaline thioglycollate fractional extraction method. Thus it has been shown that there was little increase in dialysable nitrogen using five 20-min extractions at 50°C with 0.1M thioglycollate initially at pH 10.5 followed by one extraction with the 0.1M thioglycollate at pH 12.3 (Gillespie and Lennox 1955a).

The present authors have also demonstrated that, when 2-ml portions of a 1 per cent. solution of *S*-carboxymethyl keratine 2 (Gillespie 1957) are dialysed through "Cellophane" tubing against 2 l. of 0.1M thioglycollate at pH 10.0, 10.5, 11.0, 11.5, 12.0, or 12.5 for 10 days at 2°C, no protein is lost except at the two highest pH values (12.0 and 12.5) where 5 per cent. of the protein is lost.

Additional evidence of stability was provided by the observation that the amide content of keratine 2 was not less but greater than that of the parent wool (Simmonds 1955; Leach, unpublished data) and that equilibration of wool at pH 13.3 for 24 hr at 25°C did not affect the amide content (Horner 1954). These data also strengthen the view that electrophoresis of the alkaline thioglycollate extracts of wool gives a true picture of the protein components of the fibre, or of groups of components having mobilities corresponding to keratines 1, 2, 3, and 4, and not of the products of alkaline hydrolysis of some unidentified parent protein.

#### V. ACKNOWLEDGMENT

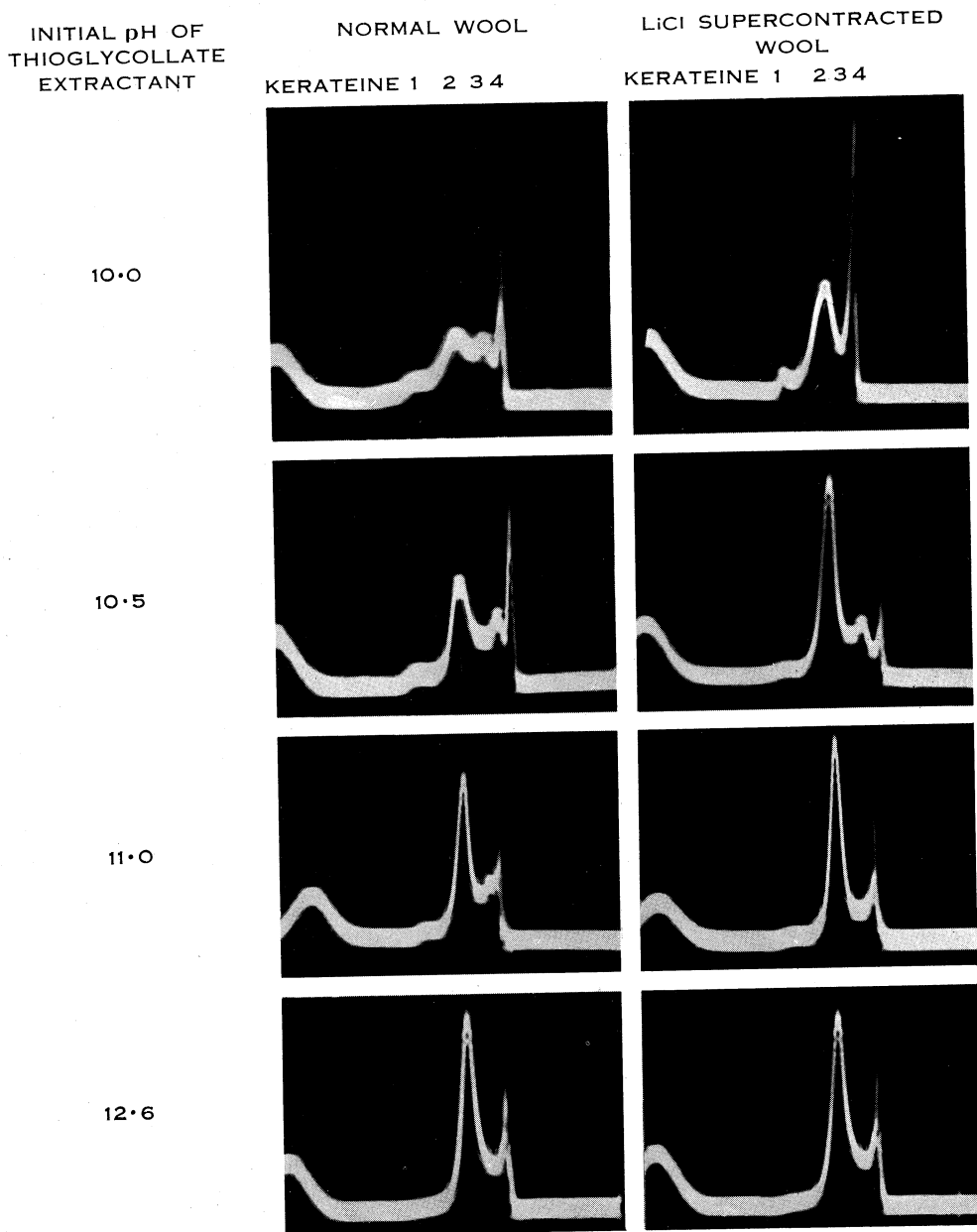
Thanks are due to W. G. Crewther of this Laboratory for supplying the wool cells.

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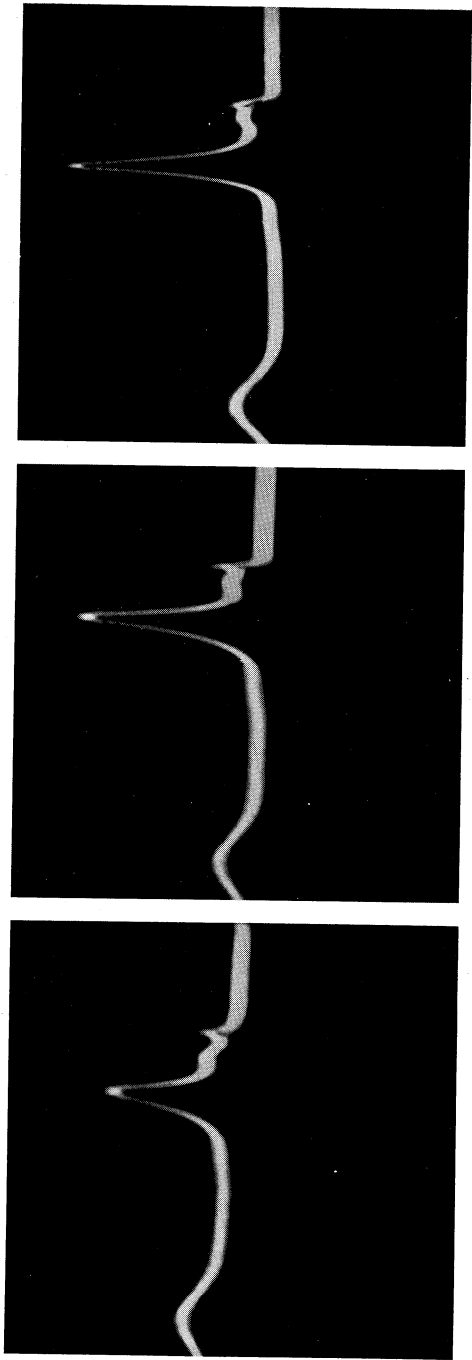
## EXTRACTION OF WOOL PROTEINS FOLLOWING SUPERCONTRACTION



Ascending boundary electrophoresis patterns of thioglycollate extracts from normal and 9M LiCl-supercontracted wool run in a thioglycollic acid-glycine-KOH buffer of ionic strength 0.22 at pH 11. Protein concentration 1.5 per cent. except for pH 10.0 extract of normal wool which is at 1.1 per cent. Period of electrophoresis 300 min except for pH 10.5 extract of normal wool (320 min) and pH 11.0 extract of normal wool (250 min).

EXTRACTION OF WOOL PROTEINS FOLLOWING SUPERCONTRACTION

PHENOL SUPERCONTRACTED WOOL



10.5

11.0

12.6

INITIAL pH OF THIOGLYCOLLATE EXTRACTANT

Ascending boundary electrophoresis patterns of thioglycollate extracts of wool supercontracted in 8M phenol. Buffer used was as for Plate 1 and period of electrophoresis was 300 min.