RELATION BETWEEN THE TYROSINE CONTENT OF VARIOUS WOOLS
AND THEIR CONTENT OF A CLASS OF PROTEINS RICH IN TYROSINE
AND GLYCINE

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Abstract

Wool samples can vary in their content of tyrosine by as much as 60%. It
has been shown that this is due to variation in their content of a heterogeneous group
of proteins which are very rich in tyrosine and glycine (about 1 residue in 5 and 1
residue in 3 respectively). Certain Lincoln wools and some felting lustre mutant
Merino wools appear to contain very little of these proteins whereas fine Merino wools
may contain as much as 12% by weight of them. As this is too much protein to be
accommodated in the cuticular membranes, the currently held site of origin of high-
tyrosine proteins, it is concluded that at least some of these proteins must be
accommodated in an unknown region of the cortex.

The variable tyrosine content of wools may be related to observed differences
in their rate of photochemical degradation and mechanicochemical properties.

I. INTRODUCTION

Since the studies of Barritt and King (1926, 1929) showing that the sulphur
content of wool can vary from 3·0 to 4·1%, values later extended by Reis (1965) to
2·7-4·2%, it has been realized that wool is a material of very variable composition.
The major part of this non-uniformity of sulphur content is due to the variable
synthesis by the follicle of specific proteins of very high sulphur content which for
convenience have been termed ultra-high-sulphur proteins (Gillespie and Reis 1966;
Gillespie, Broad, and Reis 1969; Broad and Gillespie 1970). Wool samples which
are at the lower limit of sulphur content (about 2·7%) probably contain little or no
ultra-high-sulphur proteins but in all other wools there is a linear relation between
the proportion of these proteins and the sulphur content of the wool. These proteins
have a quite distinctive amino acid composition and consequently their presence in
wool in variable amounts is reflected in variations in the content of a number of amino
acids besides cystine. Some like histidine, serine, threonine, and proline increase as
cystine increases whilst others like aspartic acid, leucine, and phenylalanine decrease
in amount (Gillespie, Broad, and Reis 1969). In this way most of the observed
variations in amino acid composition (Crewther et al. 1965) can be accounted for.
However, many amino acid analyses show that there is also a major variability in
tyrosine content which is unrelated to variations in cystine content (Ward, Binkley,
and Snell 1955; Le Roux and Speakman 1957; Crewther et al. 1965) or to alterations
in the proportion of ultra-high-sulphur proteins in wool from the one sheep (Gillespie,
Broad, and Reis 1969). Le Roux and Speakman (1955, 1957) suggested that this
variability of tyrosine content might be due to differing proportions of crystalline
and non-crystalline regions in the fibre. It seemed of interest to see whether this was

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due, by analogy with the ultra-high-sulphur proteins, to a variable synthesis of specific proteins of high-tyrosine content. Such a group of proteins are known to exist in wool (Gillespie 1960; Harrap and Gillespie 1963; DeDeurwaerder, Dobb, and Sweetman 1964; O'Donnell and Thompson 1964; Zahn and Biela 1968). The present paper describes experiments designed to examine this proposition.

II. Materials and Methods

(a) Origin and Preparation of Wool Samples

The sources of the wool samples are given in Section V. The fibre tips of the wool were discarded and the remainder cleaned by the procedure of Gillespie and Reis (1966).

(b) Preparation of Soluble Proteins

The wool samples were solubilized by alkaline reduction in the presence of urea as described previously (Gillespie and Reis 1966). This solution was clarified by centrifugation to remove an insoluble residue and after alkalylation with iodoacetate and dialysis against deionized water, the low-sulphur protein fraction along with the high-tyrosine protein fraction (partially insoluble at neutral pH) was precipitated as the zinc complex by making the solution 0.02M with zinc acetate. The pH was usually in the optimum range of 5.8–6.2. After stirring for 2 hr the precipitate was centrifuged at 5000 g for 30 min, dissolved in 0.02M sodium citrate (50 ml/g wool), and then dialysed against deionized water. It is important not to exceed these centrifugation conditions for the precipitate then becomes difficult to dissolve. These proteins will be referred to as the zinc precipitate fraction. The supernatant containing the high-sulphur proteins was made 0.02M with sodium citrate, dialysed against deionized water, and freeze-dried.

(c) Recovery of Insoluble Extraction Residue

In some experiments the residue from the extraction was suspended in deionized water (200 ml/g wool), alkylated with iodoacetate, centrifuged at 13,000 g, and the washing process repeated once. The washed residue was suspended in a small volume of water and freeze-dried.

(d) Identification of High-tyrosine Proteins by Gel Electrophoresis

The zinc precipitate proteins of wool resolve into a minimum of 15 bands when run electrophoretically at pH values near 8.6 (Thompson and O'Donnell 1964). The group of bands classed as “intermediate” in mobility by the latter authors have been recognized as containing at least some of the high-tyrosine components (O'Donnell and Thompson 1964). This procedure has been used for their identification in the present study.

Electrophoresis was carried out as follows. The gel was composed of 13.0 g starch (Connaught), 40 g urea, and 50 ml buffer (pH 8.46) composed of 8.5 mM citric acid, 75 mM Tris, and 3.7 mM sodium tetraborate. The bridge buffer (pH 8.35) contained boric acid (0.3M) and NaOH (0.06M). The gels were run in a cooled-plate apparatus at a voltage gradient of 15 V/cm for about 4 hr. At the completion of electrophoresis the gels were sliced and stained with nigrosine (0.01%) in water-ethanol-acetic acid (45:45:10 v/v).

(e) Amino Acid Analyses

The samples (20 mg) were hydrolysed for 18 hr in vacuo at 108°C with 4 ml of HCl (6N) and phenol (2.5 mM) and analysed with a Beckman-Spinco model 120C amino acid analyser.

(f) Estimation of High-tyrosine Proteins in the Zinc Precipitate Fraction

The extinction coefficient (E% cm 277 nm) was used as one measure of the proportion of high-tyrosine proteins in the zinc precipitate fraction. This involved two measurements, a total protein concentration by refractometry and an absorbance measurement at 277 nm. Because of the unusual solubility properties of these proteins and their proneness to form opalescent solutions the following procedure had to be adopted for the measurement of extinction coefficients. The dialysed zinc precipitate fraction was mixed to suspend insoluble protein and an aliquot dialysed for 24 hr at 2°C against 2 litres of pH 10.5 buffer (glycine 17.5 g, NaOH 8 g). The total
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protein concentration was measured in this dialysed solution using a Brice-Phoenix differential refractometer with the dialysate as blank, assuming $dn/dc = 1.85 \times 10^{-3}$ (Harrap and Gillespie 1963). An aliquot of this solution was then diluted with at least 10 volumes of glacial acetic acid and the absorbance measured at 277 nm using glacial acetic acid as the blank.

Using these extinction coefficients very rough estimates were made of the proportion of high-tyrosine proteins in wool samples. The following assumptions were made: that the helical low-sulphur proteins account for 50-60% of wool, that their extinction coefficient is 6.5, and that of the high-tyrosine proteins is 30.

(g) Removal of High-tyrosine Proteins from a Zinc Precipitate Fraction

Purification was accomplished by the use of an acetone fractionation originally developed by Gillespie (1960) but subsequently simplified and made much more reproducible by Dowling (personal communication). A solution of zinc precipitate fraction in deionized water was centrifuged at 40,000 $g$ for 1 hr to sediment those high-tyrosine proteins which are insoluble at neutral pH and which are responsible for the turbidity of the zinc precipitate fraction. To this solution (adjusted to 1% protein conc.) was added with stirring 2 volumes of acetone and $\frac{1}{2}$ volume of saturated ammonium sulphate. After stirring for 2 hr the precipitated proteins were collected by centrifugation, dissolved in 0.05M sodium borate, and dialysed. This precipitation was repeated once and the final precipitate of purified low-sulphur protein was dissolved in borate solution and dialysed against deionized water. High-tyrosine proteins were recovered from the acetone-ammonium sulphate supernatants by dialysis and freeze-drying.

Table 1

<table>
<thead>
<tr>
<th>Type of Sheep</th>
<th>Wool Code</th>
<th>Extinction Coefficient ($E_{1%}^{1 \text{cm}}$ 277 nm)</th>
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<tr>
<td></td>
<td>Zinc Precipitate Fraction</td>
<td>Zinc Supernatant Fraction</td>
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<tr>
<td>Lincoln*</td>
<td>568</td>
<td>6.72</td>
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<tr>
<td>Lincoln</td>
<td>593</td>
<td>6.61</td>
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<tr>
<td>Lincoln</td>
<td>594</td>
<td>6.63</td>
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<tr>
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<td>7.76</td>
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<tr>
<td>Border Leicester</td>
<td>565</td>
<td>8.22</td>
</tr>
<tr>
<td>Suffolk</td>
<td>414</td>
<td>8.57</td>
</tr>
<tr>
<td>Dorset Horn</td>
<td>572</td>
<td>9.57</td>
</tr>
<tr>
<td>Merino SNP†</td>
<td>29</td>
<td>9.82</td>
</tr>
<tr>
<td>Merino MNP†</td>
<td>9</td>
<td>8.93</td>
</tr>
<tr>
<td>Merino MPB†</td>
<td>8</td>
<td>8.87</td>
</tr>
<tr>
<td>Merino FNP*†</td>
<td>5</td>
<td>10.22</td>
</tr>
<tr>
<td>Merino*</td>
<td>265</td>
<td>10.00</td>
</tr>
<tr>
<td>Merino (Fine)</td>
<td>418x</td>
<td>10.90</td>
</tr>
<tr>
<td>Lustre mutant Merino*</td>
<td>99</td>
<td>6.62</td>
</tr>
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</table>

* Amino acid analyses of these wool samples are recorded in Table 2.
† Abbreviations refer to strain of Merino and fineness of wool: SNP, strong non-Peppin; MNP, medium non-Peppin; MPB, medium Peppin B; FNP, fine non-Peppin.

III. RESULTS

(a) Variation in the Proportion of High-tyrosine Components in the Zinc Precipitate Fraction from Different Types of Wool

The extinction coefficients of the zinc precipitate fraction prepared from a variety of different wool samples are shown in Table 1. It can be seen that they vary
from about 6.6 for some Lincoln wools to 10.2 for some Merino wools with other wools providing values in between these extremes.

These zinc precipitate fractions have been run electrophoretically in starch gels and some are shown in Figure 1. It can be seen that those listed as having low-

![Fig.1](image1)

**Fig. 1**—Gel-electrophoresis pattern of zinc precipitate proteins from different wool samples showing variations in the proportions of components with mobilities faster than component 8. (a) Lincoln No. 593; (b) lustre mutant No. MW99; (c) Merino No. 5; (d) Merino No. 19; (e) Suffolk No. 414. I.B., intermediate bands.

![Fig.2](image2)

**Fig. 2**—Gel-electrophoresis patterns showing the progressive removal of high-tyrosine proteins during the fractionation of a zinc precipitate fraction by the acetone-ammonium sulphate procedure. (a) Unfractionated; (b) supernatant from zinc precipitate fraction after centrifugation; (c) first acetone–ammonium sulphate fractionation: precipitate; (d) second acetone–ammonium sulphate fractionation: precipitate, purified low-sulphur proteins; (e) centrifuged zinc precipitate fraction: type I high-tyrosine fraction; (f) first acetone–ammonium sulphate fractionation: supernatant, type II high-tyrosine fraction.

extinction coefficients in Table 1 also contain the lowest proportion of components with mobilities faster than band 8. In these low-tyrosine preparations at least one band slower than 7, possibly 6, can be seen to be missing so that it is likely that not all the high-tyrosine components are confined to the “intermediate” range of mobilities.
The extinction coefficients of a number of zinc supernatant fractions (high-sulphur proteins) were measured and the results are recorded in Table 1. It can be seen that with the exception of the lustre mutant proteins, the extinction coefficients cover quite a small range and show no relation to those of the corresponding zinc precipitate fractions.

(b) Variations in the Tyrosine Content of Wool

Two wool samples (Lincoln 568 and lustre mutant Merino 99) which gave zinc precipitate fractions with low-extinction coefficients and two wools (Merino Nos. 265 and 5) which gave zinc precipitate fractions with high extinction coefficients were hydrolysed and analysed for their content of amino acids. It can be seen (Table 2) that between the lowest and highest values there is a 60% variation in tyrosine content. There is a good correlation between the content of this amino acid and the extinction coefficient of the zinc precipitate fraction isolated from that wool. Other correlations can also be seen, for phenylalanine and glycine closely parallel the changes in tyrosine.

### Table 2

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>Tyrosine-rich Protein*</th>
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<tr>
<td>Lys</td>
<td>2·63</td>
<td>3·60</td>
<td>2·99</td>
<td>3·63</td>
<td>2·95 4·47 2·70 4·10 0·10</td>
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<tr>
<td>His</td>
<td>0·85</td>
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<td>0·70</td>
<td>1·14</td>
<td>0·87 1·41 0·84 1·17 0·12</td>
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<tr>
<td>Arg</td>
<td>6·95</td>
<td>6·01</td>
<td>7·39</td>
<td>5·13</td>
<td>6·60 5·73 6·74 5·79 4·83</td>
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<tr>
<td>CMCys†</td>
<td>—</td>
<td>12·20</td>
<td>—</td>
<td>11·90</td>
<td>—   10·90 —   8·71 12·40</td>
</tr>
<tr>
<td>Asp</td>
<td>5·58</td>
<td>4·95</td>
<td>6·75</td>
<td>5·32</td>
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<td>Thr</td>
<td>7·09</td>
<td>6·31</td>
<td>6·50</td>
<td>6·69</td>
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<td>8·49</td>
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<td>6·37</td>
<td>8·32</td>
<td>9·19 8·67 8·74 7·87 33·00</td>
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<tr>
<td>Ala</td>
<td>5·05</td>
<td>5·47</td>
<td>5·74</td>
<td>5·72</td>
<td>5·48 5·94 5·04 6·16 0·45</td>
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<tr>
<td>CyS</td>
<td>14·30</td>
<td>1·39</td>
<td>10·90</td>
<td>1·10</td>
<td>10·70 0·72 12·20 0·82 0·00</td>
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<tr>
<td>Val</td>
<td>6·00</td>
<td>6·19</td>
<td>6·12</td>
<td>6·05</td>
<td>5·59 6·24 5·48 6·59 1·11</td>
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<tr>
<td>Met</td>
<td>0·36</td>
<td>0·58</td>
<td>0·47</td>
<td>0·64</td>
<td>0·46 0·80 0·40 0·93 0·00</td>
</tr>
<tr>
<td>Ile</td>
<td>3·15</td>
<td>3·06</td>
<td>3·62</td>
<td>3·10</td>
<td>3·02 3·27 3·00 3·77 0·52</td>
</tr>
<tr>
<td>Leu</td>
<td>6·44</td>
<td>6·23</td>
<td>7·79</td>
<td>6·58</td>
<td>7·26 6·82 6·94 7·37 5·72</td>
</tr>
<tr>
<td>Tyr</td>
<td>2·61</td>
<td>2·61</td>
<td>2·68</td>
<td>2·86</td>
<td>4·25 2·91 3·88 2·80 19·40</td>
</tr>
<tr>
<td>Phe</td>
<td>1·91</td>
<td>1·83</td>
<td>2·16</td>
<td>2·09</td>
<td>2·71 2·28 2·38 2·47 2·71</td>
</tr>
<tr>
<td>Trp‡</td>
<td>0·33</td>
<td>n.d.</td>
<td>0·36</td>
<td>n.d.</td>
<td>0·45 n.d. 0·44 n.d. n.d.</td>
</tr>
</tbody>
</table>

* Representative of certain type II high-tyrosine protein components.
† S-Carboxymethylcysteine.
‡ Estimated by a colorimetric procedure (Spies and Chambers 1949).
To exclude the possibility that tyrosine-rich proteins might be left behind in extracting some wools, the residues from the extraction of these four wool samples were also hydrolysed and analysed. It can be seen (Table 2) that the four residues are remarkably alike in composition particularly in their contents of tyrosine.

(c) Removal of High-tyrosine Proteins from a Zinc Precipitate Fraction

A zinc precipitate fraction with a very high extinction coefficient prepared from Merino wool No. 19 was fractionated by the acetone-ammonium sulphate procedure. The progressive decrease in extinction coefficient at each stage of the fractionation is shown in Table 3, finally reaching a level comparable to the lowest of the Lincoln wool proteins. These preparations have also been run in starch gel (Fig. 2) and it can be seen that the progressive decrease in extinction coefficient is accompanied by a disappearance of the bands which run faster than band 8. The very high extinction coefficients of the high-tyrosine fractions separated during this purification procedure can be seen in Table 3.

| Table 3 |
| Extinction coefficients of fractions obtained from a zinc precipitate fraction isolated from a merino wool of high tyrosine content |

Protein concentrations measured by refractometry in glycine-NaOH buffer at pH 10.5 and absorbance at 277 nm in glacial acetic acid

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Extinction Coefficient ((E_{1%}^{1 \text{ cm}} 277 \text{ nm}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zinc precipitate fraction</td>
<td>10.8</td>
</tr>
<tr>
<td>Centrifugation Supematant</td>
<td>9.8</td>
</tr>
<tr>
<td>Precipitate*</td>
<td>28.0</td>
</tr>
<tr>
<td>First acetone-ammonium sulphate fractionation Supematant†</td>
<td>30.5</td>
</tr>
<tr>
<td>Precipitate</td>
<td>7.1</td>
</tr>
<tr>
<td>Second acetone-ammonium sulphate fractionation—precipitate‡</td>
<td>6.6</td>
</tr>
<tr>
<td>Zinc precipitate fraction from Lincoln wool</td>
<td>6.6</td>
</tr>
</tbody>
</table>

* Type I high-tyrosine fraction. † Type II high-tyrosine fraction. ‡ Purified low-sulphur proteins.

IV. Discussion

This work provides confirmation of earlier observations that wools from different sheep can vary in their content of tyrosine residues. These differences are most pronounced between breeds but there are also intra-breed differences. The maximum range over which these variations occur is not known but in the present work a variation of 60% was observed. The evidence that these variations in tyrosine content are largely due to variations in the proportion of proteins which are rich in tyrosine is as follows. When wools of high tyrosine content are extracted the residue is much lower in tyrosine content than the parent wool and is similar in composition to the
residue obtained from the low tyrosine wools. It can be concluded therefore that differences in tyrosine content are due solely to differences in the extracted proteins. As the high-sulphur proteins (zinc supernatant fraction) all have similar extinction coefficients regardless of whether they originate from wools of low- or high-tyrosine content it can be concluded further that differences in tyrosine content are associated solely with the proteins in the zinc precipitate fraction. That the extra tyrosine is associated with a specific group of proteins is shown by two pieces of evidence: the zinc precipitate fraction shows fast-moving gel-electrophoretic bands with mobilities characteristic of tyrosine-rich proteins and after purification by the acetone–ammonium sulphate precipitation procedure these bands are absent from the starch-gel pattern. Furthermore the extinction coefficient of the precipitated protein is now the same as that of the zinc precipitate fraction isolated from a low-tyrosine wool. At this stage of the investigation there is no indication as to a likely cause for the variable incorporation of the tyrosine-rich proteins into wool, whether it is wholly genetic in origin or whether other factors such as diet are also involved. These factors are at present under investigation.

This work has shown that there are two classes of high-tyrosine proteins, both heterogeneous, those of limited solubility (type I) which can be isolated by centrifugation alone and those which are more soluble in the presence of the low-sulphur proteins (type II) and which are separated by an acetone fractionation. Gel electrophoresis (Fig. 2) shows that with one or two exceptions there is no overlap in components, type I being mostly the lower mobility components whereas type II contains the fractions of higher mobility. This is probably a reflection of the major difference in S-carboxymethyleysteine (CMCys) content between them, for type I with a CMCys content of 5% is similar to the low-sulphur proteins in this respect whereas type II, which contains 11% CMCys, is more nearly like certain of the high-sulphur proteins. As both types have about the same molecular weight (type I, 11,400±5%; type II, 12,300±5%) with no evidence of molecular weight inhomogeneity it can be concluded that components separating during gel electrophoresis do so not because of differences in size but because they differ in charge. It is probable that at least part of this charge heterogeneity is due to differences in CMCys content.

Over the last 15 years or so a number of workers have attempted the separation of homogeneous helical proteins from wool prior to sequence studies and most, if not all, of these preparations have been contaminated with high-tyrosine proteins (Gillespie 1960; O'Donnell and Thompson 1964). On the basis of the present studies it can be predicted that the use of a low-tyrosine wool such as a Lincoln wool for the preparation of the helical proteins would greatly simplify this work particularly if the sheep was kept on a low-sulphur diet to minimize the synthesis of the ultra-high-sulphur proteins (Gillespie and Reis 1966).

DeDeurwaerder, Dobb, and Sweetman (1964) suggested that the high-tyrosine proteins were located between the cuticular layers of the wool fibre and Andrews, Inglis, and Williams (1966) discussed the origin of these proteins in more specific terms as cementing materials from the intercellular layers of the cuticle. The amino acid analysis given by the latter workers is very similar to that of Zahn and Biela (1968), and to the analysis given in the present study (Table 2). The most marked
characteristics are their richness in glycine, tyrosine, and phenylalanine, their low content of glutamic acid, and the almost complete absence of lysine and histidine. Bradbury, Leeder, and Watt (1971), after a thorough study of the histological components of wool and the proteins which originate from them, isolated a protein from the cell membrane complex which was moderately rich in glycine and tyrosine and on this basis suggested identity with the protein fraction of DeDeurwaerder, Dobb, and Sweetman (1964). However, the analysis given by Bradbury, Chapman, and Kerry (1966) differs markedly from the analysis given by Andrews, Inglis, and Williams (1966) in having 25 times more lysine, 5 times more histidine, and 15 times more glutamic acid. Furthermore the proportion of high-tyrosine protein found in some Merino wools in the present work (approx. 12% by weight) is too large to be accommodated extracellularly, as Bradbury, Leeder, and Watt (1971) found that the total amount of cell membrane complex accounted for no more than 3.3–3.7% of the Merino wool fibre. It must be concluded that the site of origin of much of this protein is unknown but that it is almost certainly in some internal structure, perhaps the matrix. This conclusion is reinforced by the fact that echidna quills which have no scale structure contain about 32% of high-tyrosine proteins (Gillespie 1972).

There are two matters of a practical nature which may be of relevance to this problem. Wool as an apparel fibre suffers from the defect of yellowing when exposed to radiation and this is thought to be connected with photodegradation of the aromatic amino acid residues particularly tryptophan (Inglis and Lennox 1965; Inglis, Leaver, and Lennox 1966; Lennox and Rowlands 1969) in a reaction in which tyrosine is involved in the internal transfer of excitation energy (Teale and Weber 1957). At least some of the tyrosine-rich proteins are also moderately rich in tryptophan and this is reflected in the somewhat increased tryptophan content of the two tyrosine-rich wools (Table 2). It is therefore of considerable interest to know what part if any the tyrosine-rich proteins play in the yellowing process and whether their elimination or reduction in proportional amount has any effect on the stability of wool to radiation damage. Le Roux and Speakman (1955, 1957) found that the tyrosine content of wool was directly connected with certain important physical characteristics of wool. Although these workers used a rather crude estimation for tyrosine content nevertheless the mechanicochemical relations they proposed may be valid and the relation between the level of high-tyrosine proteins in wool and its mechanicochemical properties should now be re-examined.

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VI. References
