CHEMICAL CHANGES IN WOOL TREATED WITH SOLUTIONS OF IODINE

By W. G. Crewther* and L. M. Dowling*

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Summary

The chemical changes in wool associated with iodination in various solvents have been investigated. In many solvents a large proportion of the tyrosine residues is iodinated but in others this modification is negligible. Oxidation of disulphide bonds occurs in all solvents. This appears to proceed stepwise and intermediate oxidation products of cystine residues are formed in considerable amounts during iodination in solvents such as ethanol. Oxidation of sulphydryl groups and ethylation of carboxyl groups also occur in this solvent. The presence of small amounts of water in the ethanol increases the rate of reaction with the wool. Iodination in ethanol also causes a considerable decrease in solubility in solutions of urea–bisulphite, and an increase in trypsin digestibility and alkali solubility.

The results are discussed in relation to recent suggestions that the tyrosine residues of wool are confined to the amorphous regions of the fibre (Ghosh, Holker, and Speakman 1958) and that tyrosine residues which react with iodine in propanolic solutions are in the non-crystalline portions of the fibre (Haly and Feughelman 1960). Factors affecting the solubility of wool in urea–bisulphite solutions are also discussed.

I. Introduction

Although iodine has been used in protein research as a more or less specific reagent for tyrosine, the complexity of the reaction of iodine with proteins is now being more widely recognized (Ramachandran 1956). Apart from substitution reactions with the tyrosine and histidine residues of proteins, oxidation of tryptophan, serine, threonine, methionine, cysteine, and cystine residues has been demonstrated. By analogy with the action of perbenzoic acid and hydrogen peroxide on cystine (Lavine 1936), Ramachandran (1956) suggests that partial oxidation products of cystine residues of proteins may be formed during iodination. Some indication that such products are formed during the treatment of wool with bromine (Consden, Gordon, and Martin 1946) or with hydrogen peroxide (Consden and Gordon 1950) has already been obtained. More definite evidence for the formation of such partial oxidation products of cystine during oxidation of wool with peracetic acid has now been provided by Maclaren, Leach, and O'Donnell (1959).

Earlier studies of the iodination of wool have been concerned primarily with locating tyrosine residues in wool in radioautographic experiments (Richards and Speakman 1955), in X-ray diffraction studies (Fraser and MacRae 1957), or for assessing the accessibility of tyrosine residues to certain solvents (Harrison and Speakman 1958; Ghosh, Holker, and Speakman 1958; Haly and Feughelman 1960). Similarly Haly, Feughelman, and Griffith (1957) have related changes in the supercontraction behaviour of wool fibres following iodination to substitution in the tyrosine residues of wool.

* Division of Protein Chemistry, C.S.I.R.O. Wool Research Laboratories, Parkville, Vic.

In order to interpret the effects of iodination on the physical and chemical properties of wool we found it necessary to obtain more complete information concerning the reactions of iodine with wool particularly with respect to iodination in ethanol. Richards and Speakman (1955) have already reported that the histidine and tryptophan residues of wool are not modified by iodination in ethanol whereas the tyrosine residues are almost quantitatively converted to di-iodotyrosine residues. However, we considered three additional types of reaction were likely to occur: oxidation of sulphydryl or disulphide groups, ethylation of carboxyl groups catalysed by HI formed in oxidation and substitution reactions, and changes in protein configuration which would be facilitated by rupture of disulphide bonds. Particular attention has been given to these reactions during iodination in aqueous or anhydrous ethanol but comparative studies have been made with other solvents.

Earlier studies on the arrangement of tyrosine residues in the wool fibre and their importance in determining the physical properties of wool are discussed in the light of the additional chemical data concerning iodination.

II. Materials and Methods

The Corriedale 56's wool used in these experiments was cleaned by extraction three times with light petroleum, twice with cold ethanol, and several times with cold distilled water. It was dried in a stream of air at 40°C.

Unless otherwise specified non-aqueous solvents were dried and redistilled. Alcohols were dried by the method of Manske (1931), other solvents by shaking with the appropriate dessicant. For use with these solvent systems the wool was dried *in vacuo* at 40°C over P_2O_5 for 3 days. All other reagents were of "Analar" quality.

With each solvent system iodination of the wool was carried out at 20°C for periods which varied according to the rate of iodination. A liquor ratio of 100 : 1 was used for all solvents. The subsequent procedure was similar to that described by Richards and Speakman (1955) and by Harrison and Speakman (1958): thorough rinsing with clean solvent, soaking twice in fresh solvent for periods of 30 min (this was followed by rinsing in ethanol if the solvent was non-miscible with water), soaking for 24 hr in 0·1N $Na_2S_2O_3$ containing 0·2 g/l Na_2CO_3, and soaking with many changes of distilled water until the wool was free of iodide (12–14 days). The wool was then air dried and conditioned.

The tyrosine contents of treated and untreated wool samples were determined by a modification of the method of Bernhart (1938) previously described (Crewther and Dowling 1960). Approximately 14% of the tyrosine is destroyed during hydrolysis in alkali in the presence of the other amino acids of wool, and the results obtained have accordingly been corrected for this loss. The disulphide plus sulphydryl (–SS– + –SH) contents of the wool samples were estimated both by the methods of Shinohara (1936) using acid hydrolysates, and by the polarographic method of Leach (1960) using intact fibres. Sulphydryl contents were estimated either by the method of Leach (1960) or that of Burley (1956). The values quoted in this paper were obtained by the former method but similar results were obtained by the latter.

Primary amino groups in intact wool were determined by a method similar to that of McPhee (1958), the alkali solubility by the method of Harris and Smith (1936), the solubility in urea–bisulphite solution by the method of Lees and Elsworth (1956) and the solubility in alkaline solutions of thioglycollate by the method of Lennox (1956). The trypsin digestibility of the wool samples was determined following pretreatment in buffers at pH 4 and pH 11 as described by Crewther and Pressley (1958). The iodine content of treated wool samples was determined by the method described by Richards and Speakman (1955).

TABLE 1

COMPARISON OF THE EFFECTS OF IODINATION IN DIFFERENT SOLVENTS ON THE CONTENTS OF TYROSINE AND (–SS– + –SH) OF WOOL

Iodination Treatment	Time of Iodination (days)	Tyrosine (μmoles/g)	(–SS– + –SH)* (μmoles/g)	(T/S)†
Untreated	—	307	505	
0·78N I_2/ethanol	0·25	151	418	3·33
0·78N I_2/ethanol	3	49	298	2·05
0·78N I_2/propanol	6	236	450	2·10
0·78N I_2/propanol	15	206	428	2·15
0·78N I_2/acetone	7	309	482	0·00
0·78N I_2/acetone	15	310	444	0·00
0·33N I_2/CCl_4	15	277	428	0·65
0·33N I_2/CH_2Cl_2	3	243	469	2·92
0·33N I_2/CH_2Cl_2	15	126	428	3·92
0·078N I_2/0·1N KI, unbuffered	0·25	144	369	1·97
0·078N I_2/2N HCl + *c.* 0·1N KI	0·25	295	351	0·12
0·078N I_2/0·1N KI in 0·1M sodium borate at pH 9·0	0·25	120	415	3·43

* Determined on intact wool by polarographic methods.

†T = percentage decrease in tyrosine content, S = percentage decrease in (–SS– + –SH).

III. RESULTS

(*a*) *Effects of Iodination in Various Solvents on the Tyrosine and (–SS– + –SH) Contents of Wool*

Table 1 lists the tyrosine contents and (–SS– + –SH) contents of wool samples which had been treated with various solutions of iodine. With ethanol, propanol, and acetone, 0·78N iodine was used to permit comparison within the series and with the results of Richards and Speakman (1955) and Harrison and Speakman (1958). With carbon tetrachloride and methylene dichloride the concentration (0·33N) corresponds with saturation of the former solvent at room temperature. The very rapid reaction of iodine with wool in aqueous media led to the use of more dilute solutions of iodine in aqueous KI.

In all solvents a considerable proportion of the disulphide groups of the wool was oxidized. There was no obvious correlation between the loss of tyrosine and the decrease in (–SS– + –SH). Whereas prolonged treatment in I_2/CH_2Cl_2 caused iodination of about 60% of the tyrosine residues and only 15% decrease in (–SS– + –SH), treatment in I_2/acetone caused no measurable loss of tyrosine but a decrease of about 12% in the content of (–SS– + –SH). In aqueous KI the pH of the solutions influenced the relative effects on tyrosine residues and on (–SS– + –SH).

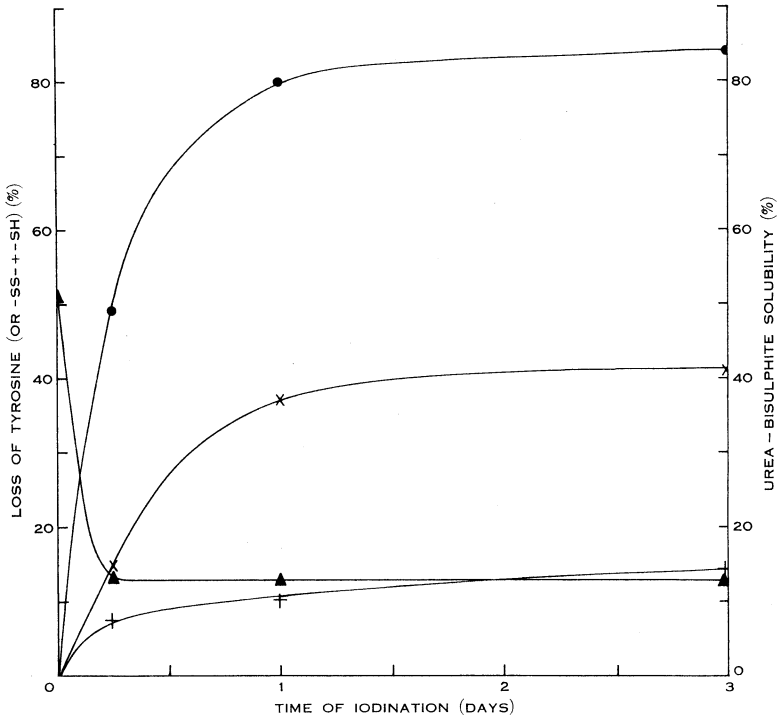

Fig. 1.—Variations in the tyrosine content, the (–SS– + –SH) content as determined by the methods of Shinohara and Leach, and the urea–bisulphite solubility of wool with time of treatment in anhydrous ethanolic iodine solution. ●Loss of tyrosine. ×Loss of (–SS– + –SH) (Leach). +Loss of (–SS– + –SH) (Shinohara). ▲Urea–bisulphite solubility.

Iodine determinations were carried out on samples of wool which had been iodinated in anhydrous ethanol for 72 hr and in anhydrous propanol for 15 days. The values obtained corresponded with conversion of 91 and 37% respectively of the tyrosine residues to di-iodotyrosine residues. As estimated by decrease in tyrosine content the values were 84 and 33% respectively (Table 1). The rates of reaction of wool with iodine in anhydrous ethanol, anhydrous propanol, and unbuffered aqueous KI are shown in Figures 1–3. The maximum decreases in tyrosine content during iodination in ethanol, propanol, and aqueous KI were 84, 33, and 50% even with prolonged reaction.

(*b*) *Oxidation of Sulphydryl and Disulphide Groups*

Table 2 shows that the sulphydryl content of wool was decreased by all of the iodination treatments investigated but none completely removed the sulphydryl groups (cf. Leveau 1959). The values obtained by the method of Burley were similar to those reported in Table 2 but the values obtained by both methods for iodinated wool samples lacked precision.

Table 1 indicates the extensive oxidation of disulphide groups which occurs during iodination in certain solvents and Table 3 compares the values for the (–SS– + –SH) contents obtained by the method of Leach using intact wool and that of Shinohara using wool hydrolysates. Wool samples which had been iodinated in ethanol

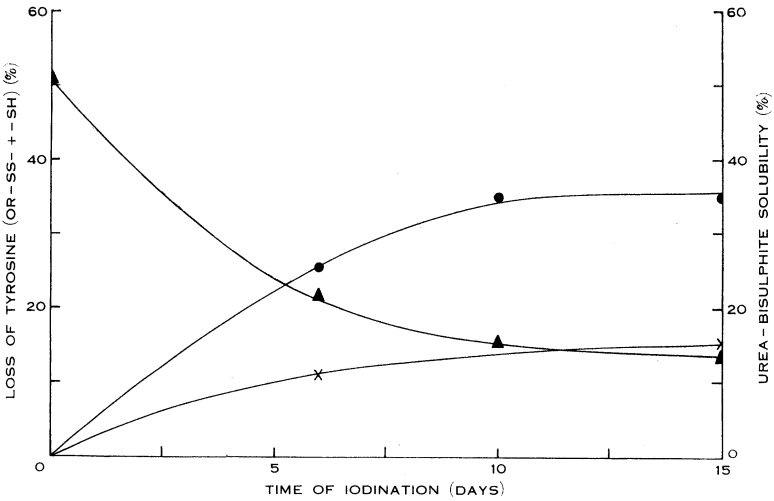

Fig. 2.—Variations in the tyrosine content, the (–SS– + –SH) content as determined by the method of Leach, and the urea–bisulphite solubility of wool with time of treatment in anhydrous propanolic iodine solution. ●Loss of tyrosine. ×Loss of (–SS– + –SH) (Leach). ▲Urea–bisulphite solubility.

or ethanol/water mixtures (Table 3) were treated with 2N HCl/2M KI solutions at 20°C overnight as described by Harris and Smith (1937). Considerable amounts of iodine were liberated and the (–SS– + –SH) content increased in some samples by more than 100%. Treatment of the wool samples with 2N HCl containing no KI did not release iodine although the (–SS– + –SH) contents increased.

(*c*) *Effects of Water on Iodination in Alcohols*

Table 3 and Figure 4 show that the presence of water in the ethanol used as solvent increased the rate of iodination of tyrosine residues and the rate of oxidation. There was also an increase in the extent of these reactions. The reaction in propanol was affected to an even greater extent by the presence of water (Table 4). The rate of reaction was greatly increased and there was a major increase in the extent of reaction.

(*d*) *Ethylation of Carboxyl Groups*

Circles of plain-weave Merino 64's fabric, each weighing 11 mg, were treated for 72 hr at 20°C with 0·78N iodine in anhydrous ethanol containing [1-^{14}C]ethanol. The iodinated samples were washed twice in the radioactive solvent for 30-min periods, then rinsed with water, treated with $Na_2S_2O_3$ solution, and washed thoroughly in the usual manner. For comparison, similar fabric circles were treated in the same solvent containing no iodine but with the addition of concentrated HCl to give a final concentration of 0·1N. The ethylation process catalysed by the HCl was complete in about 35 days. After complete removal of free ethanol, chemical analysis showed that esterification of the carboxyl groups amounted to 480 μ-equiv/g.

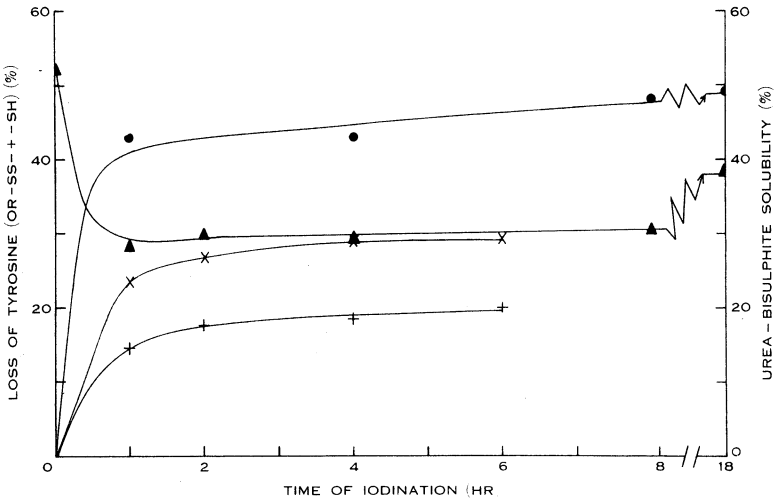

Fig. 3.—Variations in the tyrosine content, the (–SS– + –SH) content as determined by the methods of Shinohara and Leach, and the urea–bisulphite solubility of wool with time of treatment in aqueous KI_3. ●Loss of tyrosine. ×Loss of (–SS– + – SH) (Leach). +Loss of (–SS– + –SH) (Shinohara). ▲Urea–bisulphite solubility.

The radioactive count of this material was compared with that of the iodinated sample, also carefully freed of ethanol, and the estimate of ethoxy groups in the wool obtained in this way was 298 μ-equiv/g. Further repeated washing of the iodinated sample in water and acetone did not decrease the count. Direct chemical determination of ethoxy content gave a value of 192 μ-equiv/g.

(*e*) *Trypsin Digestibility*

Wool which had been iodinated in undried ethanol for 3 days was digested by solutions of crude trypsin even after soaking at 30°C for 20 hr at pH 4 (Table 5). The original wool after similar soaking did not lose weight during digestion. Pre-soaking at pH 11 resulted in very extensive digestion of the iodinated wool and, as previously reported (Crewther 1956), a small loss of weight in the uniodinated wool. The effect was much less if anhydrous ethanol was used as solvent during

iodination and with anhydrous propanol or unbuffered aqueous KI as solvents the digestibility was very small.

(*f*) *Solubility and other Data*

Iodination of wool in ethanol or propanol causes the solubility in urea–bisulphite solution to fall from about 50% to about 13% (Table 6). This occurs rapidly in ethanol but much more slowly in propanol (Figs. 1 and 2). With unbuffered aqueous KI as solvent the urea–bisulphite solubility falls to a minimum even more quickly than in ethanol, and then slowly increases (Fig. 3). Iodination in ethanol causes a similar

TABLE 2

EFFECTS OF IODINATION IN VARIOUS SOLVENTS ON THE SULPHYDRYL CONTENT OF WOOL

Sulphydryl content determined by the method of Leach (1960)

Iodination Treatment	Time of Iodination	Sulphydryl Content (μmoles/g)
Untreated	—	25·1
0·78N I_2/ethanol	6 hr	13·9
0·78N I_2/ethanol	72 hr	13·6
0·78N I_2/ethanol, 10% water	6 hr	10·9
0·78N I_2/propanol	15 days	19·5
0·078N I_2/0·1N KI, pH 6	6 hr	19·5
0·078N I_2/0·1N KI, pH 9	6 hr	14·6

decrease in solubility in alkaline thioglycollate solutions but iodination in unbuffered aqueous KI has no measurable effect on the solubility in this reagent (Table 6). The alkali solubility of wool is increased to some extent by iodination in ethanol (Table 6) and there is also a very small increase in primary amino groups from 190 to 202 μ-equiv/g.

IV. DISCUSSION

(*a*) *Chemical Changes Caused by Iodination of Wool*

Apart from the iodination of tyrosine residues, the most important reaction of iodine with wool appears to be with the disulphide residues (Tables 1 and 3). The discrepancies between the estimates of (–SS– + –SH) by the method of Shinohara, using acid hydrolysates of the wool samples, and that of Leach, using intact fibres (Table 3; Figs. 1 and 3), indicate the presence of intermediate oxidation products of disulphide groups. This is confirmed by the increase in the values obtained by the latter method and the release of free iodine after treating the fibres with HCl/KI.

Maclaren, Leach, and Swan (1960) have shown that both "cysteine sulphinic acid" and the thiolsulphonate derived from cystine undergo disproportionation

during acid hydrolysis to produce cystine, cysteic acid, and possibly small amounts of other products. Each mole of the thiolsulphonate produced 0·52 mole of cystine, each mole of the sulphinic acid produced 0·16 mole of cystine. The former result most closely corresponds with the equation

$$5\ \text{–S.SO}_2\text{–} + 2\ \text{H}_2\text{O} \rightarrow 3\ \text{–S.S–} + 4\ \text{–SO}_3\text{H},$$

the theoretical yield of disulphide per mole of –S.SO_2– being 0·6 mole. The yield of disulphide from the thiolsulphinate group would be proportionately greater than 0·52 (theoretical 0·8) and that from more oxidized intermediates lower than 0·52 mole.

TABLE 3

COMPARISON OF THE (–SS– + –SH) CONTENT OF ACID HYDROLYSATES AND UNHYDROLYSED SAMPLES OF IODINATED WOOL

Iodination Treatment	Time of Iodination	(–SS– + –SH) Content (μmoles/g) of:	
		Hydrolysate (Shinohara)	Intact Fibre (Leach)
Untreated	—	519	504
0·078N I_2/0, 1N KI	6 hr	407	366
0·33N I_2/CCl_4	15 days	505	427
0·33N I_2/CH_2Cl_2	3 days	487	465
0·78N I_2/acetone	15 days	472	460
0·78N I_2/ethanol	72 hr	419	290
0·78N I_2/ethanol, 0·1% water	72 hr	421	254
0·78N I_2/ethanol, 1·0% water	72 hr	382	219
0·78N I_2/ethanol, 10% water	72 hr	262	106

Because of this disproportionation in acidic or alkaline solutions it is not possible to determine the cysteic acid content of the intact wool nor is a direct determination of the thiolsulphinate or thiolsulphonate content possible. The ratio $(H-I)/(I_0-I)$, where H = the disulphide content of the hydrolysate, I = the disulphide content of the intact iodinated fibre, and I_0 = the disulphide content of untreated wool, provides an estimate of the molar yield of disulphide produced during hydrolysis. The values so obtained for iodination in anhydrous ethanol, and ethanol containing 0·1, 1·0, and 10% water are 0·65, 0·71, 0·60, and 0·41 respectively. These figures represent minimum values since the formation of cysteic acid during iodination would give a correspondingly low value of this ratio. This indicates that the thiolsulphinate group is one of the intermediates in the oxidation of the disulphide bonds. Iodination in CCl_4 or CH_2Cl_2 also gives rise to considerable amounts of oxidation intermediates whereas oxidation in aqueous KI or acetone proceeds largely to completion (Table 3).

Little is known of the nature of the reaction of sulphydryl groups of the wool with iodine. Some sulphenyl iodide groups may be formed (Fraenkel-Conrat 1955; Cunningham and Nuenke 1959) but as Fraenkel-Conrat has found that such groups are very reactive it is improbable that they would survive the treatment with thiosulphate solution. Sulphydryl groups would probably be oxidized to the disulphide (Fraenkel-Conrat 1955) if their position in the protein permitted this reaction but some may also be converted to sulphonic acid groups.

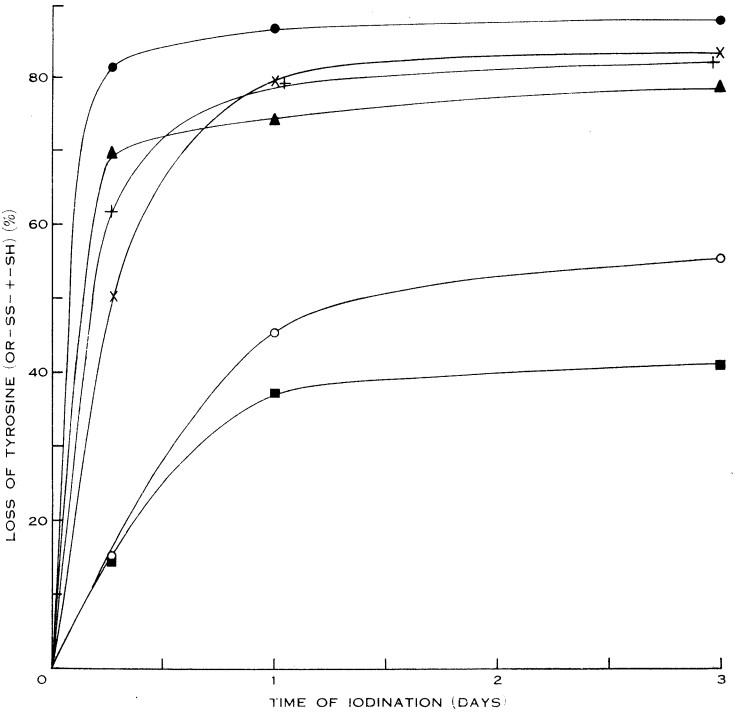

Fig. 4.—Effect of water on the reaction of the tyrosine residues and disulphide groups of wool with iodine in ethanolic solution. (–SS– + –SH) contents determined by the method of Leach.

Loss of Tyrosine	Loss of (–SS– + –SH)
× Anhydrous ethanol	■ Anhydrous ethanol
+ 1% aqueous ethanol	○ 1% aqueous ethanol
● 10% aqueous ethanol	▲ 10% aqueous ethanol

The values obtained for the ethoxy contents of wool fabric after iodination in ethanol leave no doubt that some 200 μ-equiv. of carboxyl groups per gram of wool were esterified. The discrepancy between the value obtained by direct analysis and that obtained by the radiotracer technique is attributable to the dimensional instability of the fabric which results in high values. This technique serves merely to confirm the presence of considerable amounts of ethoxy groups in the wool. Under similar conditions of time and temperature in ethanol containing 0·1N HCl

the ethoxy content of the fabric reaches only about 100 μ-equiv/g (Springell, personal communication). This unexpectedly high value for esterification cannot be accounted for completely in terms of the production of HI during oxidation and substitution; these reactions would give a final concentration of about 0·02N HI in the liquor.

(*b*) *The Urea–Bisulphite Solubility of Iodinated Wool*

The decrease in solubility in this reagent observed when wool has been subjected to various chemical or physical treatments has been attributed to formation of lanthionine (Lees and Elsworth 1956), to sulphydryl–disulphide interchange (Kessler and Zahn 1958), and to configurational changes in proteins (Swan 1959). Leveau

TABLE 4

EFFECT OF WATER ON THE IODINATION OF WOOL IN PROPANOL

Iodination was at 20°C

Solvent for Iodination	Time of Iodination (days)	Tyrosine Content (μmoles/g)	Solvent for Iodination	Time of Iodination (days)	Tyrosine Content (μmoles/g)
Untreated	—	307	Propanol + 1·0% water	3	221
Anhydrous propanol	3	283	Propanol + 1·0% water	6	159
Anhydrous propanol	6	227	Propanol + 10% water	3	42
			Propanol + 10% water	6	35

(1959) has already reported a decrease in urea–bisulphite solubility when wool is iodinated in ethanol and attributes this to strengthening of the hydrogen bonds between tyrosine side-chains and ionized carboxyl groups. This explanation predicts a direct relationship between solubility and the extent of iodination of tyrosine residues in various solvents. No such relationship has been observed (Tables 1 and 6; Fig. 3). In addition this explanation cannot account for the decrease in solubility in alkaline thioglycollate solution (Table 6) which results from iodination in ethanol. Under the conditions of this test the di-iodotyrosine residues would be completely ionized and therefore incapable of hydrogen bonding with ionized carboxyl groups.

Unfortunately the unlikely possibility that lanthionine is formed during the iodination cannot be checked since partial oxidation products of cystine produce small amounts of lanthionine during acid hydrolysis (Maclaren, unpublished results). The other alternatives are equally improbable. Although the wool proteins appear to have been disordered by iodination in ethanol containing small amounts of water this effect is insignificant in anhydrous ethanol (Table 5); yet the solubility in urea–bisulphite solution decreases to much the same value whether the solvent is anhydrous or not. Hence there is no reason to attribute insolubility to configurational changes in the wool proteins. Likewise sulphydryl–disulphide interchange is inhibited by oxidation. The solubility of the wool proteins is known to depend largely on

their net negative charge (O'Donnell 1954; Gillespie 1956). This would be influenced by oxidation of disulphide groups, ethylation of carboxyl groups, iodination of tyrosine groups, and the final pH of the urea solutions. Changes in solubility cannot therefore be attributed to a single chemical reaction of iodine with the wool.

(c) *Accessibility of Tyrosine Residues in Wool*

Table 1 shows that the substitution of tyrosine residues and the oxidation of disulphide groups proceed at different relative rates in different solvents. The use of this type of data as an index of the ability of solvents to diffuse into wool (Harrison and Speakman 1958) is therefore open to doubt since, for example, different conclusions would be drawn according as the iodination of tyrosine or the oxidation of disulphide was taken as the index of accessibility of the fibre.

TABLE 5

TRYPSIN DIGESTIBILITY OF IODINATED WOOL

Iodination Treatment	Time of Iodination	pH of Pretreatment	Digestibility* (%)
Untreated	—	4·0	−1·0
		11·0	1·5
0·78N I_2/ethanol (undried)	1 hr	4·0	1·0
0·78N I_2/ethanol (undried)	72 hr	4·0	23·8
		11·0	53·8
0·78N I_2/ethanol (anhydrous)	72 hr	4·0	−0·1
		11·0	16·0
0·78N I_2/propanol	15 days	4·0	−1·2
0·078N I_2/KI	6 hr	4·0	−0·3
		11·0	1·8

* Negative values indicate a gain in weight after treatment with the enzyme. This probably results from adsorption of the enzyme.

The incomplete iodination of tyrosine residues in the various solvents may be the result of (1) very slow reaction of the residue with iodine, (2) the establishment of an equilibrium, or (3) the inaccessibility of part or all of the tyrosine to the reagent due to its incorporation in an ordered structure or because of steric effects of other residues. With aqueous KI_3, I_2/ethanol, and I_2/propanol, constant levels of iodination of tyrosine residues were reached but in each case the extent of iodination was different. If a difference in accessibility of crystalline and non-crystalline regions of the fibre were the factor chiefly responsible for determining the final level of iodination, similar levels of iodination would be expected in all solvents. The results suggest that in these solvents the establishment of an equilibrium is responsible for limiting the extent of iodination of tyrosine residues; this applies particularly with solutions of I_2 in aqueous KI where changes in pH influence the final extent of iodination (Table 1). The effects of small amounts of added water on the extent of iodination in ethanol and propanol (Tables 3 and 4) support this view.

The results have a bearing on the conclusions of Ghosh, Holker, and Speakman (1958) and those of Haly and Feughelman (1960) regarding the location of tyrosine residues in the wool fibre. The former authors conclude that the tyrosine residues of wool are located in the "amorphous regions" of the wool fibre and use the accessibility of 96% of the tyrosine residues of wool to iodine in ethanol (not anhydrous) as evidence for this assumption. Haly and Feughelman, on the other hand, suggest that the partial iodination of tyrosine in propanol/I_2, taken in conjunction with the absence of 33 Å repeat on the X-ray diffraction pattern, is evidence that only tyrosine residues in the matrix or less ordered regions are iodinated in this solvent.

The conclusion of Ghosh, Holker, and Speakman would be valid only if it were known that the crystalline regions of the fibre are too closely packed to allow ingress of iodine and that the reaction with iodine does not cause configurational changes in the wool proteins, particularly in the crystalline regions.

TABLE 6

EFFECTS OF IODINATION OF WOOL ON ITS SOLUBILITY IN STANDARD REAGENTS

Iodination Treatment	Time of Iodination	Urea–Bisulphite Solubility (%)	Alkaline Thioglycollate Solubility (%)	Alkali Solubility (%)
Untreated	—	51	39	8·5
Ethanol/I_2	3 days	13	20	13·2
Propanol/I_2	15 days	13	—	—
Aqueous KI_3	1 hr	28	40	—

The evidence that iodination in absolute ethanol can cause a marked increase in trypsin digestibility (Table 5) suggests that configurational changes have taken place in the proteins. Furthermore the X-ray diffraction evidence (Fraser and MacRae 1957) that iodination of tyrosine residues gives rise to a new sharp spot corresponding with a repeat along the meridian of 33 Å, together with sharpening of the spot of 25 Å and a noticable decrease in sharpness of the high angle pattern (MacRae, personal communication), indicates in a positive manner an association of tyrosine with the crystalline regions of the fibre and suggests some disordering of the α-helices. There is therefore no sound basis for the assumption that tyrosine residues occur only in the amorphous regions of the fibre. The analogy with silk which Ghosh, Holker, and Speakman (1958) use is unsound since the crystallites of wool are largely in the form of α-helices whereas those of silk form β-sheets which do not accommodate tyrosine residues (Marsh, Corey, and Pauling 1956).

The location of the di-iodotyrosine residues in wool treated with propanolic iodine will be considered in a later paper.

V. ACKNOWLEDGMENTS

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