3. Vitamin E may be present in small amounts. The evidence is doubtful; if tocopherol is present it is outside the range of detection of normal spectroscopic measurement. It is estimated that the amount of tocopherol cannot exceed 0.03 % of the sebum.

4. Two fractions from a 'sterol-like' substance provided by MacKenna *et al.* (1952) have been shown to both give a blue colour with trichloroacetic acid (λ_{max} 660 m μ .) and with antimony trichloride in chloroform (λ_{max} 664 m μ .). One shows λ_{max} 235 m μ . and the other λ_{max} 278 m μ . (weak) as well.

5. Cholesterol, squalene, a hydrocarbon, m.p. 56°, and an unidentified substance, m.p. 79–81°, have been isolated.

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The Reactivity of Radiomimetic Compounds

1. CROSS-LINKING OF PROTEINS

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Within the last five years a number of different compounds has been shown to produce biological effects similar to those of X-rays. Radiomimetic activity has been defined by Loveless (1951) as a highly specific effect upon the resting cell producing an alteration of the genetic material which is revealed by chromosome breakage and rearrangement in subsequent divisions. Radiomimetic compounds are powerful growth inhibitors and have found limited use in the chemotherapy of malignant disease. Several have been demonstrated to be both carcinogenic and mutagenic (Haddow, 1951). Chemically, all the compounds in this class can be considered as alkylating agents (Loveless, 1951); they include sulphur mustard and nitrogen mustards, epoxides, ethyleneimines and *O*-methanesulphonyl derivatives of hydroxy compounds. Haddow, Kon & Ross (1948) demonstrated that, in the bischloroethylamine (nitrogen mustards) series, the struc-

tural condition requisite for biological activity is the presence of two halogenoalkyl groups. It has since been found that this requirement holds throughout the whole series and that, with the exception of a few isolated cases, only compounds with two alkylating groups in the molecule are active in the intact animal (Hendry, Homer, Rose & Walpole, 1951; Haddow, private communication). In tests *in vitro*, unifunctional compounds are capable of bringing about radiomimetic effects, but only at concentration 50 to 100 times those required for corresponding multifunctional compounds (see Bieseke, Philips, Thiersch, Burchenal, Buckley & Stock, 1950; Loveless & Ross, 1950).

These observations led Goldacre, Loveless & Ross (1949) to propose the hypothesis that the radiomimetic compounds exert their biological action by cross-linking genetic material such as chromatid threads. This hypothesis could not be tested directly,

but has found support in the findings that mustard gas cross-links nucleic acid (Elmore, Gulland, Jordan & Taylor, 1948) and alginic acid (Deuel & Neukom, 1949) and that bisepoxides cross-link alginic acid and pectin (Deuel, 1947) and wool (Capp & Speakman, 1949; Fearnley & Speakman, 1950).

To determine if a correlation exists between biological activity and the ability to cross-link proteins, a representative selection of radiomimetic compounds and some multifunctional but inactive substances was examined.

The two proteins studied were crystalline bovine serum albumin and wool. The soluble protein was treated in aqueous solution and cross-linking was measured directly by determining the change in molecular weight by light scattering. In the case of wool, which is already extensively cross-linked by disulphide bonds, the formation of additional cross-links can be determined only after the disulphide links have been broken.

EXPERIMENTAL

Materials

Crystalline bovine serum albumin was obtained from Armour Laboratories, Lindsey St., London, E.C. 1, and found to be electrophoretically homogeneous in potassium phosphate buffer, pH 7.2, ionic strength 0.1. Root ends of Lincoln wool fibres were used after purification by successive Soxhlet extractions with ethanol and ether.

Compounds not commercially obtainable were prepared in the Organic Chemistry Department of the Chester Beatty Research Institute and kindly supplied by Mr G. M. Timmis, Dr W. C. J. Ross and Mr J. L. Everett. The dihalogen derivatives of dinitrobenzene were kindly provided by Imperial Chemical Industries Ltd. For the compounds shown in Table 2, the methods of preparation are given as follows: compound no. 1, Ward (1935); no. 3, Ross (1949); no. 7, Everett & Kon (1950). Compounds nos. 11–16 were prepared by the action of methanesulphonyl chloride on the corresponding alcohols (Timmis, 1950, 1951).

Treatment of wool fibres

Wool fibres (1 g.) were suspended in 25 ml. water, NaHCO_3 , borate or acetate buffer (depending on the pH required) containing 3×10^{-3} moles of the reagent. If the reagent was not sufficiently soluble, the excess was present as solid. Solution of the less water-soluble compounds in aqueous acetone or ethanol did not lead to more extensive reaction and these mixed solvents were therefore not used. The reaction mixture was shaken at 40° for 1 day and then left standing for 2 days at the same temperature. The mustards and mesyloxy compounds (see Table 2) hydrolyse in water with the liberation of acid, and it is therefore difficult to maintain the pH constant. High concentration of buffer cannot be used, since all the radiomimetic alkylating compounds readily react with anions (see, for example, Ross, 1950), which compete with the proteins for the reagent. To study the reactions at different pH values a compromise had to be made between pH stability and the extent of the competing reaction; 0.4 M–0.5 M-sodium borate or acetate

buffers and NaHCO_3 were therefore used. With NaHCO_3 the pH remained steady at 8.2, but with the other buffers pH changes of as much as 1 unit occurred. Epoxides hydrolyse without change in pH, and in the case of the ethyleneimines the pH change is small, so that 0.1 M buffer was sufficient.

Modified wool fibres

The amino groups of wool were acetylated by the I.G. Farben process (Alexander & Whewell, 1947), and 90% of the $\epsilon\text{-NH}_2$ groups appeared to have reacted in this way. (This value was determined by treating the fibres with 1-fluoro-2,4-dinitrobenzene and estimating the amount of dinitrophenyllysine formed.) The carboxyl groups were esterified by refluxing wool fibres in anhydrous 0.1 N-methanolic HCl for 4 hr. (Alexander, Carter, Earland & Ford, 1951).

Cross-linking of wool fibres

Speakman (1947) showed that a wool fibre contracts in length after treatment with a solution of NaHSO_3 at 100° ('supercontraction'), but that this contraction was prevented if new non-disulphide cross-links were introduced. This method is not, however, completely unambiguous, since the extent of contraction depends also on the relative number of charged groups in the fibre. For example, thorough esterification of the carboxyl groups brings about a diminution in the degree of contraction (Blackburn & Lindley, 1948). This difficulty was eliminated by investigating the action of the corresponding uni- and bi-functional compounds in parallel experiments which enables effects due to esterification only and to cross-linking to be differentiated. Contraction is also diminished without cross-linking by depositing large quantities of polymers within the fibres. As some of the compounds studied are known to polymerize (Hendry *et al.* 1951), the extent of reaction was so adjusted that the increase in weight of the wool fibres was not greater than 5% (except for the halogeno-2,4-dinitro compounds, which cannot polymerize), since this amount of polymer does not affect fibre contraction. The bisulphite test may fail to detect cross-links if these are not stable to the severe conditions employed in the test, and for this reason the contraction and solubility of fibres after oxidation with peracetic acid was determined. This reagent, used at room temperature, confines its attack to the disulphide bonds and after 90% of these have been oxidized, the fibre contracts in length (Alexander, 1951) and dissolves in 3 N-aqueous ammonia leaving only the subcuticle (10% of the whole fibre). The reaction with peracetic acid is relatively mild, and, even after contraction, the fibre retains its α -keratin structure, whereas bisulphite at 100° brings about complete disorientation. Only by making these several tests and taking the precautions outlined is it felt that cross-linking can be unambiguously established and distinguished from other possible effects.

Bundles of fibres were tied at each end with cotton thread, and the distance between the tie bands measured. The fibres were then immersed in 5% (w/v) NaHSO_3 , maintained at 100°, withdrawn after 1 hr., rinsed and dried between filter papers; the length was again measured. The contraction was expressed as percentage change in length. The contraction in peracetic acid was determined after treatment for 25 hr. at 22° in 1.6% peracetic acid prepared by the method of Greenspan (1947). The solubility of the fibres after oxidation with peracetic acid was determined by

treating a weighed bundle of fibres as above, immersing in 0.3 N aqueous ammonia for 24 hr. at room temperature and filtering the residue on a coarse sintered-glass filter. All determinations were carried out in duplicate. Control samples of fibres treated only in buffer were tested simultaneously. Satisfactory reproducibility was obtained as long as fibres from the same batch were used. Treatment of wool fibres at pH > 9 at 40° for 3 days leads to extensive formation of lanthionine cross-links which prevent contraction in bisulphite (Speakman, 1947), or in peracetic acid (Alexander, 1951), and solubilization of the oxidized wool in dilute NH_3 . For this reason it was impossible to carry out reactions at pH > 9. The small number of cross-links introduced at 40° at pH 9, though not sufficient to influence contraction, can diminish the solubility of the oxidized fibre (see Table 1), and this is therefore an extremely sensitive method of detecting cross-links.

Determination of molecular weight by light scattering

The reaction of the radiomimetic compounds with bovine serum albumin was examined at two concentrations of protein, 0.5 and 2%, and with 10^{-2} and 2×10^{-2} M solutions of the reagents (4×10^{-2} M solution of compound no. 1) respectively in 0.1 M-sodium phosphate buffer to maintain the pH at 7. (In the experiments with epoxides no buffer was present.) The protein and reagents were carefully filtered through porcelain candles with a pore size of 0.5 μ . diameter, mixed together in sterile vessels and allowed to react at 40° for 5 days. The reaction mixture was cooled to room temperature and the light scattered at right angles measured for various dilutions made with filtered 0.1 M-phosphate buffer to maintain constant ionic strength. The intensity of the light (I) scattered by the protein was compared with that scattered by pure nitrobenzene and the results expressed as the relative scatter, $\tau = I_{\text{protein}}/I_{\text{nitrobenzene}}$. From a graph of c/τ against c (where c is the concentration of protein in g./ml.) the mol.wt. was determined from the intercept at zero concentration. This method has been used by Edsall, Edelhoch, Lontie & Morrison (1950). Their value of the molecular weight for the untreated protein was accepted as a standard and the other molecular weights determined by reference to this value. The same molecular weight was obtained for solutions of protein immediately after mixing and after keeping for 5 days at 40° indicating that no aggregation of the untreated protein occurred, as was found by Bier & Nord (1949) with egg albumin. During filtration through 0.5 μ . filters, 10–20% of the protein of the stock 4% solution of protein was retained by the filter. This ensured complete clarification, but made it impossible to filter a heterogeneous reaction product since the larger particles would be preferentially retained.

RESULTS

Cross-linking of soluble proteins

Comparison of Figs. 1 and 2 shows that cross-linking occurred only in protein solutions containing 2% protein, even though in the more dilute (0.5%) solution twice the proportion of reagent to protein was used. An increase in molecular weight, i.e. cross-linking, is indicated when the intercept on the ordinate is less than that for untreated protein. This

behaviour is similar to that found in other systems; cross-linking of alginic acid by mustard, first observed by Deuel & Neukom (1949), was found to occur only in relatively concentrated solutions of the polymer. With the sodium salt of polymethacrylic acid it has also been shown that the reaction with nitrogen mustards is almost wholly intramolecular at polymer concentrations of 0.1% or less, but that in more concentrated solutions cross-linking leading to gel formation readily takes place (Alexander, unpublished observations).

The nitrogen mustard and the bisepoxide cross-linked serum albumin while the dimethanesulphonyl compound failed to do so, although it is a very effective cross-linking agent for wool fibres (see p. 182). Propylene oxide seems to have had a degrading effect, which is being further investigated. The pronounced change in slope of the c/τ against c curve of the protein treated with both the chloroethyldimethylamine and the bischloroethylmethylamine indicates that the charge on the protein has been increased (Edsall *et al.* 1950), although a change in molecular weight was only produced by the bifunctional compound. The change in gradient is also observed in the reaction of the nitrogen mustard with a 0.5% protein solution when no cross-linking occurred (see Fig. 1), and it can be concluded that the nitrogen mustard has reacted intramolecularly.

Epichlorhydrin and 2:4:6-tris-(ethyleneimino)-s-triazine (Table 2, compound no. 9) are both very effective cross-linking agents for serum albumin, and reaction with these compounds under the conditions used in the above experiments leads to rapid precipitation of the protein. By reducing the protein concentration to 0.1% and allowing it to react with 10^{-2} M-triazine, the degree of cross-linking was restricted and the complex remained in solution. Since, however, the triazine itself polymerizes to give an insoluble product, the solution of the cross-linked protein was faintly opalescent and turbidity measurements could not be made. The polymer could not be separated by filtration since the cross-linked protein molecules were too large to pass through the fine pores, which indicates that they were of high molecular weight.

Cross-linking of wool fibres

The results obtained with twenty different compounds are given in Table 2. The corresponding treatments in buffer solution only, are given in Table 1. A surprising feature is the inability of the aliphatic nitrogen mustard (compound no. 2) and the sulphur mustard (compound no. 4) to cross-link wool fibres, although these compounds have been shown to cross-link other systems. The effect of the nitrogen mustard was examined under other conditions of pH and concentration besides those given in

Table 2, but no evidence for cross-linking was found in any of these experiments. The conditions of the test are unlikely to bring about rupture of the

Fig. 1. Molecular weight determinations by light scattering of dilutions of a 0.5% (w/v) solution of bovine serum albumin after treatment with radiomimetic compounds ($1 \times 10^{-2} \text{ M}$). For details see text.

new bond, which would then not be revealed, since cross-linking was well established by the methods used for the chemically closely related compounds nos. 3, 9 and 10. To eliminate altogether the possi-

found this method suitable for detecting cross-linkage formation and it has been used by us for examining fibres treated with all the compounds shown in Table 2. The results so obtained were in every case in accord with those given by the peracetic acid and bisulphite tests, but are not recorded since it was impossible to obtain satisfactory quantitative reproducibility and also because the rationale of the test is not yet understood. The difference in the reaction of the aliphatic nitrogen mustard (compound no. 2) with a soluble protein and wool cannot be explained. All the reactions with proteins involve a number of simultaneous and competitive reactions with the buffer solution, and profound differences between a homogeneous and a heterogeneous system are therefore not surprising.

The aromatic nitrogen mustard (compound no. 3), although capable of cross-linking wool fibres, is not nearly as effective as epichlorohydrin (no. 6) and the bisepoxide (compound no. 7). Epichlorohydrin derives its bifunctional character from its chlorine atom, which can react under mild conditions with amino groups. Alternatively, after epichlorohydrin has reacted via its epoxy group, elimination of hydrochloric acid may occur to form a new epoxy group and this could provide a second reaction centre. Although this reaction has been shown to take place under relatively mild conditions (Alexander *et al.* 1951), it is unlikely to occur at pH 7.

Fig. 2. Molecular weight determination by light scattering of dilutions of a 2% (w/v) solution of bovine serum albumin after treatment with radiomimetic compounds ($2 \times 10^{-2} \text{ M}$, except for $(\text{CH}_3)_2\text{N}.\text{CH}_2\text{CH}_2\text{Cl}$ which was $4 \times 10^{-2} \text{ M}$). For details see text.

bility that the new cross-links are broken in subsequent treatments, the contraction of fibres in aqueous phenol was examined. For the compounds mentioned above, this test also failed to reveal the presence of new cross-links. Elöd & Zahn (1944)

The polyethyleneimines readily cross-link wool and compound no. 9 is outstanding. These substances were originally developed in Germany for cross-linking regenerated cellulose fibres, and in this application compounds nos. 9 and 10 found the

widest use (Alexander & Whewell, 1947). All the bifunctional mesyloxy derivatives, with the exception of compound no. 12, cross-link wool; compound no. 15 is more effective than compound no. 14, although both substances have similar chemical reactivities (Timmis, 1951). The failure of compound no. 12 to bring about cross-linking cannot be attributed to its low reactivity (Timmis, 1951) since treatment for 7 days, which is sufficient for complete hydrolysis of the compound, did not result in cross-linking.

Table 1. *Contraction and solubility of wool fibres after treatment with buffer solution for 3 days at 40°*

Buffer (pH)	Contraction after reaction with		Percentage of oxidized fibre insoluble in ammonia
	Bisulphite (%)	Peracetic acid (%)	
Distilled water	27.9	19.0	12
pH 2	32.6	13.4	12
pH 4	30.4	12.8	13
pH 7	30.0	12.5	26
pH 9	24.8	11.6	40
NaHCO ₃ (pH 8.2)	32.4	10.5	15

Changes of pH seem to affect the cross-linking reaction of all the different types of compounds in the same way (compare compounds nos. 3, 7, 9 and 13). No cross-linking occurs in treatments at or below pH 4, and the reaction appears to have an optimum at pH 7. Solutions more alkaline than pH 9 could not be used since these brought about the formation of lanthionine cross-links.

Chemical considerations suggest that the amino- and carboxyl-groups in wool are most likely to react with the radiomimetic compounds. In an attempt to determine which of these are involved, a selected number of the compounds was examined with wool samples in which the carboxyl and amino groups were blocked by esterification and acetylation, respectively. Table 3 shows that with the exception of the very effective 2:4:6-tris-(ethyl-eneimino)-*s*-triazine (compound no. 9), none of the compounds cross-linked acetylated wool, but in nearly all cases their effectiveness was not impaired by esterification. Only the mesyloxy compound did not react with esterified fibres.

Alderhalden & Stix (1923) and Sanger (1945) demonstrated that 1-halogeno-2:4-dinitrobenzenes react with many of the reactive groups in proteins. Recently, Zahn (1951) showed that 1:5-difluoro-2:4-dinitrobenzene brought about a measure of cross-linking in wool. This observation has been confirmed and the corresponding dichloro and dibromo compounds (compounds nos. 18–20) were found to act similarly. 1-Fluoro-2:4-dinitrobenzene (compound no. 17) can bring about similar changes

(i.e. diminished contraction and solubility after disulphide bond breakdown), though to a lesser extent than the bifunctional compounds, and is the only unfunctional compound to do so.

DISCUSSION

All the bifunctional radiomimetic compounds examined can cross-link proteins, though not necessarily in the same system. The results do not, however, lend support to the cross-linking hypothesis of radiomimetic activity (Goldacre *et al.* 1949), since the cross-linking tests used are unable to differentiate between biologically active and inactive multifunctional compounds. Thus, although the trisethyleneimino compound (no. 9) is both the most highly biologically active compound and one of the best cross-linking agents, the dihalogenodinitrobenzene derivatives and epichlorhydrin have no radiomimetic activity in the intact animal (Haddow, private communication), but are powerful cross-linking agents for wool and serum albumin. The lack of correlation between ability to cross-link and biological activity is best shown in the series of bifunctional mesyloxy compounds (nos. 11–16). Timmis (1951) found that the butanediol derivative (no. 14) has the highest biological activity and that this falls with decreasing chain length; introduction of an acetylenic bond (in compound no. 15) or substitution as in compound no. 16 removes all biological activity without altering significantly the chemical reactivity, but these two substances are among the most effective cross-linking agents examined. The ability of compound no. 15 to effect such powerful cross-linking may perhaps be ascribed to the linear structure of the molecule imposed by the triple bond, which prevents intramolecular reaction. This type of reaction is known to occur with the saturated compounds of this series (e.g. no. 14), and may involve ringing round on an amino group (Timmis, 1951), or reaction with adjacent groups on the same macromolecule (cf. Alexander, 1952). The failure of the ethylene glycol ester (compound no. 12) to cross-link wool is difficult to explain as in this case, also, steric factors would favour inter- rather than intramolecular reaction.

The only unfunctional compound (no. 17) which brings about changes comparable to cross-linking is one containing a 2:4-dinitrobenzene group in addition to an alkylating group. The apparent cross-linking action probably results from the strong secondary forces by which dinitrobenzene nuclei are held to one another, or to groupings already present in the wool fibre. A comparable effect occurs when wool is reduced followed by alkylation of the sulphhydryl groups (Patterson, Geiger, Mizell & Harris, 1940). The strength of the wool fibre after

Table 2. *Cross-linking of wool fibres by radiomimetic and related compounds*

Reagent no.		pH of solution	Contraction after reaction with		Percentage of oxidized fibre insoluble in ammonia
			Bisulphite (%)	Peracetic acid (%)	
Nitrogen and sulphur mustards					
1	$(\text{CH}_3)_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl}$	5.8	32.0	14.8	—
2	$\text{CH}_3 \cdot \text{N} \cdot (\text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl})_2$	5.8	29.6	11.4	26
		8.2 (NaHCO_3)	31.2	14.5	19
3	$p\text{-CH}_3\text{O} \cdot \text{C}_6\text{H}_4 \cdot \text{N} \cdot (\text{CH}_2 \cdot \text{CH}_2\text{Cl})_2$	4 (approx.)	26.4	8.8	15
		7 (approx.)	29.3	11.6	58
		8.2 (glycine-NaOH)	15.4	14.1	48
4	$\text{S} \cdot (\text{CH}_2 \cdot \text{CH}_2 \cdot \text{Cl})_2$	* 8.2 (NaHCO_3)	25.1	11.0	39
Epoxides					
5		7 (water only)	27.2	14.7	14
6		7 (water only)	3.8	2.3	75
7		7 (water only)	0.9	1.5	78
		9	1.0	—	76
		2	25.9	—	22
Ethyleneimines					
8		4	27.1	11.9	—
		7	24.7	15.3	13
9		4	30.7	13.9	—
		7.5	1.8	—	86
		9	2.0	—	68
10	$\left[\begin{array}{c} \text{CH}_2 \\ \diagup \quad \diagdown \\ \text{N} \cdot \text{CO} \cdot \text{N}(\text{CH}_2)_3 \\ \diagdown \quad \diagup \\ \text{CH}_2 \end{array} \right]_2$	7	12.9	6.1	—
Methanesulphonyloxy derivatives (X is $\text{CH}_3\text{SO}_2\text{O}$ —)					
11	$\text{X} \cdot (\text{CH}_2)_3 \cdot \text{CH}_3$	8.2 (NaHCO_3)	24.5	13.1	—
12	$\text{X} \cdot (\text{CH}_2)_2 \cdot \text{X}$	8.2 (NaHCO_3)	25.1	13.9	—
13	$\text{X} \cdot (\text{CH}_2)_3 \cdot \text{X}$	4	25.6	9.2	11
		7	6.5	3.5	76
		8.2 (NaHCO_3)	5.0	2.4	88
14	$\text{X} \cdot (\text{CH}_2)_4 \cdot \text{X}$	8.2 (NaHCO_3)	7.7	11.7	62
15	$\text{X} \cdot (\text{CH}_2\text{C}\equiv\text{C} \cdot \text{CH}_2) \cdot \text{X}$	8.2 (NaHCO_3)	0.0	13.4	74
16	$\text{X} \cdot \begin{array}{c} \text{NO}_2 \\ \\ \text{CH}_2 \cdot \text{C} \cdot \text{CH}_2 \\ \\ \text{CH}_3 \end{array} \cdot \text{X}$	8.2 (NaHCO_3)	1.5	13.2	63

* Applied from 50% ethanol.

Table 2 (cont.)

Reagent no.	Halogeno-2:4-dinitrobenzenes*	pH of solution	Contraction after reaction with		Percentage of oxidized fibre insoluble in ammonia
			Bisulphite (%)	Peracetic acid (%)	
17		8.2 (NaHCO ₃)	17.8	14.0	48.5
18		8.2 (NaHCO ₃)	5.6	9.0	80.5
19		8.2 (NaHCO ₃)	12.8	11.3	73.0
20		8.2 (NaHCO ₃)	3.0	11.4	68.6

* Applied from 75% ethanol.

treatment is much less when a short-chain unfunctional alkylating agent such as methyl bromide is used, than when long-chain groups (e.g. heptyl), capable of interaction by secondary forces with

radiomimetic compounds can be replaced by a group capable of powerful interaction by secondary forces.

The results with chemically modified wool fibres (see Table 3) show that the amino groups, but not the carboxyl groups, play an important part in the cross-linking reaction and the cross-links may be formed between pairs of amino groups. Chemical considerations suggest that all radiomimetic compounds react by a carbonium ion mechanism and that they can react only with amino groups which are unionized and carboxyl groups which are dissociated (Ross, 1949). This is the case in model systems such as polymethacrylic acid and the polymer of ethyleneimine (Alexander, unpublished observations); in these, cross-linking occurs only with the ionized form of the former and the undissociated form of the latter polymer.

The optimum pH range found for cross-linking wool (7–8) would suggest reaction with carboxyl rather than with amino groups, since the majority of the latter are in the ionized and therefore unreactive state. The contrary result obtained would indicate that the chemical reactivity of these groups may not

Table 3. Cross-linking of modified wool fibres by radiomimetic compounds

Reagent (numbered as in Table 2)	Contraction after reaction with bisulphite	
	Acetylated fibres (%)	Esterified fibres (%)
None	28.2	25.0
No. 7	31.2	9.2
No. 6	28.5	1.7
No. 9	6.4	5.5
No. 13	27.3	26.5

other groups, are introduced. It is interesting in this connexion that *N*-2:4-dinitrophenyl-ethyleneimine is one of the few unfunctional compounds which has a biological activity comparable to that of multifunctional compounds (Hendry *et al.* 1951), and it may be that one of the chemically reactive groups in

be correctly represented as —NH_3^+ and —COO^- and that in fact they behave in chemical reaction as —NH_2 and —COOH groups near the isoelectric point, where they neutralize one another. This suggestion is supported by the observation (Rutherford, Patterson & Harris, 1940) that isoelectric proteins can be esterified with diazomethane, which in simple systems only reacts with unionized carboxyl groups.

SUMMARY

1. To examine the hypothesis that the radiomimetic alkylating agents function by a cross-linking mechanism, the action of a representative group of these compounds has been studied with serum albumin in solution and with wool.

2. It was shown from molecular weight determinations using light scattering that the serum albumin is cross-linked in a 2% (w/v) solution by the nitrogen mustards, bisepoxides and tris-(ethyleneimino)triazine.

3. Examination of the physical properties of wool fibres after reaction with twenty radiomimetic and related compounds showed that cross-linking can be brought about by most of these substances.

4. There appears to be no correlation between the biological activity of a compound and its effectiveness as cross-linking agent in the systems examined.

5. Experiments with acetylated and esterified wool fibres indicate that the cross-links involve a reaction with amino groups.

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