We can now add one further figure to our balance sheet for the metabolism of benzene at 0.5 g./kg.

	%
Benzene eliminated unchanged	39
Benzene oxidized (and excreted as glucuronide and ethereal sulphates)	21
Benzene eliminated as phenylmercapturic acid	1

Preliminary results have shown that another metabolite of benzene, muconic acid, accounts for at least a further 1 % of the dose.

SUMMARY

1. The excretion of phenylmercapturic acid by rabbits receiving benzene has been studied.

2. The presence of phenylmercapturic acid in urine after administration of benzene has been

shown by developing a specific turbidimetric method of estimation, based on the formation of phenylmercuric mercaptide from phenylmercapturic acid by alkaline hydrolysis.

3. The iodometric method for the determination of mercapturic acid has been shown to be influenced by diet and by hydroxyquinol, a metabolite of benzene, and may give high results when small amounts of mercapturic acids have to be estimated in urine.

4. By the turbidimetric method about 1 % of the benzene fed at a dose level of 0.5 g./kg. and about 0.8 % at 1 g./kg. was excreted as phenylmercapturic acid, which was found in the urine for 2–3 days after dosing.

5. Some arylcysteines and mercapturic acids have been synthesized and their ultraviolet absorption spectra recorded.

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Esterification of the Carboxyl Groups in Wool

By P. ALEXANDER,* D. CARTER, C. EARLAND AND O. E. FORD

Research Department, Wolsey Ltd., Leicester

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The carboxyl groups in wool were partially methylated with diazomethane by Rutherford, Patterson & Harris (1940) and with methyl sulphate and methyl iodide by Blackburn, Carter & Phillips (1941). In both cases titration showed that not more than 50 % of the carboxyl groups were esterified and that the reaction was non-specific and methylation of other groups occurred. Blackburn & Phillips (1944) reported that a mixture of methanol and acetic anhydride esterified the carboxyl groups, in part at least, but that some of the terminal amino groups in lysine and the peptide imino linkage were simultaneously acetylated. Fraenkel-Conrat & Olcott (1945) showed that anhydrous alcohols containing a catalytic quantity of mineral acid reacted speci-

fically with the carboxyl groups in proteins without modifying any other group. Methanol containing 0.1N-hydrochloric acid was used by Blackburn & Lindley (1948) to introduce 0.8 % of methyl groups into wool; this is equivalent to masking 65 % of the carboxyl groups.

Fraenkel-Conrat (1944) found that 1:2-epoxides readily esterified carboxyl groups in proteins and showed that, although epoxides also combine with amino groups, their basic nature is not suppressed and the reaction is therefore suitable for studying the effects of blocking the carboxyl group on the properties of proteins. Schlack (1947) discovered that the affinity of acidic dyes for wool was greatly increased by reacting the latter with an epoxide, and it will be shown that this results from the suppression of the ionization of the carboxyl groups.

* Present address: Department of Chemistry, Imperial College of Science and Technology, London, S.W. 7.

In this paper a quantitative study of the esterification of wool with alcohols and epoxides, the accessibility of wool to different alcohols and the stability of the ester formed are reported.

The findings of Speakman & Hirst (1933) on the mechanical properties of wool as a function of pH indicate that in the isoelectric region, approximately from pH 6 to 8, the free amino and carboxyl groups in wool exist largely in the zwitterion condition, $-\text{NH}_3^+ \dots \text{COO}^-$. These groups, present in approximately equal numbers, mutually balance their electrical charges and probably exert an electrostatic influence on one another which decreases the swelling and increases the strength of the fibre. On combination with acid (e.g. hydrochloric acid) the configuration of this so-called salt link is changed to $-\text{NH}_3^+ \dots \text{Cl}^- \dots \text{HOOC}-$ and a 'Gegenion' enters the wool to bring about electrical neutrality (Alexander & Kitchener, 1950). This ion is held in the electric double layer and can take part by mass effects in ion-exchange reactions. When the carboxyl group is esterified 'Gegenions' have to be present to balance the charge of the corresponding ionized amino groups even when the fibres are in equilibrium with neutral solutions. The number of carboxyl groups esterified can therefore be determined from the reduction in the maximum acid-combining capacity of the wool since a salt link after esterification, i.e. $-\text{NH}_3^+ \dots \text{Cl}^- \dots \text{ROOC}-$ no longer combines with acid. It is essential that the 'Gegenion' should not be a hydroxyl ion since this, of course, would act as a base and by an ion-exchange process consume acid and give an erroneous value for the true acid-combining value of the protein. To ensure that all the 'Gegenions' were chloride ions in this investigation, the wool was repeatedly washed with solutions of potassium chloride before determining the combination with hydrochloric acid. This was particularly important in the experiments on the saponification of the esters by alkali since the first reaction was an exchange of the chloride ions by hydroxyl ions. If wool after treatment with alkali was not washed with salt solution but in distilled water only, an acid-combining value corresponding to untreated wool was obtained. However, when the esterified wool, after reaction with alkali, was equilibrated with a salt solution, then the degree of saponification could be determined from the increase in acid-combining power.

EXPERIMENTAL

Materials. For single fibre experiments Lincoln wool was used and for all other experiments knitted fabric made from high quality Merino wool.

The alcohols were dried over CaO followed by distillation from the respective diester of phthalic acid. Epichlorhydrin was obtained from Shell Chemicals Ltd., propylene oxide from Petrocarbon Ltd. and ethylene oxide from Imperial

Chemical Industries Ltd. Glycidol was prepared by reaction of equivalent quantities of α -monochlorhydrin dissolved in isopropanol and conc. aqueous NaOH. Styrene and *iso*-eugenol epoxide were prepared by oxidation of the corresponding olefin with perbenzoic acid and peracetic acid respectively.

Esterification. When completely anhydrous conditions were required, gaseous HCl was passed into the alcohol; otherwise, conc. HCl was used. Wool dried in an air oven at 110° was treated with the alcohol at a ratio of solution to wool of 50 : 1 (v/w) in a flask fitted with reflux condenser. The epoxides were applied from 5% (w/v) aqueous solution at 95° at a solution to wool ratio of 30 : 1 (v/w).

Combination of wool with acid. The wool was equilibrated to pH 5 before measuring the acid-combining power. After esterification the wool was freed from acid by washing in running tap water for 2 hr., followed by shaking ten times for 2 min. each with distilled water and then four times with *N*-KCl. In this way acid is only consumed in the back titration of the carboxyl groups. After this washing the wool was placed in a solution of 0.02 *N*-HCl containing *N*-KCl at a ratio of solution to wool of 50 : 1; in this medium combination of wool with acid is at a maximum. With vigorous shaking equilibrium was attained in less than 4 min. and the amount of acid combined was determined by titration. Previous investigators who did not agitate the wool system (e.g. Speakman & Hirst, 1933) found it necessary for attainment of equilibrium to leave the wool in contact with acid for 2 days. However, Alexander, Gough & Hudson (1949a) showed that the rate of most reactions of ions with wool is controlled by diffusion through the liquid and is speeded up enormously by efficient agitation. When the flask containing the wool and acid was placed into an efficient shaker the time to reach equilibrium was reduced from about 2 hr. to less than 4 min. With very dilute solutions of acid more than 12 hr. were necessary to attain equilibrium without agitation, but the reaction was invariably complete after 15 min. in the shaker.

Titration curves. These were obtained by the method of Steinhart & Harris (1940) with the modification that the solutions were placed in a shaker when equilibrium was reached in 15 min. The importance of speeding up the reaction is particularly important for the high pH part of the curve, since no degradation took place during the short periods the wool had to be in contact with alkali. Desorption of acid cannot be speeded up to the same extent and the washing procedure described is necessary.

Rate of dyeing curves. The wool was fixed to a frame revolving at 300 rev./min. and the exhaustion of the dye bath determined colorimetrically (Alexander & Hudson, 1950).

The alkali solubility of the wool was determined by the method of Harris & Smith (1936). The load-extension measurements were made as described by Speakman (1947) on a modified fibre balance.

RESULTS

Esterification with alcohols catalysed by mineral acids

Effect of concentration of acid. Simple kinetic considerations suggest that the rate of esterification is proportional to the concentration of hydrogen

ions which for strong acids is the same as the concentration of acid. From Fig. 1 it can be seen that this relationship appears to hold approximately for ethylation with hydrochloric acid as the catalyst. Analogous results were obtained with methanol and *n*-butanol, the only other alcohols which were examined in detail. Concentration of acids greater

Fig. 1. The effect of different concentrations of HCl in ethanol containing 5% (v/v) of water on the rate of esterification of wool at 78°.

than 0.2N could not be used since these begin to hydrolyse the wool at the temperatures used. Sulphuric acid in concentrations of 0.1N or lower is as effective as hydrochloric acid of the same normality, but at higher concentrations the catalytic effect of sulphuric acid is less, probably because it is incompletely ionized at low pH values. The reversible formation of alkyl hydrogen sulphate may also contribute to the decreased activity of sulphuric acid at high concentrations.

Fig. 2. The effect of temperature on the rate of esterification of wool by ethanol containing 2% of water and 0.2N-HCl.

Effect of temperature. The rate of esterification is dependent on temperature, and at 25° it was found

that only 10 and 30% of the carboxyl groups had reacted with ethanol containing 0.1N-hydrochloric acid in 1 and 6 days respectively. With methanol the reaction was somewhat faster and under the same conditions 55% of the carboxyl groups were esterified in 6 days.

In Fig. 2 the rates of ethylation at three temperatures are compared. The great increase in rate on raising the temperature from 60 to 78° is, in all probability, due not only to the speeding up of the reaction but also to the increased rate of removal of water from the wool by the vigorous agitation which occurs at the boiling point.

The influence of water. From Table 1 it can be seen that there is no significant difference in the rate of methylation on changing from a completely anhydrous medium to a solution containing 1% of water, but that with 5% of water there is a very significant decrease.

Table 1. Effect of water on rate of esterification of wool at 60° with methanol containing 0.1N-HCl

Water content (%, v/v)	Acid combined (m-equiv./100 g. wool) after		
	0.5 hr.	1 hr.	2 hr.
0	56	40	31
0.6	54	37	32
1	52	36	33
5	64	41	—
8	78	69	61

Esterification with different alcohols. Fig. 3 shows the rate of reaction of different alcohols under completely anhydrous conditions either at 100° or at the

Fig. 3. Rate of esterification of wool with the following anhydrous alcohols containing 0.1N-HCl; methanol at 65°, *n*-propanol at 97°, *n*-butanol at 100°.

boiling points of the alcohol. In every case the curve flattened out after 4 hr. and after 6 hr. all the carboxyl groups capable of reacting had been esterified. In this way the proportion of carboxyl groups which

are available for esterification by alcohols of varying molecular weights and complexities were determined (see Table 2).

Table 2. *Maximum esterification of wool at 100° with different anhydrous alcohols in the presence of 0.1N-HCl or at the boiling point of the alcohol, if below 100°*

(Time of reaction 6 hr. In every case minimum acid-combining value was reached after 4 hr. of reaction, cf. Fig. 3.)

Alcohol	Acid-combining value of wool (m-equiv./100 g. of wool)	Proportion of total carboxyl groups available for esterification (%)
Methanol	28	67
Ethanol	36	56
<i>n</i> -Propanol	38	54
<i>iso</i> Propanol	70	15
<i>n</i> -Butanol	50	39
<i>iso</i> Butanol	65	21
<i>sec.</i> -Butanol	70	15
<i>n</i> -Amyl alcohol	65	21
Ethylene glycol	40	51
Glycerol	44	46
Pentane-1:5-diol	65	21
Benzyl alcohol	72	12
Cetyl alcohol dissolved in xylene	72	12

The value for the maximum amount of esterification (i.e. for the minimum acid-combining value) for each alcohol was independent of the concentration of acid, but increased slightly in the presence of 0.5 % of water in the alcohol (see Table 3). With more than 2 % of water the rate of the reaction was found to decrease sharply and the wool after reaction was seen to be degraded, probably owing to acid hydrolysis.

Table 3. *Effect of water content of alcohol on maximum esterification of wool*

(Conditions as in Table 2.)

Alcohol	Water content (% v/v)	Acid-combining value of wool (m-equiv./100 g. of wool)
<i>n</i> -Propanol	0	44
	0.5	39
	2	41
<i>n</i> -Butanol	0	57
	0.5	50
	2	53

The esterification can, of course, be formulated as an equilibrium

and it might be considered that the reason why only 40 % of the carboxyl groups can be esterified with

n-butanol as opposed to 70 % with methanol is not related to accessibility of carboxyl groups in the wool, but arises from different equilibrium constants in the two cases. However, if the extent of the reaction were governed by equilibrium considerations, the addition of 0.5 % of water to the butanol would reduce the number of carboxyl groups esterified to less than 1 %. This is not the case, and it can therefore be concluded that steric factors in the wool are responsible for the differences observed between the alcohols.

Hydrolysis of peptide bonds. Fraenkel-Conrat & Olcott (1945) showed that no hydrolysis of peptide bonds occurred with 0.1N-hydrochloric acid in methanol at room temperature. The following experiments indicate that there was no substantial peptide bond breakdown in wool under anhydrous conditions when the same reagent was used at 65° for 4 hr., 70 % of the carboxyl groups being esterified. (1) The ester groups were readily saponified by dilute ammonia and the original acid-combining power was restored. If main chain degradation had taken place new carboxyl groups would have been formed, the acid-combining value being increased. (2) The work necessary to stretch a fibre in acid when salt links are broken was not significantly changed on esterification (see Table 10). (3) The solubility of the wool in alkali was only increased from 11 to 13 % on esterification. Both the elastic properties and the solubility in alkali are very sensitive tests for main chain degradation and the small changes produced by the treatment can probably be ascribed to the blocking of the carboxyl groups.

If the reaction is not carried out under anhydrous conditions, peptide bond hydrolysis occurs, the solubility in alkali is increased and the work to stretch a fibre in acid decreased.

Esterification with 1:2-epoxides

Table 4 shows that of the epoxides examined only epichlorhydrin is capable of esterifying more than 10 % of the available carboxyl groups in wool. The

Table 4. *Combination of wool with acid after treatment with different epoxides in 5 % (w/v) aqueous solution at 95° for 2 hr.*

Substance used	Acid-combining value (m-equiv./100 g. of wool)
None	81.6
Ethylene oxide	81.4
Propylene oxide	77.4
Glycidol	78.7
<i>iso</i> Eugenol oxide	75.6
Styrene oxide	72.0
Epichlorhydrin	51.0

reaction was complete after 2 hr. in every case and a lower acid-combining figure than that given in

Table 4 was not obtained with any epoxide even after very prolonged treatment. These results are in contrast with those of Fraenkel-Conrat (1944) who found that ethylene oxide, propylene oxide and epichlorhydrin are equally reactive with globulins. Differences in the rate of hydrolysis of the epoxides in aqueous solution cannot explain the great superiority of epichlorhydrin as an esterifying agent for the carboxyl groups in wool, since in every case at least 50 % of the epoxide originally employed was still present at the end of the experiment. Though the epoxides combine at different rates with organic acids (Brønsted, Kilpatrick & Kilpatrick, 1929), epichlorhydrin does not stand out in the order of reactivities and there is no correlation with the results obtained on wool.

The effect of temperature and of concentration of epichlorhydrin on the extent of the reaction is seen from Table 5. An esterification of approximately

Table 5. *Effect of concentration of epichlorhydrin and of temperature on rate of esterification of wool*

Concentration of epichlorhydrin (%, w/v)	Temp. (°)	Acid combined (m-equiv./100 g. of wool) after		
		0.5 hr.	1 hr.	2 hr.
		—	—	—
5	95	—	52	51
	70	—	54	54
1	95	73	67	64
	70	80	74	70
0.3	95	78	75	75
	70	—	77	75

40 % (corresponding to an acid-combining value of 51 m-equiv./100 g.) seems to be the maximum attainable with epichlorhydrin, as a higher value was not obtained under any experimental conditions.

In distilled water the pH of the epichlorhydrin solution is approximately 6 and this remained sensibly unchanged throughout the reaction. Experiments in which the solutions were adjusted with sulphuric acid and phosphate buffers to different pH values showed that the optimum pH range was between 5 and 7 for reaction with carboxyl groups.

Extensive reaction with groups other than carboxyl was indicated from a weight increase of

9–10 % on treatment with epichlorhydrin. The cystine content of the wool was unchanged and this proved that the disulphide bond had not taken part in the reaction. After hydrolysis the concentration of the basic amino-acids and tyrosine was the same as that of untreated wool. From this it cannot be concluded that the free amino and phenolic groups did not react with epichlorhydrin, since compounds would be hydrolysed by the treatment with acid. The setting power of wool is reduced when the amino groups have been reacted with 1:2:4-fluorodinitrobenzene (Farnworth & Speakman, 1948). After reaction with epichlorhydrin wool could no longer be given a permanent set (Table 6), whereas after esterification with alcohols the setting power of fibres is increased (Blackburn & Lindley, 1948). This indirect evidence indicates reaction of the epoxide with amino groups which was shown to occur readily with soluble proteins by Fraenkel-Conrat (1944). In view of the results of Fraenkel-Conrat (1944), reaction of epichlorhydrin with phenolic groups in the wool is probable; but this could not be proved, since phenolic groups cannot be determined in unhydrolysed wool.

Saponification of esterified carboxyl groups

It was found that saponification was complete in less than 1 hr. in 0.1N-sodium hydroxide at room temperature, but no saponification occurred even after prolonged contact with distilled water at 100°. Washing esterified wool under tap water of pH approx. 7.8 caused some saponification after extended periods (Table 7). The rate of saponification of methylated wool in 0.1N-ammonia and a soap solution is shown in Fig. 4.

Although the rate of esterification of different alcohols decreased with increasing molecular weight, there was no corresponding difference in the rate of saponification. The esters formed with the different alcohols examined were readily saponified, although the esters containing groups of greater molecular weight appeared to react somewhat more slowly. However, it is doubtful whether these differences are significant (Table 8).

The esters are much more stable to acid than to alkali and there was no indication of hydrolysis after 3 days in 0.1N-hydrochloric acid at room tempera-

Table 6. *Effect of epichlorhydrin treatment on setting power of single wool fibres.*
Fibres held stretched 30 % for 1 hr. in water at 100°

Treatment	Percentage retained after releasing fibres in water at 100° for			
	2 min.	15 min.	30 min.	60 min.
None	19.5	17.8	17.0	16.4
	18.3	13.7	11.1	9.0
Epichlorhydrin treated (42 m-equiv./100 g. of wool)	8.6	1.5	-0.7	-4.5
	7.7	2.8	0.3	-2.1

ture. The esters are, however, hydrolysed under conditions used for hydrolysing peptide bonds in wool (i.e. 5*N*-hydrochloric acid at 120°).

Fig. 4. Rate of saponification of wool which had esterified with methanol (0.1 *N*-HCl) in the following solutions: 0.1 *N*-NH₃ at 60°, —○—; 0.1 *N*-NH₃ at 25°, —×—; 0.5% soap solution at 60°, —①—.

Table 7. *Hydrolysis of methylated wool on keeping for different periods under running tap water*

Time under tap water (hr.)	Acid-combining value (m-equiv./100 g. of wool)	
	Exp. 1	Exp. 2
6	—	31
9	25	—
18	28	42
54	46	60
135	71	75

Effect of alkali on wool treated with epichlorhydrin

Wool which had been esterified with epichlorhydrin and then reacted with alkali was found to combine very much more slowly with acid, even under conditions of violent agitation, than untreated wool, while wool esterified with alcohols, whether it has been in contact with alkali or not, as well as epichlorhydrin-treated wool which has not been in contact with alkali, showed maximum acid combination after 4 min. (see p. 630) and no further acid was consumed on standing. It seemed possible that the slow additional acid uptake of wool esterified with epichlorhydrin and then treated with alkali results from the following series of reactions:

Table 8. *Hydrolysis of different esters of wool by treatment with 0.1 *N*-NH₃ at 60° for 30 min.*

Substance used for esterification	Acid-combining value of wool (m-equiv./100 g.)	
	Before hydrolysis	After alkaline hydrolysis
(Untreated wool)	81.7	82.1
Methanol	32.6	70.4
	40.7	72.5
Ethanol	50.6	71.8
	70.0	78.1
<i>n</i> -Propanol	49.0	72.5
<i>iso</i> Propanol	68.7	78.2
<i>n</i> -Butanol	58.1	72.8
Ethylene glycol	61.5	74.9
Pentane-1:5-diol	73.7	79.2
<i>iso</i> Eugenol oxide	75.6	81.2
Styrene oxide	72.0	82.0
Benzyl iodide*	50.7	68.6
Cetyl iodide†	49.8	81.9

* pH 8 phosphate buffer containing 5% (v/v) benzyl iodide shaken with wool for 2 hr. at 95°.

† 5% solution (w/v) in *isopropanol* for 2 hr. at 70°.

In agreement with this mechanism it was found that between 80 and 90% of the calculated quantity of chloride ions were liberated on treating epichlorhydrin-treated wool with 0.1 *N*-ammonia at 60° for 3 min. Moreover, experiments with the model substance, α -monochlorhydrin, showed that ring formation was complete with 0.1 *N*-sodium hydroxide in less than 2 min., whereas complete acid hydrolysis of the epoxide in 0.1 *N*-hydrochloric acid at 25° required 5 hr.

It was found (Table 9) that after contact for only 4 min. with dilute alkali such as 0.02*M*-borax or 0.1 *N*-ammonia, the acid-combining value of epichlorhydrin-treated wool after 20 hr. in acid was increased to that of untreated wool although no such increase occurred after 4 min. in acid. From these results it can be concluded that little saponification occurred, but that the probable epoxide formation was complete during the 4 min. reaction with alkali.

Hence, the difference between the quantity of acid consumed by epichlorhydrin-treated wool after 4 min. and after 24 hr. was taken as a measure of the number of epoxide groups in the wool.

The rate of saponification of epichlorhydrin-treated wool is seen from the rate of increase in the

Table 9. *Formation of epoxide groups in wool treated with epichlorhydrin by reaction with alkali*

Alkali used	Time of treatment at 60° (min.)	Acid-combining value (m-equiv./100 g. in acid) for		m-equiv. of epoxide in 100 g. wool
		4 min.	20 hr.	
0.1N-NH ₃	0	53.0	53.4	0.4
	2	65.4	79.6	14.2
	10	77.0	85.5	8.5
	30	80.0	86.5	6.5
	60	82.7	86.9	4.2
0.05 M-Borax	0	53.6	53.9	0.3
	2	62.6	82.3	19.7
	10	69.9	81.7	11.8
	30	73.7	84.8	11.1

4 min. acid-combining value and the number of epoxide groups present from the difference of the two acid-combining values. By short treatment with alkali virtually complete conversion to epoxide can be obtained without hydrolysis of the ester. The rate of saponification of epichlorhydrin-treated wool is, within experimental error, the same as that of wool esterified with simple alcohols (Table 9).

Fig. 5. Rate of dyeing of esterified and ordinary wool in a solution containing 200 mg. of azo geranine 2GS (Colour Index 637) only (—) and 250 mg. of Orange II and 0.1N-HCl (---); temperature 25°; solution to wool ratio 150 : 1; wool rotated at 200 rev./min. —×—, untreated wool; —□—, wool esterified with methanol (acid-combining power 46 m-equiv./100 g.); —○—, wool esterified with methanol (acid-combining power 25 m-equiv./100 g.).

Properties of esterified wool

Dyeing with simple acidic dyes takes place by an ion-exchange process in which the inorganic anion

held by a charged amino group is replaced by the dye anion of greater affinity (Alexander & Kitchener, 1950) and can only take place when some carboxyl groups are masked. In ordinary wool anion exchange is possible only in acid solutions, when the carboxyl groups have combined with a proton, but when some of the carboxyl groups are esterified the corresponding amino groups retain a positive charge in solutions of pH less than 10 and carry a 'Gegenion' which can take part in ion exchange. In agreement with this concept it is seen from Fig. 5 that in neutral solution only the esterified wool takes up dye, whereas in acid solution both esterified and untreated wool dye equally readily. Basic dyes such as methylene blue are bound as 'Gegenions' to charged carboxyl groups and as a result it is found that esterified wool at all pH values takes up much less of these dyes than untreated wool.

Esterification of the carboxyl groups with alcohols results in the same increase in the wash-fastness of dyes on wool which Schlack (1947) found after reaction with epichlorhydrin. Simple acidic dyes sorbed from acid solution lose affinity for the wool in neutral solutions when the ionized carboxyl groups neutralize the amino groups, but the slow diffusion of the big dye ions makes the diffusion out of the fibre a slow process. In esterified wool, however, the carboxyl groups do not compete with the dye anions for the amino group and the wool only loses its affinity for the dye anion in alkaline solution when the amino groups lose a proton and become un-ionized.

The titration curve of methylated wool is shown in Fig. 6, and the combination of the unblocked carboxyl groups with acid occurs at the same pH and is affected by neutral salt in the same way as in untreated wool. The combination of alkali with esterified wool, however, is shifted to lower pH values and shows a smaller influence of salt, probably because the amino groups no longer form part of a salt linkage and can now titrate as free base. Since combination with alkali is complete in 2 min. at room temperature no saponification occurs during the titration at pH less than 12. The acid-combina-

tion curve of wool treated with epichlorhydrin is exactly the same as that of methylated wool.

The work necessary to stretch wool fibres in acid solution is less than that in neutral solutions, and the difference is thought by Speakman & Hirst (1933) to represent the contribution of the salt links

Fig. 6. Titration curve of ordinary wool (----) and wool esterified with methanol (0.1 N-HCl) (—). —○—, titrated in a solution containing acid or alkali only; —○—, titrated in a solution containing N-KCl.

to the strength of the fibre. In Table 10 it is seen that this difference is decreased on esterification and that the reduction is directly related to the decrease in acid-combining power.

The esterification of carboxyl groups by amyl alcohol and butanol adds further proof that the concept of a maximum pore size put forward by Speakman is erroneous and that the dry wool fibre is accessible to large molecules although the rate of penetration is slow. From consideration of the kinetics of diffusion of both molecules and ions from aqueous solutions, Alexander, Gough & Hudson (1949b) showed that a model involving micropores containing free liquid was untenable and suggested that the wool fibres behaved as a homogeneous gel. The fact that the number of carboxyl groups available for esterification by alcohols varies with the molecular weight of the latter requires a modification of this picture. It is interesting that not all the carboxyl groups in wool are accessible even to the small molecule, methanol, which was shown by Fraenkel-Conrat & Olcott (1945) to esterify completely a number of proteins. The reason why alcohols of high molecular weight are capable of esterifying more carboxyl groups in the presence of low concentrations of water (Table 3) can either be due to the fact that increased swelling makes more groups accessible or that increased degradation by acid (see p. 632) leads to an opening of the structure. The failure of epoxides other than epichlorhydrin to react with the carboxyl groups in wool, an effect not observed with the proteins studied by Fraenkel-Conrat (1944), also requires elucidation since

Table 10. *The difference in the work needed to stretch a fibre in a phosphate buffer at pH 7 and in 0.1 N-HCl after different degrees of esterification*

(Work required for untreated wool in pH 7 buffer taken as 100.)

Treatment	Acid-combining value (m-equiv./100 g.)	Work required to stretch fibres 30% in		Difference in work to stretch in buffer at pH 7 and 0.1 N-HCl (%)
		Buffer at pH 7	0.1 N-HCl	
None	81.6	100	59.8	41.2
Epichlorhydrin	54.0	80.5	58.5	32.0
Methylation	44.4	76.7	57.6	28.6
	73.3	91.6	58.0	37.0

DISCUSSION

Speakman (1930, 1931) deduced from the elastic properties of fibres in different alcohols that wool is not accessible to molecules larger than propanol, but is readily penetrated by methanol and ethanol. He also concluded that in the presence of water the fibre can be penetrated by much larger molecules than in the absence of water. King (1947) showed that alcohols of higher molecular weight than ethanol are capable of diffusing into the wool fibre, but that the diffusion coefficients are so small that no penetration would be detected at room temperature in the period given by Speakman.

steric factors are obviously not operative in this case.

An explanation for the differences in accessibility may be found in the recent separation of wool into a fraction of high molecular weight and low cystine content and a fraction of low molecular weight and of high cystine content (Alexander & Earland, 1950). Preliminary investigations show that the low molecular weight fraction is exceptionally rich in acidic amino-acids. This low molecular weight fraction acts as a cement between micelles made up of the high molecular weight molecules and the carboxyl groups in the two phases would, of course, vary in accessibility.

The rate of hydrolysis of esters in solution is highly dependent on the nature of the alcohols, e.g. hydrolysis of methyl acetate is twice as fast as that of ethyl, *n*-propyl and *n*-butyl acetate and six times as fast as that of isopropyl acetate (Smith & Olsson, 1925). The fact that the different wool esters hydrolyse at approximately the same rate suggests that the rate-controlling step in the heterogeneous reaction may not be the actual hydrolysis but a diffusion process.

SUMMARY

1. The effect of concentration of acid catalyst, temperature and water on the esterification of the carboxyl groups in wool with alcohols has been studied.

2. The number of carboxyl groups capable of being esterified decreases with increasing molecular weight of the alcohol.

3. The esters formed are readily saponified by alkali and the rate of this reaction is independent of the alcohol used.

4. Of a number of 1:2-epoxides examined only epichlorhydrin was found to esterify more than 10 % of the carboxyl groups of wool. The ester of monochlorhydrin formed reacts with dilute alkali at room temperature to form an epoxide which is combined in the wool.

5. The effect of esterifying the carboxyl groups on the titration curve, dyeing behaviour and elastic properties of wool have been investigated.

6. It is shown that the combination of acids with wool can be greatly speeded up by efficient agitation and that equilibrium can be established in a few minutes.

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Blood Glutathione and Non-glucose Reducing Substances in Diabetes

By ELIZABETH K. B. ILLING, C. H. GRAY AND R. D. LAWRENCE

Department of Chemical Pathology, King's College Hospital, Denmark Hill, London, S.E. 5

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A relation between glutathione and experimental diabetes was first suspected when Leech & Bailey (1945) observed a simultaneous fall in blood alloxan and glutathione following the injection of diabetogenic doses of alloxan into rabbits. Lazarow (1946, 1947) subsequently demonstrated that glutathione and other sulphhydryl compounds such as cysteine and BAL (2:3-dimercaptopropanol) were capable of

protecting the rat against the diabetogenic activity of alloxan. More recently Conn, Louis & Johnston (1949) have shown that the intravenous administration of reduced glutathione inhibited the temporary diabetes produced by the administration of adrenocorticotrophic hormone (ACTH).

It is possible that the great variation observed by Mosenenthal & Barry (1946) in the non-glucose reducing