of phosphocreatine have been determined in specimens of about 0.1 g. brain without chemical separation.

2. After death, a very rapid fall in phosphocreatine and rise in inorganic phosphate takes place in the brain of guinea pig as of other species.

3. On slicing brain cortex and allowing the slices to respire with glucose under normal metabolic conditions, the inorganic phosphate falls and the phosphocreatine rises until the concentrations approach those normal to the tissue *in vivo*.

4. The concentrations reached vary with the metabolic mixture.

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The Reaction of Oxidizing Agents with Wool

3. THE INFLUENCE OF THE MORPHOLOGY ON THE RATE OF REACTION

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A detailed study of the kinetics of the reaction between wool and aqueous chlorine solutions at pH 2 by Alexander, Gough & Hudson (1949) has shown that the rate is controlled either by diffusion through a film of liquid surrounding the fibre or by diffusion within the fibre, depending on the conditions of treatment. The rate of the reaction increases with increasing agitation up to a limiting stirring rate, after which it becomes constant. At this point the temperature coefficient changes sharply, showing that a change in mechanism has occurred. The

following observations show that the rapid rate at high stirring speeds with chlorine at pH 2 is not governed by the actual chemical reaction but by the diffusion of the chlorine to the site of reaction where it reacts instantaneously:

(1) The quantity of chlorine which has reacted increases with time according to a parabolic law which is to be expected for diffusion into the fibres under the experimental conditions employed.

(2) The observed activation energy (12.5 kg. cal./mol.) is the same as that found for a wide variety of

reactions, e.g. dyeing, ion exchange (Alexander & Hudson, 1950) and oxidation with acid potassium permanganate (Alexander & Hudson, 1949). The one feature common to all these processes is diffusion within the fibre.

(3) The reaction between chlorine in acid solution and the amino-acids cystine and tyrosine, which are preferentially oxidized in wool, is extremely rapid compared with the rate of reaction with wool.

(4) The rate of reaction with wool in which all the disulphide bonds have been changed to $-C-S-C-$ linkages is the same as that for untreated wool in which the disulphide bond is oxidized preferentially (see p. 24).

(5) On interrupting a kinetic run, a break in the curve is observed which is typical for diffusion-controlled processes (see p. 23).

The reaction of chlorine in acid solution is very similar to the oxidation by potassium permanganate at pH 2 (Alexander & Hudson, 1949), and in both cases diffusion appears to proceed through a homogeneous medium. Complete penetration of chlorine occurs, as is shown by the removal of all the cystine in acid solutions. The fibre behaves as a uniform gel of relatively low water content, although the actual fibre is thought to consist of a complex network of small micropores filled with solvent through which diffusion occurs. This behaviour of the fibre towards the diffusing molecules is interesting as the morphological structure of wool is known to be extremely complex, consisting of various layers differing in accessibility (see below). It will be shown that the diffusion of chlorine and permanganate in alkaline solution is affected by the variation of structure within the fibre, which, however, does not affect diffusion from acid solution.

EXPERIMENTAL METHODS

The measurement of the rate of reaction has been described in detail elsewhere (Alexander, Gough & Hudson, 1949) and will only be summarized here. Purified wool samples, after immersion in the appropriate buffer solution for 24 hr., were fitted uniformly round a frame stirrer and rotated at known speeds in a known volume of Cl_2 solution at the required pH. Samples of solution were withdrawn periodically and analysed iodometrically. Reactions at pH < 8 were conducted in sealed flasks, but in alkaline solution open beakers were employed as no loss of Cl_2 occurs. The following buffer solutions of ionic strength, approx. 0.1, maintained the pH of solution constant during the reaction:

pH 3 and 4. Potassium hydrogen phthalate and H_2SO_4 .

pH 5 and 6. Sodium acetate and acetic acid.

pH 7-10. KH_2PO_4 and NaOH.

pH 11. 0.1M- Na_2CO_3 .

The volumes of the buffer components used depended on the alkalinity of the standard NaClO stock solution.

In stating the concentrations of the reactants, three variables must be specified, namely, the quantity of Cl_2 in solution, the volume of solution and the weight of fabric used.

In the work described here, the ratio of weight of fibres to volume of solution was kept constant at 1:150 and the concentration of Cl_2 is expressed as percentage by weight of the weight of wool.

The wool was pretreated in various ways in several of the experiments as follows:

Potassium cyanide. The wool fabric was first wetted out in water overnight and then immersed in a 0.7% solution of KCN and boiled for 30 min. The fabric was next washed until no further reaction for CN^- ions was observed in the washings, and finally placed in the appropriate buffer for 24 hr. According to Cuthbertson & Phillips (1945) the cystine is converted almost quantitatively to lanthionine by this treatment. It was found that in the present case the cystine content of wool decreased from 10.08 to 0.72%, corresponding to a 93% loss of cystine.

Chlorine at pH 2. The wool, previously wetted out in water, was allowed to react with a solution of Cl_2 (5 and 10% of the weight of wool) in dilute H_2SO_4 at pH 2, until no Cl_2 remained in solution. After washing well in running water the fabric was left overnight in the pH 10 buffer.

Partial acid hydrolysis. The samples of wool were sealed in tubes containing N-HCl and placed in an electric oven at 80°. After 8 hr. the samples were withdrawn and wetted out in the buffer at pH 10.

Potassium hydroxide. Samples of fabric (2 g.) were dipped for 30 sec. into a solution of 2.5 g. KOH in 1000 ml. of white spirit and 100 ml. of *n*-butanol at room temperature. Under these conditions reaction is confined to the extreme outside of the fibres. The samples were then washed in running water and immersed overnight in the buffer at pH 10.

RESULTS

Effect of pH on rate

It is observed from Fig. 1 that the rate of reaction remains almost constant from pH 2 to 7, but rapidly decreases from pH 8 to 11. In the absence of a more

Fig. 1. Effect of the pH of the Cl_2 solution on the rate, given as half-life value ($t_{0.5}$), for untreated (A) and KCN-treated wool fibres (B). Broken curve: effect of pH on the concentration of OCl^- ions, given as percentage of oxidizing agent present. Stirring rate 600 rev./min.; temp. 0°; 10 g. Cl_2 /100 g. wool.

satisfactory method of comparing rates of reactions proceeding by different mechanisms the half-life values are used. The change in composition of the

chlorine solution with pH is also included in the graph and is seen to run parallel with the decrease in rate. The constant rate of reaction in the pH range 2-7, where the ratio of chlorine to hypochlorous acid changes considerably, is explained by the similar diffusion coefficients of the two species, as the reaction is controlled by diffusion within the fibre. In addition, the penetration of neutral molecules would be unaffected by electrical changes on the surface and within the fibre, except indirectly by the change in swelling with pH of solution. That the half-life values remain almost constant shows that swelling has a negligible effect. Above pH 6, however, the solution contains increasing proportions of hypochlorite ions, so that over the pH range 7-9 the reaction is probably governed by the concentration of hypochlorite ions and hypochlorous acid. It appears from Fig. 1 that the rate of reaction with the hypochlorite ions is slow compared with the hypochlorous acid reaction. It has been shown that the rate is first order with respect to the initial chlorine concentration in acid solution (Alexander, Gough & Hudson, 1949). Thus, if it is assumed that the rate of the reaction with hypochlorous acid is greater than that of the hypochlorite ion reaction, approximate rate constants can be calculated neglecting the latter reaction as follows:

$$k = k_0 [\text{HOCl}],$$

where k = observed velocity constant and k_0 = velocity constant with 100% HOCl in solution. Table 1 includes such values for pH 6-9. Thus, at pH 7 and 8 reaction proceeds mainly by diffusion of hypochlorous acid through the fibre, but for pH values greater than 8 the rate of reaction is greater than it would be if hypochlorous acid alone reacts.

Table 1. Comparison of observed half-life values with those calculated on the assumption that the reaction is due to hypochlorous acid alone

(Temp. 0°.)			
pH	HOCl (%)	$t_{0.5}$ (obs.) (min.)	$t_{0.5}$ (calc.) (min.)
6	97	3.1	3.1
7	67	4.5	4.5
8	23	12.0	13.0
9	3	43.0	100.0

The properties of the treated wool and the nature of the reaction products support the conclusion from this kinetic evidence that the reaction at pH 8 is controlled mainly by reaction with hypochlorous acid. Thus, reaction at pH 8, but not at pH 10, leads to the complete conversion of cystine to cysteic acid at pH 2 (Alexander, Hudson & Fox, 1950). The extent of damage to the fibre, measured by decrease in strength, solubility in alkali and removal of scale structure, is of the same order for samples treated

at pH 2 as at pH 8, whereas it is much less if the wool sample is chlorinated at pH 10. In addition, wool treated at pH 2 and 8 is resistant to shrinkage, but fabric treated at pH 10 felts almost to the same extent as an untreated sample (Alexander, 1950).

At pH 9-11 the solution is composed almost entirely of hypochlorite ions, but the rate of reaction decreases continually. In this pH range, therefore, reaction is due entirely to the hypochlorite ion and the rate is highly pH-dependent. This process is discussed further in the final section.

Effect of stirring

The change in rate with pH is reflected in the effect of stirring on reaction rate at various acidities. Thus above pH 8 the reaction is found to be almost independent of stirring, although below pH 8 the

Fig. 2. Effect of stirring on the rate of reaction ($t_{0.5}$) of Cl_2 solutions at pH 3 (A) and pH 10 (B); 10 g. Cl_2 /100 g. wool; temp. 0°.

rate is highly dependent on stirring (Fig. 2). Thus, if diffusion is the rate-determining step in alkaline solution, the diffusion of hypochlorite ions through the fibre must be slower than the diffusion in solution to the surface and also much slower than the diffusion of chlorine and hypochlorous acid through the fibre. It is observed that at pH 7 and 8, when reaction may be controlled by hypochlorous acid and hypochlorite ions, the rate is more dependent on stirring than at pH 9 but less so than at pH 2-6. In addition, with low concentrations (0.5% on weight of wool or 0.001N) at pH 10 the reaction becomes more dependent on stirring, showing that here the rate may be partly dependent on the rate of diffusion of ions to the surface.

Effect of concentration

Change in concentration affects the rate of reaction in different ways at pH 2 and 10 (Table 2). At pH 2 the rate suddenly decreases for concentrations less than 10% chlorine and remains con-

stant, which shows the change in mechanism from diffusion through the fibre to diffusion in solution.

Table 2. *Effect of concentration of chlorine on rate of reaction at various pH values at 0° and 600 rev./min. stirring*

pH	(Fibres, 1 g. in 150 ml. of solution.) Cl ₂ concn. (g./100 g. wool)					
	25	15	10	7	3	1
	<i>t</i> _{0.5} (min.)					
2	3.2	2.5	2.7	—	1.6	1.7
4	—	—	3.4	—	2.0	2.1
8	—	—	11.5	—	5.2	4.6
10	60.0	—	71.0	65	57.0	44.0

At pH 10, however, the half-life values (*t*_{0.5}) are approximately constant for concentrations greater

examination of the form of the rate curves for various concentrations leads to an explanation of this change in *t*_{0.5}. The rate curve at pH 10 (see Fig. 3B and 4A) consists of two distinct portions corresponding to an initial rapid reaction followed by a slower process which forms the major portion of the reaction. The range of the initial rapid reaction is seen to increase with decreasing concentration, and can be detected with difficulty with 10 % chlorine (Fig. 4A). This corresponds to an approximately constant quantity of chlorine, independent of initial concentration, and is most likely due to adsorption on and reaction with the surface and near surface of the fibres. From the curve for 0.5 % chlorine, assuming the value of 10⁴ sq.cm./g. for the surface area obtained from adsorption data (Rowen & Blaine, 1947) it is found that if the initial reaction is confined to the surface, each hypochlorite ion is accommodated in approx. 10 A.². This reasonable figure shows that the initial limited portion of the rate curves can be accounted for by reaction confined to the surface.

The major portion of the rate curve for concentrations greater than 1 % chlorine is found to follow a logarithmic law (Fig. 5; see also Discussion, p. 25),

Fig. 3. Rate curves for experiments at 600 rev./min. and 0° for the following treatments: A, 4 g. Cl₂/100 g. wool at pH 10 interrupted after 20 min.; B, 0.5 g. Cl₂/100 g. wool at pH 10 interrupted after 30 min.; C, 10 g. Cl₂/100 g. wool at pH 2 interrupted after 2 min.

Fig. 4. Rate curves at pH 10 with 10 g. Cl₂/100 g. wool at 600 rev./min. and 0°. A, untreated wool; B, pretreated with 10 g. Cl₂/100 g. wool at pH 2 (curve for pretreatment with 5 g. Cl₂/100 g. wool is identical); C, pretreated with KCN.

than 5 % chlorine, showing that the rate is proportional to the concentration. For lower concentrations the value of *t*_{0.5} decreases continuously showing that the rate of reaction becomes almost independent of the initial chlorine concentration. An

Fig. 5. Graphs of $\log [C_0/(C_0 - x)]$ against t at 0° for 1, 4 and 25 g. Cl₂/100 g. wool at pH 10; stirring at 600 rev./min. C_0 is the initial concentration of OCl⁻ ion and $(C_0 - x)$ the concentration at time t .

which shows that the rate of reaction is proportional to the hypochlorite concentration. In support of this it is found that the curves plotted in the form of percentage decomposition against time can almost be superimposed if allowance is made for the initial rapid reaction. It is, therefore, the influence of the initial surface reaction which causes the continual increase in *t*_{0.5} values with increasing concentration, reaching a constant value when the initial reaction is negligible.

Activation energies

Table 3 includes values of the apparent activation energies (*E*) for pH 9 and 10 calculated from the simple Arrhenius relation. For the more concentrated solutions, these values are calculated from *t*_{0.5} values, whereas in the case of the very dilute

solutions E was calculated from $t_{0.5}$ and from initial rates, identical values being obtained. This indicates that the initial reaction and the major reaction are subject to the same restrictive influences. The average value of 10.4 kg. cal./mol. is somewhat less than the corresponding value in acid solution (12.5), which is additional proof that different mechanisms operate in acid and in alkaline solution.

Table 3. *Experimental activation energies for reaction at pH 9 and 10*

pH	Cl (%)	Temp. (°)	$t_{0.5}$ (min.)	E (kg. cal./mol.)
9	0.5	0	11.6	10.9
		17	3.6	
	4.0	1.5	33.0	10.5
		16.9	12.0	
10	0.5	0	31.0	10.0
		19.5	8.9	
		25.9	6.1	
	4.0	0	60.0	10.4
		19.5	19.7	
		25.9	11.2	

Interrupted runs

In some experiments at pH 2 and 10, the piece of fabric was withdrawn from the solution after a given time and kept out of contact with the solution for sufficient time to allow any diffusion gradient to decrease considerably. The fabric was then rapidly immersed in the solution and the rate of reaction determined as before. If the reaction is controlled by diffusion in the fibre, the rate on continuation will be greater than the rate immediately prior to the interruption, due to the establishment of a large diffusion gradient. Such behaviour has been observed in several cases, e.g. the reaction between potassium permanganate with wool at pH 2 (Alexander & Hudson, 1949), anion exchange (Kunin & Myers, 1947) and cation exchange (Kressman & Kitchener, 1949). The results of similar experiments are shown in Fig. 3, in which typical examples at pH 2 and 10 are compared. It is noted that with 10% chlorine at pH 2 a definite break in the rate curve is obtained, thus providing further evidence that this reaction is controlled by diffusion through the fibre. At pH 10, however, no discontinuity is observed, even for an initial concentration of 0.5% chlorine, which is also the case in the oxidation of wool by potassium permanganate at pH 9.2. These observations show that in alkaline solution the rate of oxidation is not controlled by diffusion through the fibre treated as a homogeneous medium.

Preliminary treatments

Significant results were obtained by pretreating the fibres with a variety of reagents known to modify various groups in the wool. Thus, treatment with

boiling potassium cyanide solution increases the rate of reaction with hypochlorite solution at pH 10 considerably (Fig. 1). Although the rate is greatly increased at pH 9 and 10, it is unaffected by pretreatments at pH 2, 4 and 6, which also supports the conclusion that the rate in acid solution is controlled by diffusion through the fibres. The increase in rate at pH 9 and 10 is not due specifically to the removal of cystine, as it is observed that acid hydrolysis without attack on disulphide bond increases the rate (see Table 4).

Table 4. *Effect of various pretreatments on the subsequent rate of chlorination with 10% chlorine at 0° and pH 10*

(Pretreatment at room temperature unless otherwise stated.)

Treatment	$t_{0.5}$ (min.)
Untreated	71
N-HCl, 80° for 8 hr.	5.2
5% Cl_2 , pH 2	10.4
10% Cl_2 , pH 2	10.0
KOH in butanol for 30 sec.	64.0

In addition, it is known that chlorine at pH 2 also reacts with the amino groups of the fibre, and eventually with the polypeptide chain. Treatment with this reagent increases the rate of subsequent chlorination at pH 10 considerably. Finally, experiments with potassium permanganate at pH 1 and 9.2 show that even mild treatments in acid solution increase the rate of reaction with sodium hypochlorite solution at pH 10. Pretreatment with alkaline permanganate solution, however, has no effect on the rate (see Table 5). Although reaction

Table 5. *Effect of pretreatment with potassium permanganate solutions at 25° on the subsequent rate of chlorination with 10% chlorine at pH 10 and 25°*

KMnO ₄ concentration (g./100 ml.)	Time of pretreatment	$t_{0.5}$ (min.)
Solution containing N-H ₂ SO ₄		
0	0	14
0.1	2 min.	10
0.1	10 min.	4
0.5	1 min.	4
0.5	5 min.	4
1.0	10 sec.	6
1.0	1 min.	4.5
Solution at pH 9.2 (borax buffer)		
0.1	1 hr.	15
0.1	18 hr.	13
0.5	5 min.	15
0.5	30 min.	11

with permanganate at pH 1 is extensive, only 25–30% of the total cystine can be oxidized (Alexander, Hudson & Fox, 1950) so that modifi-

cation of the disulphide bond alone is not responsible for these greatly increased rates. Pretreatment with ethanolic potassium hydroxide, which was shown by Lindberg, Philip & Gralén (1948) to remove the very thin outermost layer of the wool (the epicuticle), does not influence the rate of alkaline chlorination (Table 4).

DISCUSSION

The experimental data presented here and in previous papers shows that the rate-determining process of the reaction of wool with oxidizing agents in acid solution is the diffusion of molecules through the solid to reaction sites, accompanied by rapid reaction. The penetration of diffusing molecules is thought to proceed through channels of molecular dimensions containing solvent, and to be retarded by the molecular chains of the fibre leading to a high activation energy of the process. Although the amphoteric nature of protein fibres results in changes in the molecular structure and distribution of electrical charges within the fibre with change in pH of the solution, the kinetics of reaction at pH 2–7 can be interpreted as isotropic diffusion through a uniform gel.

The rate of reaction decreases continually from pH 8 to 11, although the available chlorine exists almost totally as hypochlorite ions. This decrease in rate is accompanied by an increase in neutralization of amino groups (approx. 25 % neutralized in the presence of 0.1M-electrolyte, the buffer concentration used at pH 11; see Steinhardt & Harris, 1940), which suggests that the reduced rate is due to electrical repulsion between negative carboxyl ions and hypochlorite ions diffusing within the fibre. The experimental results, discussed in the previous section, show that the rate is not controlled by diffusion through a uniform medium. Most of the kinetic data would be explained if the rate in alkaline solution were governed by the actual chemical reaction. In this case the rate would probably be of the first order so long as the wool was in considerable excess; the rate would be independent of stirring; no breaks in the interrupted curves would be expected; and the energy of activation could well be less than that of the fibre diffusion-controlled process at pH 2. This possibility is not substantiated by the following considerations. The kinetics of oxidation with alkaline permanganate solution are very similar to the kinetics of alkaline chlorination. Thus, on changing the pH from 2 to 9.2, the rate of reaction is greatly reduced, and becomes almost independent of stirring. The kinetic curve changes from parabolic to logarithmic, and in interrupted treatments there is no discontinuity at pH 9.2, although this is very marked at pH 2. This close similarity between the effects of the two dissimilar oxidizing agents suggests that the rate is not controlled by chemical

decomposition. Moreover, the two reagents react quite differently with wool both in acid and in alkaline solution (Alexander, Hudson & Fox, 1950), e.g. alkaline permanganate does not react with the disulphide bond, unlike alkaline hypochlorite.

The data on the rate of alkaline chlorination after the various pretreatments are difficult to interpret if the rate is governed by chemical reaction. Thus, if the increased rate is due to general degradation with an increased number of available sites for reaction, the effect of a pretreatment at pH 2 with 10 % chlorine (when 43 % cystine has reacted) would be expected to be greater than that with 5 % chlorine (when 22 % cystine has reacted). The results in Table 4 show that this is not the case. Similarly, both a mild and a severe acid permanganate pretreatment accelerate a subsequent alkaline chlorination to the same extent. This indicates that modification of the outermost portion of the fibres is a major factor influencing the rate, and moreover the observed kinetics could be explained if the rate-controlling step were diffusion through a surface obstruction. Recent researches on the morphology of the wool fibre have established the presence of a membrane near the surface which indicates the validity of such a treatment.

Various workers (cf. Zahn, 1950) have produced evidence for the existence of a continuous tube-like membrane ('subcuticle') extending the whole length of the fibre, and variously estimated as representing 2–5 % of the weight of the wool fibre. As a result of microscopic examination, the material was thought to be located in the fibre between the outer scales and the cortical cells. However, the methods of isolation involved severe degradation and its identification as a specific unit within the fibre was doubtful. Lindberg *et al.* (1948) have detected, with the aid of the electron microscope, a thin layer about 100 Å. thick (i.e. approximately 0.1 % of the wool as a whole) on the surface of the fibre and called this the 'epicuticle'. The epicuticle, which is probably fatty material, can be largely removed by prolonged ether extraction, or more satisfactorily by rapid immersion in potassium hydroxide in white spirit. Removal of the epicuticle increases the rate of adsorption of acids, although it is shown in the previous section that the rate of the alkaline hypochlorite reaction is not affected by the potassium hydroxide treatment. The epicuticle does not, therefore, affect the rate of penetration of hypochlorite ions and removal of the epicuticle is not responsible for the increased rate after various pretreatments.

Mercer, Lindberg & Philip (1949) questioned the existence of the subcuticle as isolated by Zahn and others, and believe that the tubes were a mixture of epicuticle and underlying scales in varying degrees of degradation. The existence of a protein component of increased chemical resistance, representing 7–10%

of the wool in the form of a tube similar to the subcuticle of Zahn (1950), was established by Alexander & Earland (1950) as follows. The fibres were treated with peracetic acid, which reacts in wool almost exclusively with cystine until all the cystine had been oxidized. On subsequent treatment with ammonia the major part of the fibres dissolved, leaving a 7–10% residue in the form of cylindrical tubes identical in external form with the original fibres. An X-ray photograph of the soluble protein in powder form, obtained by precipitation from the ammoniacal solution with dilute acid, shows this to be in the α -configuration, whereas the insoluble tubes give a β -picture (Alexander, Earland & Happey, 1950). The β -fold provided a much stronger structure than the α -material because of the closer packing and better inter-chain hydrogen bonding, and probably protects the elastic interior of the fibre.

The position of this membrane with respect to the scales cannot be determined from visual and electron microscopic observation and the term 'subcuticle' may well be misleading. It is certain, however, that it represents the major part of the highly complex cuticle and it seems possible that it is a skin below the scales which surrounds the cortex. For the purpose of interpreting the kinetics of oxidation with alkaline chlorine and alkaline permanganate solutions, the fibres will be treated as a homogeneous gel surrounded by a resistant membrane. That the resistant membrane lies close to the fibre surface is shown by the observation (see p. 23) that the preliminary rapid surface reaction constitutes only about 1% of the total possible reaction.

If the hypochlorite ions do not modify the membrane during the course of the reaction, the rate of transport P across the membrane of thickness l and area of membrane permeable to hypochlorite ions A_m is given by

$$P = A_m D/l (C_i - C_m),$$

where C_i and C_m are the concentrations of free hypochlorite ions on either side of the membrane and D the diffusion coefficient. If it is assumed that diffusion and reaction within the fibre are rapid processes (i.e. $C_m \sim 0$) and C_i is equal to the hypochlorite ion concentration in solution, then

$$D = \frac{lV}{A_m t} \ln \frac{C_0}{C_0 - x},$$

where V is the volume of solution, C_0 the initial concentration and $(C_0 - x)$ the concentration at time t . This logarithmic relation between time and concentration is obeyed approximately in alkaline solutions (Fig. 3). In a process of this type, no discontinuity in the rate curve would occur on interrupting a reaction.

Damaging or opening the structure of the membrane must lead to an increase in the rate of reaction

with alkaline hypochlorite as the available area permeable to the ions A_m is increased, which explains the effect of the different pretreatments. The prevention of felting shrinkage in wool by oxidizing agents is caused by disruption of the cuticle which need not necessarily be removed (Speakman & Goodings, 1926; Alexander, 1950). It would appear more than a coincidence that acid chlorine and acid permanganate solutions, which both effectively reduce the tendency of wool to felt, are effective in increasing the rate of reaction of alkaline hypochlorite, unlike alkaline permanganate and, of course, alkaline hypochlorite itself, which do not prevent felting (Alexander, Carter & Hudson, 1949).

Pretreatment with potassium cyanide, leading to the rupture of all the disulphide bonds, and partial hydrolysis both open up the membrane, thus rendering it more permeable, and hence increase the rate of reaction with alkaline hypochlorite. In acid solution chlorine initially attacks the membrane which then no longer restrains the diffusion, so that movement within the bulk of the fibre becomes the rate-controlling step. As the retardation of the membrane is due to its electrical charge which repels like charged ions, the preliminary diffusion of uncharged molecules such as chlorine and hypochlorous acid is not reduced by the presence of the membrane, so that diffusion appears to proceed through a homogeneous medium.

The driving force of the diffusion process is the concentration gradient of the hypochlorite ions, which is maintained at a high value by the complete removal on one side of the membrane due to chemical reaction. The negative membrane potential (Teorell, 1949), which cannot be measured directly, opposes the diffusion, but remains approximately constant as the solution in contact is maintained at constant pH during the reaction. In addition, chloride ions resulting from the reduction of the hypochlorite ions diffuse out at the same rate as the latter diffuse in and therefore no accumulation of ions can modify the charge on the membrane.

The continual decrease in the rate of chlorination with increasing pH after the ionization of hypochlorite is complete shows that the retarding influence of the membrane is due to electrical interaction between diffusing anions and the charged carboxyl groups in the membrane. As the pH is increased the amino groups become unionized (i.e. OH^- ions are combined) and the fibre becomes negatively charged by the carboxyl groups which had previously formed part of a salt link. There is some evidence (Alexander & Kitchener, 1950) that the outside groups of the fibre titrate preferentially, so that the charge on the membrane at a given pH may be greater than is indicated from the overall titration curve.

The individual negative charges within the fibre do not extend over great distances as they are neutralized by an electrical double layer containing an excess of cations (e.g. Na^+). The fibre is therefore freely permeable to anions so long as the number of free carboxyl groups is small, as is shown by the fact that with cyanide-treated wool, in which the membrane has been opened up, the rate of chlorination is the same at pH 8 as at pH 2. At pH 10 electrical interaction between the anions and the wool begins to be detectable and the rate of chlorination of cyanide-treated wool decreases.

SUMMARY

1. The kinetics of the reaction of wool with aqueous chlorine solution of pH less than 8 are explained by the uniform diffusion of chlorine or hypochlorous acid through the swollen fibres. It has been shown that this process is not rate-determining in solutions of pH greater than 8.

2. The form of the kinetic curve, the effect of various pretreatments on the rate, and the temperature coefficient of the process, all show that the alkaline reaction is not controlled by the actual oxidation process, but can be interpreted in terms of diffusion across a surface barrier.

3. Recent morphological researches on wool fibres have established the existence of two membranes, one of which, the epicuticle, is situated on the extreme outer surface, whilst the other forms part of the cuticle and is referred to as the subcuticle. It is shown that the presence of the epicuticle does not affect the rate of diffusion of the hypochlorite ion. In alkaline solutions the negatively charged subcuticle retards the penetration of anions, so that diffusion through this membrane determines the overall rate of reaction.

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The Biosynthesis of Choline Methyl Groups by the Rat

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It has been known for some time that the supply of choline or betaine to a methionine-free diet enables the white rat to use homocystine as the sole sulphur-containing amino-acid for growth purposes (du Vigneaud, Chandler, Moyer & Keppel, 1939). This effect has been shown to be due to the utilization of the methyl groups of choline for the biological synthesis of methionine from homocystine (Simmonds, Cohn, Chandler & du Vigneaud, 1943). The reverse reaction, i.e. the transfer of the methyl group

of methionine to choline, also occurs (du Vigneaud, Cohn, Chandler, Schenk & Simmonds, 1941). The mechanism of the transfer of labile methyl groups has been elucidated by feeding methionine containing deuterium and ^{14}C in the methyl group. The choline methyl groups were found to contain both isotopic tracers in the same ratio as in the methionine fed, demonstrating the transfer of the methyl group as such (Keller, Rachele & du Vigneaud, 1949).