although the prior formation of an unstable intermediate is not excluded.

4. The poor conversion of esters of D-galacturonic acid into compounds of L-galactonic acid appears to be due mainly to the reversible inhibition of the enzyme during the course of the reaction, and to a lesser extent to the instability of the galactonic acid derivative formed. The substance responsible for the inhibition of the enzyme has not been identified.

5. The low affinity of the enzyme for methyl D-galacturonate (K_m , 10^{-2} M) suggests that this is not the naturally occurring substrate.

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Further Studies on the Reactions of Disulphides with Sodium Sulphite

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In a previous paper (Cecil & McPhee, 1955*b*) the reactions of oxidized glutathione, cystine and some cystine derivatives were described. It was found that SO_3^{2-} ions react according to the equation

but that HSO_3^- ions do not react. Since the reaction with R.S^- is reversible (Footner & Smiles, 1925) the equilibrium

has also to be taken into account. It was found that the negatively charged disulphides react much more slowly than do those with no net charge. Thus with oxidized glutathione and cystine there is an optimum pH at which the observed rate of reaction is maximal. Below the optimum the observed rate falls off because of decreasing SO_3^{2-} concentration, and above it because of increasing negative charge on the disulphide molecule due to dissociation of the amino groups.

This paper describes similar studies on a number of other compounds, including homocystine and two positively charged compounds, bis-(β -aminoethyl)

disulphide (cystamine) and bis-(γ -aminopropyl) disulphide (homocystamine). It has been found, as was expected from the previous results, that the positively charged compounds react much more rapidly than those with no net charge. Homocystine and its derivatives react more slowly than the corresponding cystine derivatives, but otherwise show the same general behaviour. The application of this work to the disulphide groups of proteins is discussed.

The theoretical descriptions of disulphide-sulphite reaction and equilibrium mixtures have been given in detail (Cecil & McPhee, 1955*b*), and the same notation will be used here.

EXPERIMENTAL

Reagents

Cystamine. A sample of the dihydrochloride was kindly supplied by Horlicks Ltd., Slough, Bucks. (Found: Cl, 30.7; $\text{C}_4\text{H}_{14}\text{N}_2\text{S}_2\text{Cl}_2$ requires Cl, 31.6%). Standard solutions of the dinitrate were made by adding the calculated amount of AgNO_3 to solutions of the hydrochloride in 0.1N- HNO_3 , filtering off the AgCl and making the filtrates up to the required volumes.

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Cystine hydantoin. This was prepared from cystine by the method of Hess (1934). Standard solutions were made up in 0.01 N-HNO₃.

Homocystine hydantoin. This was prepared from homocystine by the method of Karabinos & Szabo (1944), m.p. 201–202° (corr).

Diformylhomocystine. This was prepared from homocystine by the method described by Fruton & Clarke (1934) for diformylcystine. (Found, C, 37.2; H, 4.9; N, 8.7; C₁₀H₁₄O₄N₄S₂ requires C, 37.1; H, 4.9; N, 8.6%.)

Homocystamine. Homocystamine dihydrobromide was prepared by the method of Schöberl & Kawohl (private communication from Professor A. Schöberl; also Kawohl, 1953). Standard solutions of the dinirate were made up as described for cystamine.

Methods

The other reagents used and the experimental details of the analytical method and kinetic experiments have been described previously (Cecil & McPhee, 1955*a, b*). Briefly, kinetic experiments were carried out in buffered solutions over the pH range 4–11.5, samples were withdrawn at measured time intervals and the reactions stopped by bringing the pH to 1.8–2.2 with HNO₃ or H₂SO₄. The thiol concentrations were then found by potentiometric titration with AgNO₃.

The disulphide concentrations used were between 0.4 and 1.5 mM, and the sulphite concentration was varied from approximately equimolar with disulphide to a 40-fold molar excess.

Na₂SO₃ was used as its own buffer whenever possible, i.e. when the sulphite was present in greater than 10-fold excess over disulphide, in the pH range 6.0–7.4. Other buffers used were acetate, phosphate, borate and carbonate.

Determination of dissociation constants. Samples of carbonate-free solutions of cystine hydantoin, homocystine hydantoin and cystamine were titrated potentiometrically with standard carbonate-free NaOH solution in a closed titration flask, with a Cambridge Alki glass electrode. Oxygen-free N₂ was bubbled through continually and served to keep the solution stirred. The glass electrode was standardized against a hydrogen electrode.

RESULTS

Estimation of the disulphides

The methods of estimation for homocystine and five other disulphides (by allowing them to react with Na₂SO₃ and titrating the thiol formed with AgNO₃)

have been reported (Cecil & McPhee, 1955*a*), and methods for five more disulphides are summarized in Table 1. The corresponding thiols have not been studied as pure substances but only as disulphide-sulphite reaction mixtures. No errors were found for the titrations in acid solution of β -aminoethanethiol (cysteamine), (which was titrated in H₂SO₄, not HNO₃, as the latter medium gave flat, asymmetric titration curves), γ -aminopropanethiol (homocysteamine) or cysteine hydantoin. With homocystine hydantoin, errors which varied slightly with pH occurred below pH 4.3. In the range of pH 1.9–2.3 the error was +3% and was constant over the range of thiol and buffer concentrations used. Similarly, *N*-formylhomocystine gave a +1% error over the pH range 2.0–2.3. This error varied considerably with pH, rising to +10% at pH 3.2 and 8% at pH 1.75. It was unchanged in the presence of borate or carbonate. As before (Cecil & McPhee, 1955*b*), correction factors were applied to the titrations done during kinetic experiments.

Homocystine showed unusual behaviour in acid solution, the titration error varying continuously with pH and sulphite concentration. With a 20-fold molar excess of sulphite over thiol (in solutions acidified with HNO₃), the titration error varied from +10% at pH 1.7 to +2% at pH 2.3. At pH 2.2 the error varied from 0 when the sulphite excess was less than fivefold to +12% when the excess was 100-fold. Thus a series of control titrations with homocystine at an identical titration pH had to be carried out after each kinetic experiment to find the correction factors to be used; these varied in any one experiment since the sulphite:thiol ratio varied continuously. The errors were of the order of 3–10%, being 1–2% higher in the presence of borate or carbonate than when the salts were absent.

Dissociation constants of groups in the disulphide molecules

The dissociation constants of cystamine and homocystine hydantoin were calculated from the titration data by the method of Simms (1926). With

Table 1. *Estimation of disulphides*

Temperature of reaction was 60° for diformylhomocystine; the other compounds were allowed to react at room temp. In each case the titration with AgNO₃ was done at the reaction pH.

Substance	Reaction conditions with Na ₂ SO ₃			Titres (% of weighed concn.)
	Moles of Na ₂ SO ₃ /mole of disulphide	pH	Time allowed for reaction (min.)	
Cystamine	10–20	6.5–7.0	<1	97
Homocystamine	10–20	6.5–7.0	<1	97
Cystine hydantoin	15–25	6.5–7.0	<1	100
Homocystine hydantoin	20–30	6.5–7.0	10	99
Diformylhomocystine	30–40	7.0–7.5	30	100

Table 2. *Alkaline dissociation constants of the disulphides*

Temp.	Cystamine		Homocystine*		Homocystine hydantoin	
	pK'_1	pK'_2	pK'_3	pK'_4	pK'_1	pK'_2
25°	8.6	9.5	8.5	9.4	8.7	9.7
0	9.3	10.2	9.2	10.1	9.1	10.1

* From the data of Rykjan & Schmidt (1944).

his notation, the dissociation constants at 20° of homocystine hydantoin were found to be pK'_1 8.65, pK'_2 9.68, and of cystamine pK'_1 8.69, pK'_2 9.61. The dissociation constants of cystine hydantoin could not be determined, since this compound began to decompose above about pH 9 at 20°, and a titration curve could not be obtained. pK_1 was approximately 8.1.

The dissociation constants used in the calculations to be described are given in Table 2. It was assumed that NH_3^+ groups have a heat of ionization of +11 000 cal. (Cohn & Edsall, 1943) and that the hydantoin ring structure has a heat of ionization similar to that of imidazole compounds, which is approximately +7000 cal. (Cohn & Edsall, 1943).

Reaction of diformylhomocystine

The pK values of the carboxyl groups of diformylhomocystine would be expected to be similar to those of dithiodipropionic acid and diformylcystine (i.e. less than about 5.5), so that kinetic experiments at pH 6.5 refer, within the experimental limits, to the completely ionized form.

Above pH 9, the reaction is reversible and all the sulphite is in the form of SO_3^{2-} . Rate constants (k_a) for the forward reactions between ionized diformylhomocystine and SO_3^{2-} ions at 0° and 25° were found from experiments in the pH range 9.2–11.2; at 0°, $k_a = 0.57 \pm 0.04$ l. mole⁻¹/min.⁻¹ and at 25°, $k_a = 3.9 \pm 0.2$ l. mole⁻¹/min.⁻¹. These figures are the means and standard deviations of at least sixteen determinations of k_a from a minimum of four kinetic experiments. Rate constants from experiments in borate and carbonate buffers were the same within experimental error.

The reaction is complex below pH 9, when the equilibrium $HSO_3^- \rightleftharpoons H^+ + SO_3^{2-}$ must also be considered, and apparent initial rate constants were estimated as before (Cecil & McPhee, 1955*b*). It was again found that the rate constant for reaction with HSO_3^- ions was negligible, and that the change in apparent rate constant with pH was described by the equation

$$k' = \frac{k_2 K_\phi}{K_\phi + [H^+]}, \quad (1)$$

where k' is the apparent rate constant at a given hydrogen-ion concentration, k_2 is the rate constant for reaction with SO_3^{2-} ions (i.e. k_a above) and K_ϕ is

Table 3. *Reaction of diformylhomocystine with sodium sulphite at 25° and pH below 9*

pH	Extrapolated value for apparent rate constant	
	k' (l. mole ⁻¹ min. ⁻¹)	pK_ϕ calc.
6.45	1.3	6.75
6.85	2.0	6.83
7.20	2.8	6.80

the dissociation constant of HSO_3^- . Some results are given in Table 3, a mean value for pK_ϕ of 6.8 again being found from equation (1) from the experimental values of k' and k_2 .

From measurements of the equilibrium concentrations of thiol in reaction mixtures at pH values below 9, the results in Table 4 were calculated as shown previously (Cecil & McPhee, 1955*b*). As with the three other thiol compounds studied, the SH group of *N*-formylhomocystine has a heat of ionization of approximately +6000 cal./mole⁻¹.

Reactions of homocystine, homocystine hydantoin and cystine hydantoin

The kinetic expressions applying to these reactions, which are complicated by the presence in the disulphide molecules of groups whose state of ionization change in the pH range used, have been derived (Cecil & McPhee, 1955*b*). The rate constants for the reactions with SO_3^{2-} ions (k_a) of the zwitterion form of homocystine and the uncharged forms of homocystine hydantoin and cystine hydantoin were calculated from equation (1), by taking the value of K_ϕ (1.6×10^{-7}) found in experiments with other disulphides and using the apparent rate constants (k') measured in the pH range 4.8–7.0. The results are summarized in Table 5, and the mean values for k_2 are given in Table 9.

Above pH 9, the apparent rate constants (k'') of the forward reactions with SO_3^{2-} ions decrease as the pH is increased, the results for homocystine and homocystine hydantoin being shown in Table 6. (Kinetic experiments with cystine hydantoin could not be done at pH values above 9 owing to decomposition of the disulphide.) In both series of experiments, the results in borate were consistent with those in carbonate, as shown by the small range of values calculated for the forms of net charge -1

Table 4. *Results from equilibrium studies on the reaction of diformylhomocystine with sodium sulphite*

Temp.	<i>I</i>	$\text{R.S.S.R} + \text{SO}_3^{2-} \xrightleftharpoons{K_1} \text{R.S}^- + \text{R.S.SO}_3^-$		ΔG° (cal. mole ⁻¹)	ΔS° (cal. mole ⁻¹ deg. ⁻¹)
		$10^2 K_1$	ΔH° (cal. mole ⁻¹)		
0°	0.09–0.11	0.41 ± 0.01	+7100	+2600	+15
25	0.08–0.11	1.23 ± 0.02	—	—	—
$\text{R.SH} \rightleftharpoons \text{R.S}^- + \text{H}^+$					
		pK_{SH}			
0	0.09–0.11	10.37 ± 0.03	+5800	+13 600	–26
25	0.08–0.11	9.98 ± 0.02	—	—	—

Figures given in the third column are the means and standard deviations of at least six calculated values.

Table 5. *Reactions of homocystine, homocystine hydantoin and cystine hydantoin with sodium sulphite at pH below 9*

Substance	Temp.	pH	Extrapolated value for apparent rate constant, k'	Rate constants with SO_3^{2-} , k_2
			(l. mole ⁻¹ min. ⁻¹)	(l. mole ⁻¹ min. ⁻¹)
Homocystine	0°	4.85	0.2	18
	0	6.42	5.5	18
	0	6.63	6.5	16
	0	6.92	11	19
	25	4.88	2	167
	25	5.63	11	172
	25	5.98	20	152
	25	6.42	40	136
	25	6.63	60	150
	25	6.63	60	150
Homocystine hydantoin	0	5.26	0.2	7
	0	5.84	0.7	7
	25	4.91	0.75	58
	25	5.38	2	54
	25	5.49	2.5	54
	25	6.22	12	57
Cystine hydantoin	0	5.30	1.3	42
	0	6.32	10	40
	25	4.79	3	304
	25	4.92	4	306
	25	5.51	14	285
	25	5.80	25	274

Table 6. *Reactions of homocystine and homocystine hydantoin with sodium sulphite at pH values above 9*

Substance	Temp.	pH	Apparent rate constant, k'
			(l. mole ⁻¹ min. ⁻¹)
Homocystine	0°	9.30	8.0
	0	9.96	2.4
	0	10.41	0.75
	0	11.12	0.45
	25	9.20	36.0
	25	9.70	14.6
	25	10.10	5.3
	25	11.10	2.3
	25	11.10	2.3
Homocystine hydantoin	0	10.04	1.5
	0	10.80	0.86
	25	9.20	20.5
	25	9.83	9.1
	25	10.32	6.8
	25	11.07	5.0

Table 7. *Reactions of cystamine and homocystamine with sodium sulphite in acetate buffers at I 0.03*

Substance	Temp.	pH	Extrapolated value for apparent rate constant, k' (l. mole ⁻¹ min. ⁻¹)	Rate constants with SO_3^{2-} , k_2 (l. mole ⁻¹ min. ⁻¹)
Cystamine	0°	4.58	4	660
	0	4.75	5.5	620
	0	5.32	20	620
	25	4.56	40	6900
	25	4.58	40	6800
	25	4.68	50	6600
	25	5.39	250	6600
Homocystamine	0	4.76	2	220
	0	5.08	4	210
	0	5.40	9	230
	0	5.53	11	215
	25	4.52	14	2700
	25	4.62	17	2600
	25	5.05	45	2600
	25	5.21	60	2400

which are given in Table 9. (These were calculated from the experiments in Table 6.) The rate constants for the forms of net charge -2 which were found from experiments in carbonate buffers are also given in Table 9.

Experiments were not done at high enough pH values for the equilibrium constants for the reactions of homocystine and homocystine hydantoin (in forms of net charge -2) with SO_3^{2-} ions to be found.

Reactions of cystamine and homocystamine

The rate constants for the reactions of cystamine (net charge +2) and homocystamine (net charge +2) with SO_3^{2-} ions were found from experiments in acetate buffer (*I* 0.03) over the pH range 4.5-5.5, K_a being taken to be 1.6×10^{-7} as above. The results are shown in Table 7, the mean k_2 values being given in Table 9.

The presence of buffer salts markedly affects the rates of these two reactions. Increasing the ionic strength lowers the rate constants for the reactions, and also phosphate, borate and carbonate inhibit the reaction. It has been found (Cecil & McPhee, 1955b) that rate constants for the cystine SO_3^{2-} reaction were lower in carbonate than borate, but a similar effect has not been observed with other compounds except the two now being discussed. The inhibitory effect of phosphate is shown by the fact that a value for k_2 of 3900 l. mole⁻¹ min.⁻¹ was found at *I* 0.03 in phosphate, compared with 6700 l. mole⁻¹ min.⁻¹ in acetate at the same ionic strength (Tables 7 and 8). The effect of ionic strength is illustrated by experiments in phosphate, when increasing the ionic strength from 0.03 to 0.05 lowered k_2 from 3900 to about 2400 l. mole⁻¹ min.⁻¹ (Table 8); also by an experiment done in acetate, with the ionic strength brought to 0.10 by the addition of sulphate, k_2 then being 1800 l. mole⁻¹

Table 8. *Reaction of cystamine with sodium sulphite in various buffers at 25°*

pH	<i>I</i>	Extrapolated value for apparent rate constant, k' (l. mole ⁻¹ min. ⁻¹)	Rate constants with SO_3^{2-} , k_2 (l. mole ⁻¹ min. ⁻¹)
Phosphate buffer			
6.17	0.05	450	2300
6.17	0.03	750	3900
6.46	0.05	800	2500
Acetate with sulphate added			
5.32	0.10	57	1800
Borate buffer Apparent rate constant k'' (l. mole ⁻¹ min. ⁻¹)			
9.20	0.06	630	
9.55	0.09	180	
9.90	0.10	100	
Carbonate buffer			
9.41	0.06	96	
9.91	0.07	77	
10.49	0.09	37	

min.⁻¹. The k'' values found at pH values above 9 in borate and carbonate are too low for calculations of the rate constants for the various charged forms of cystamine to be made. Negative values for these rate constants were obtained even when a value of about 2000 l. mole⁻¹ min.⁻¹ was used for the rate constant for the form of net charge +2. Experiments shown in Table 8 also indicate that, as with cystine, reaction is slower in carbonate than borate; for example, at pH 9.9, k'' is 100 l. mole⁻¹ min.⁻¹ in borate, but is 77 l. mole⁻¹ min.⁻¹ in carbonate.

Homocystamine showed the same general behaviour as cystamine.

DISCUSSION

Table 9 summarizes the kinetic data so far obtained on the reactions of disulphides with SO_3^{2-} ions, the influence of the net charge of the disulphide molecules on the rate constants being clearly demonstrated. Homocystine and its derivatives react more slowly than the corresponding cystine compounds, the largest differences being between the rate constants for the zwitterion forms of homocystine and cystine, which differ by a factor of about 7. However, the values of k_2 for all the homocystine derivatives are of the same order of magnitude as for the corresponding cystine derivatives, and it is clear that the most important factor governing the rates of reaction of disulphide bonds with SO_3^{2-} ions is the net charge in the immediate vicinity of the bonds. Total charge also influences the rate constants to a certain extent, uncharged cystine derivatives (diacetylcystine ester and the uncharged form of cystine hydantoin) reacting more closely than the zwitterion form of cystine; similarly, the uncharged form of homocystine hydantoin reacts more slowly than the zwitterion form of homocystine.

The work of Schöberl & Ludwig (1937) and Kassel & Brand (1938) is in qualitative agreement

with that presented here, except for the reaction of cystamine with sulphite. They found that cystamine reacted more slowly than cystine, although Kassel & Brand (1938) observed that cystine ester did react more rapidly than cystine. Much higher ionic strengths were used in both these sets of experiments than in ours, which is a possible reason for the different results.

It is significant that the reactions of simple disulphides with potassium cyanide follow the same general pattern as the reactions with sulphite (Schöberl & Ludwig, 1937). The reacting species is almost certainly CN^- ions, and reactivity will be governed by charged groups in the disulphide molecules, although the variations in rate constants might be expected to be less than is the case with divalent SO_3^{2-} ions. Similarly, the reduction of disulphide bonds by thiol compounds involves, in alkaline solution at least, the attack of RS^- ions, so that variations in reactivity of disulphide bonds toward, say, alkaline thioglycollate (Walker, 1925) could be due to nearby charged groups. Coulomb forces will also influence the reactivity of disulphides toward OH^- ions (Tarbell & Harnish, 1951).

This work has been done at relatively low ionic strengths ($I < 0.15$) where Coulomb forces exert their maximum effect. At high ionic strengths the

Table 9. Summary of kinetic data

Substance	Rate constants with SO_3^{2-} , k_2 (l. mole ⁻¹ min. ⁻¹)		Net charge on molecule	Activation energy, E_a (cal.)	Frequency factor, pZ_0 , at 25°, (l. mole ⁻¹ sec. ⁻¹)	Entropy of activation, $\Delta S^\ddagger$ (cal. mole ⁻¹ deg. ⁻¹)
	(25°)	(0°)				
Cystamine	6700†	630†	+2	15 300	1.6×10^{13}	+1
Diacetylcystine (ester)	265	37	0	12 800	1×10^{10}	-14
Cystine hydantoin	290	40	0	12 800	9×10^9	-14
Diformylcystine (ionized)	9	1.5	-2	11 500	4×10^7	-25
Dithiodipropionic acid (ionized)	1.1	0.13	-2	13 500	9×10^7	-24
Cystine‡						
2+, 2-	1100	140	0	13 300	9×10^{10}	-10
1+, 2-	(5-70)	(0.05-5)	-1	—	—	—
0+, 2-	3§	0.5§	-2	11 600	2×10^7	-27
Homocystamine	2600†	220†	+2	16 000	1.2×10^{13}	+1
Homocystine hydantoin	56	7	0	13 500	6×10^{10}	-10
	(12-15)	(1.5-2)	-1	—	—	—
	4.5§	0.7§	-2	12 000	4×10^7	-24
Diformylhomocystine (ionized)	3.9	0.57	-2	12 400	7×10^7	-24
Homocystine‡						
2+, 2-	155	18	0	13 900	3×10^{10}	—
1+, 2-	(20-30)	(1-1.5)	-1	—	—	—
0+, 2-	2.0§	0.4§	-2	10 400	1.3×10^6	-32
Oxidized glutathione‡						
2+, 4-	160	22	-2	12 800	6×10^9	-15
1+, 4-	(40-50)	(2-4)	-3	—	—	—
0+, 4-	10§	2§	-4	10 400	4×10^6	-29

† Values for k_2 in acetate buffers at I 0.03.

‡ Numbers of positive and negative charges/molecule in different states of ionization.

§ Values for k_2 in carbonate buffers.

differences in rate constants may not be so large, but the overall effects observed are so great that the differences would be expected still to exist.

Application to proteins. The variations in rates observed are large enough to show up as qualitative differences for disulphide bonds in proteins. For example, with a 20-fold molar excess of sulphite over disulphide at pH 7 at room temperature, the cystamine reaction is complete in less than 1 min., the cystine reaction is complete in 15 min., but the diformylcystine reaction takes over 30 hr. to reach completion. Thus under a standard set of conditions, protein disulphide bonds which had either no net charge or a net positive charge in their immediate environment would react readily with sulphite, whereas those associated with net negative charges would appear not to react. The pH range normally used in the study of proteins is about 4–6, where negative charges would be due to CO_2^- groups, but at higher pH values ionization of tyrosine OH groups would be expected to influence the rates of reaction of neighbouring disulphide bonds. Lindley & Phillips (1947) have suggested that the variations in reactivity of disulphide bonds in wool toward sulphite could be due to their environments, the reactive bonds being associated with carboxyl and acid amide side-chains, and the unreactive bonds having non-polar environments. However, the present work indicates that nearby carboxyl groups would make disulphide bonds unreactive toward sulphite, a non-polar environment allowing them to react readily unless steric hindrance were the limiting factor.

It is interesting that all the disulphide bonds of wool can react with oxidizing agents which are neutral molecules, but that only about 30 % of the total will react if the oxidizing agents are negative ions (Alexander, Hudson & Fox, 1950). Further, the rate of reaction with MnO_4^- ions decreases with increasing pH, so that it appears that charged protein groups play some part in these reactions.

When an ionic reactant is involved, it might be anticipated that Coulomb forces from nearby charged groups would greatly influence the rates of reaction of protein functional groups. However, the effect has rarely been considered in protein chemistry and the possible magnitude of such effects in typical reactions used for studying proteins does not appear to have been previously demonstrated. Similar studies on characteristic reactions of other amino acids and their simple derivatives would give information essential to an understanding of the chemistry of proteins. For example, 1-fluoro-2,4-dinitrobenzene reacts readily with both histidine and its methyl ester to form bisdinitrophenyl derivatives, but reaction is very slow with α -acetylhistidine and α -benzoylhistidine methyl ester (Sanger, 1945). Thus environment will

influence the reactivity of protein imidazole rings toward FDNB, 1-fluoro-2,4-dinitrobenzene, and variations in reactivity have been observed (Porter, 1950).

Interactions between various protein functional groups (due to Coulomb forces, hydrogen bonding, etc.) would be expected to cause variations in the ionization constants of any one type of group (Laskowski & Scheraga, 1954), and there is evidence that the pK values of the tyrosine OH groups of native egg albumin may vary (Crammer & Neuberger, 1943). Rate constants for reactions involving different charged forms of the side chains would be considerably different. For example, the rate constant for the reaction of the OH form of tyrosine with hypiodous acid is about 1000 times lower than that for the O^- form (Li, 1942). Similarly, the rates of oxidation of dithiol compounds depend on the pK values of the SH groups (Barron, Miller & Kalnitsky, 1947). Thus variation in the state of ionization of groups of a particular type at a given pH would be one of the factors leading to the apparent non-reactivity of protein functional groups under a given set of reaction conditions.

Configurational changes in protein structure, such as are involved in denaturation or in the stretching of fibres, will change the environments of many of the functional groups, thereby bringing about general differences in reactivity. For example, disulphide bonds which react slowly with SO_3^{2-} ions in a native protein (due to nearby CO_2^- groups) may react readily in the denatured state. (The converse is also true, so that, even though more disulphide bonds may be detected after denaturation, those found do not necessarily represent the total number in the protein.) Again, changes in pK values of groups on denaturation, as occurs with the tyrosine OH groups of egg albumin (Crammer & Neuberger, 1943), will cause a different reactivity if similar reaction conditions are used. It is always possible that groups may appear non-reactive after denaturation. For example, diisopropyl fluorophosphate (diisopropyl phosphorofluoridate) reacts with native chymotrypsin but with neither denatured enzyme nor with any of the constituent amino acids under the same conditions (Jansen, Nutting, Jang & Balls, 1950). It is likely that a study of the reaction of diisopropyl fluorophosphate with derivatives of certain amino acids would indicate which enzyme group is involved in this reaction, and also the reason for its apparent increased reactivity in the native protein.

SUMMARY

1. The methods of estimation and the kinetics of the reactions with sodium sulphite of the following disulphides are described: cystamine, homocyst-

amine, homocystine, cystine hydantoin, homocystine hydantoin, diformylhomocystine.

2. It has been found that positively charged compounds containing disulphide bonds react much faster with SO_3^{2-} ions than do disulphides with no net charge.

3. The pK of the thiol group of *N*-formylhomocystine was calculated from equilibrium data. This group has a heat of ionization of approximately + 6000 calories.

4. The influence of chemical environment on the reactivity of protein functional groups is discussed.

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Changes in the Nucleic Acid Content of Ganglion Cells during Chromatolysis and after Stimulation

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This paper is concerned with the changes in total nucleic acid and deoxyribonucleic acid (DNA) in the superior cervical ganglion of the rabbit after section of the postganglionic fibres or stimulation of the preganglionic fibres.

In peripheral nerve it has been shown (Logan, Mannell & Rossiter, 1952*b*) that after section of the nerve the total nucleic acid increases in the distal stump to a maximum at about the sixteenth day after section and that this rise in total nucleic acid is due largely to a marked increase in the ribonucleic acid (RNA) fraction. Similar, but less marked, changes can be detected during the first 48–96 hr. after nerve section, and furthermore it has been shown that intense stimulation of the normal nerve trunk produces a reduction in the DNA fraction while leaving the total nucleic acid unchanged (Causey & Stratmann, 1956). Hydén (1943), using Caspersson's ultraviolet-absorption technique, showed a decrease in the RNA content

of the anterior-horn cells of the spinal cord after intense motor activity.

It was apparent that if the more simple chemical method of nucleic acid estimation was to be applied to nerve-cell bodies, a more uniform and discrete group of cells than those used by Hydén would have to be employed. The cells of the superior cervical ganglion were used. The proportion of nerve cells to supporting tissues and fibres is high, and it is possible to subject the cells both to section of the nerve processes by cutting the postganglionic fibres and to excitation by stimulation of the preganglionic trunk.

EXPERIMENTAL

Rabbits of about 2.5 kg. body weight were used throughout; no selection was made for breed or sex. All survival operations were carried out under Nembutal (pentobarbitone sodium, B.P.) and ether anaesthesia. The specimens were dissected out under urethane anaesthesia. The superior