The frontier electron density distribution parallels the observed order of reactivity toward electrophilic attack, namely, meso > 1 > 2 which the π-valence electron density fails to do, unless one omits from consideration the high density at the non-perimeter atoms. Yet the ease with which the π-valence electrons are brought to the position of electrophilic attack varies, according to the implications of equation 1, in the order meso > 1 > 2 > 13 for which the self polarizabilities[5] are $\pi_{9,9} = 0.526$, $\pi_{1,1} = 0.454$, $\pi_{2,2} = 0.411$ and $\pi_{13,13} = 0.331$. In general in alternant hydrocarbons the success of the frontier electron density distribution in reflecting the experimental data is paralleled by the failure of the π-valence electron density to do likewise, unless one excludes from consideration the large densities at the non-perimeter atoms as in anthracene. It is clear, therefore, that considerable care must be exercised in the deduction of physical interpretations from correlations between experimental data and static π-electron configurations. It is vital to relate static configurations to changes in configuration, an investigation which advances strong support for equation 1 in preference to the frontier electron concept. Equation 1 also illustrates that in a polarization process the ease of provision of a pair of electrons at the position of attack depends not upon the density of this pair in the unperturbed molecule, but rather upon the total density q_r, or better still, upon the total π-electron configuration. Thus the process concerns both the appearance of a pair at the position of attack and the simultaneous removal of all other π-electron pairs, two complementary processes which cannot be separated. Hence in the unperturbed ground state q_r and $\pi_{r,r}$ are the appropriate criteria and not the density of a particular pair.

Finally the details of the polarization process provide a link between the two methods of approximation used for the description of the reactions of conjugated molecules, namely, the method of equation 1 and the alternative transition state method of Wheland.[6] The numerical correlation observed hitherto is now consolidated by the obvious physical connection indicated by the change in electron configuration implicit in (1) toward that used in Wheland's method. Numerically the π-valence electron density in benzene, for example, increases at the position of attack from 0.333 in the ground state to 1,378 for the change in coulomb integral $\delta\alpha_r = 2\beta$, a change which has been used to represent the effect of the neutral oxygen atom in a conjugated molecule[4,7] and which, therefore, does not appear to be excessive as a representation of the effect due to the net positive charge carried by a neighboring electrophilic reagent. Thus the π-valence electron density achieves a high degree of localization at the position of attack for a relatively small perturbation.

Nucleophilic reactions can be considered in exactly the same way as for electrophilic. The details of the charge shifts are less interesting since the whole system of π-electrons is removed from the position of attack, as in an analogous classical problem. Nevertheless the charge shifts take place in accordance with the physical and quantum mechanical principles enumerated earlier.

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[5] H. C. Longuet-Higgins and C. A. Coulson, *J. Chem. Soc.*, 971 (1949).

[6] G. W. Wheland, THIS JOURNAL, **64**, 900 (1942).

[7] G. W. Wheland and L. Pauling, *ibid.*, **57**, 2086 (1935).

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Equilibrium Constants of the Reaction between Sulfite and Oxidized Glutathione

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RECEIVED OCTOBER 16, 1954

The reversible reactions between sulfite or bisulfite with oxidized glutathione (GSSG + $SO_3^=$ ⇄ GS^- + $GSSO_3^-$, GSSG + HSO_3^- ⇄ GSH + $GSSO_3^-$) have been studied quantitatively in the *p*H range 12.4 to 5.2. From the results, four equilibrium constants for the reactions involving $SO_3^=$ and the various charge types of oxidized and reduced glutathione and glutathione sulfonate and one constant for the reaction of one charge type with HSO_3^- have been calculated. The heat of reaction was estimated from the values of the equilibrium constants at 12 and 25°. The results were compared with data for the analogous reactions between sulfite with cystine and with dithiodiglycolic acid.

In the course of investigations on the reactivity of disulfide groups in proteins and peptides it appeared of interest to study in a quantitative way the reaction between oxidized glutathione and sulfite and to compare the results with the data previously reported[1] for the analogous reactions of sulfite and cystine and dithiodiglycolic acid.

The reaction between oxidized glutathione (GSSG) and sulfite was studied at various *p*H and temperatures. Equilibrium constants and the heat of reaction have been calculated from results obtained by the polarographic method of analysis.

Materials.—Oxidized glutathione was prepared from glutathione in the reduced state by a method described in a previous paper.[2] Sodium sulfite was a C.P. reagent grade Merck product. All the other chemicals were C.P. reagent grade products.

Stock solutions of oxidized glutathione were 0.02 *M* in GSSG. The stock solutions were analyzed for GSSG by the polarographic method[2] and also by amperometric mercurimetric titration at the dropping mercury electrode as indicator electrode.[3] Stock solutions of sulfite were prepared with air-free water and were 0.2 to 0.5 *M* in sodium sulfite. They were analyzed for sulfite iodometrically. Only freshly prepared sulfite solutions were used.

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[1] W. Stricks and I. M. Kolthoff, THIS JOURNAL, **73**, 4569 (1951).

[2] W. Stricks and I. M. Kolthoff, *ibid.*, **74**, 4646 (1952).

[3] W. Stricks, I. M. Kolthoff and N. Tanaka, *Anal. Chem.*, **26**, 299 (1954).

The composition of the stock solutions used for the preparation of the buffers was essentially identical with that given previously.[1]

Experimental

Current voltage curves were measured at 12 and 25° with the manual apparatus and circuit described by Lingane and Kolthoff[4] and automatically with a Heyrovsky type self-recording Sargent polarograph, model XII. The diffusion current of reduced glutathione (denoted as GSH) was measured manually. All potentials were measured against the saturated calomel electrode (S.C.E.). Oxygen was removed from the solutions in the cell with purified nitrogen and during an experiment an atmosphere of nitrogen was maintained over the solution.

The characteristics of the capillary were: m = 2.02 mg. sec.$^{-1}$, t = 3.83 sec., $m^{2/3}t^{1/6}$ = 2.00 mg.$^{2/3}$ sec.$^{-1/2}$ (open circuit) the height of the mercury column was 80 cm.

The pH was measured with a Beckman pH meter, model H2. A general purpose blue label glass electrode was used for all pH measurements. The pH of each solution was determined at the temperature specified in Table I.

Appropriate volumes of air-free stock solutions of oxidized glutathione and sulfite were added to a given volume of an air-free buffer solution in an electrolysis cell. The diffusion current of reduced glutathione was measured in the mixture after equilibrium was attained. Equilibrium was found to be established faster the higher the pH of the mixture. Thus at 25° at pH 5 equilibrium was established after about six hours while at pH higher than 8 the final state was attained within a few minutes. Diffusion current constants of reduced glutathione were determined at −0.3 and −0.2 volt in solutions of the same composition as the equilibrium mixture but in the absence of the disulfide and the sulfonate. The values obtained were used for the calculation of the GSH concentration in the equilibrium mixtures.

In contrast to cysteine and thioglycolic acid[1] the diffusion current of reduced glutathione at constant ionic strength (μ = 1) was found to be practically independent of the pH within a pH range of 12.2 to 5.2. At pH 5.2 the GSH concentration should not be higher than 8×10^{-4} M since at larger concentrations reduced glutathione gives a poorly defined anodic wave which is preceded by a maximum. Cysteine gives under no conditions a true diffusion current at pH 5.[1] The diffusion current of GSH was found to decrease slightly with increasing sulfite concentration. For example a glutathione solution which was 10^{-3} M in GSH at pH 12.15 (μ = 1) gave diffusion currents of 2.63 and 2.53 μa. at sulfite concentrations of 0.02 and 0.10 M, respectively. The temperature coefficient of the diffusion current of GSH was determined to be about 2.4% per degree between 12 and 25° and therefore of the same order of magnitude as that of cysteine and thioglycolic acid.

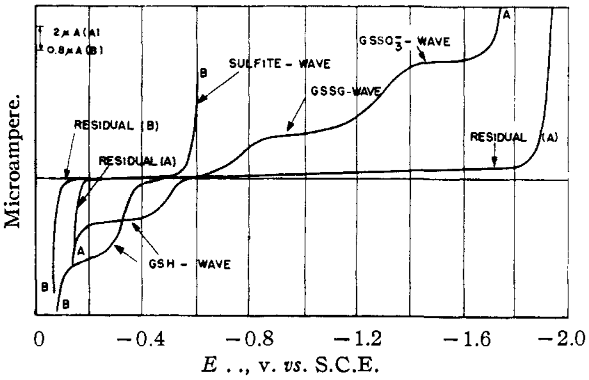

Fig. 1.—Polarograms at 25° of equilibrium mixtures which were originally: (A) 2×10^{-3} M in GSSG and 0.5 M in Na_2SO_3 in an ammonia buffer (0.1 M NH_3, 0.1 M NH_4Cl, 0.9 M KCl, 2.5×10^{-4} M thymol, pH 9.23); (B) 10^{-3} M in GSSG, 0.025 M in Na_2SO_3 in a phosphate–citrate buffer (0.11 M Na_2HPO_4, 0.045 M citric acid, 0.66 M KCl, pH 5.22).

Results and Discussion

The polarogram of an equilibrium mixture composed of 2 ml. of 0.5 M sodium sulfite solution and 18 ml. of 2.2×10^{-3} M GSSG solution in an ammonia buffer of pH 9.23 (2.5×10^{-4} M in thymol) is shown in Fig. 1A. Thymol, which has no effect on the GSH- and $GSSO_3^-$ wave, served to suppress the maximum of the GSSG wave. The polarogram resembles that obtained with cystine, except that the glutathione sulfonate wave is much better defined than the cysteine sulfonate wave which did not have a well defined diffusion current region under the same experimental conditions. A polarogram of a mixture at pH 5 is shown in Fig. 1B. In solutions of pH lower than 7 sulfite is reduced at the dropping mercury electrode[5] and the waves of GSSG and $GSSO_3$ are masked by the sulfite wave. It is seen from Fig. 1B that the diffusion current region of reduced glutathione is not affected by the sulfite wave.

From the polarographically found concentration of reduced glutathione the apparent over-all equilibrium constants of the reactions

$$GSSG + SO_3^{=}\ (SO_3H^-) \rightleftharpoons GS^-(GSH) + GSSO_3^- \quad (1)$$

were calculated for a given mixture.

Experimental results and calculated values of the apparent constant K_{app} in mixtures of ionic strength one and of various pH and temperatures are listed in Table I. Experiments in solutions of pH markedly higher than 12 could not be carried out with GSSG since the peptide rapidly decomposes in strongly alkaline medium to form reduced glutathione.

No experiments were carried out at pH smaller than 5 because of losses by escape of sulfur dioxide. The values of the apparent equilibrium constant in Table I have been calculated by introducing total concentrations for GSSG, $GSSO_3^-$, reduced glutathione and sulfite in equation 1A

$$K_{app} = \frac{[\Sigma\text{red. glutathione}][\Sigma GSSO_3^-]}{[\Sigma GSSG][\Sigma\ \text{sulfite}]} \quad (1A)$$

where red. glutathione and sulfite are present as GS^- and $SO_3^{=}$ ions at pH greater than 8 and as GSH and SO_3H^- ions at pH 5.

The dissociation of the sulfhydryl group in reduced glutathione and of the amino group in reduced and oxidized glutathione and in glutathione sulfonate and of sulfite (to bisulfite) vary between pH 5 and 11; therefore the apparent over-all constant K_{app} varies with pH.

The apparent acid–base contants of reduced and oxidized glutathione have been reported recently.[6]

The individual acid–base constants of reduced glutathione were estimated from these dissociation constants[6] using a simplified method recently described.[7] The variation of the acid–base constants of reduced and oxidized glutathione and of the sulfonate with temperature was estimated from the apparent heat of ionization $\Delta H'$ of the sulfhydryl and amino group in the same way as for cysteine.[1]

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[4] J. J. Lingane and I. M. Kolthoff, THIS JOURNAL, **61**, 825 (1939).

[5] I. M. Kolthoff and J. J. Lingane, "Polarography," Interscience Publishers, New York, N. Y., 1952, p. 559.

[6] N. C. Li, O. Gawron and G. Bascuas, THIS JOURNAL, **76**, 225 (1954).

[7] W. Stricks and I. M. Kolthoff, *ibid.*, **75**, 5673 (1953).

TABLE I

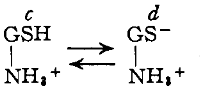
APPARENT EQUILIBRIUM CONSTANT (EQUATION 1) OF THE OXIDIZED GLUTATHIONE–SULFITE SYSTEM AT 12 AND 25°

Ionic strength of all mixtures $\mu = 1 \pm 0.1$.

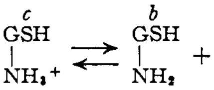
Temp., °C.	Initial concn. of GSSG, *M*	Initial concn. of sulfite, *M*	*p*H	i_d of red. glutathione, μa.	$\Sigma_{\text{red.}}$ glutathione, *M*	K_{app}	No. of expt.
12	2×10^{-3}	0.05	12.41	1.96	9.86×10^{-4}	0.020	19
12	10^{-3}	.05	12.41	1.23	6.2×10^{-4}	.020	20
12	10^{-3}	.075	11.61	1.32	6.76×10^{-4}	.019	21
12	2×10^{-3}	.05	11.55	1.86	9.60×10^{-4}	.018	22
12	10^{-3}	.05	11.55	1.17	6.06×10^{-4}	.019	23
12	2×10^{-3}	.05	10.90	2.19	1.05×10^{-3}	.024	24
12	10^{-3}	.05	10.90	1.35	6.43×10^{-4}	.023	25
25	2×10^{-3}	.05	12.15	3.23	1.24×10^{-3}	.041	13
25	10^{-3}	.05	12.15	1.91	7.34×10^{-4}	.041	12
25	2×10^{-3}	.10	12.12	3.69	1.46×10^{-3}	.040	16
25	10^{-3}	.10	12.12	2.09	8.29×10^{-4}	.040	15
25	2×10^{-3}	.05	11.62	3.09	1.21×10^{-3}	.038	18
25	10^{-3}	.05	11.62	1.85	7.25×10^{-4}	.039	17
25	10^{-3}	.02	11.30	1.46	5.72×10^{-4}	.039	9
25	2×10^{-3}	.05	11.25	3.05	1.20×10^{-3}	.037	8
25	10^{-3}	.05	11.25	1.82	7.17×10^{-4}	.037	7
25	2×10^{-3}	.10	11.20	3.59	1.45×10^{-3}	.039	11
25	10^{-3}	.10	11.20	2.03	8.20×10^{-4}	.037	10
25	10^{-3}	.02	10.20	1.59	5.97×10^{-4}	.046	6
25	2×10^{-3}	.05	10.15	3.27	1.24×10^{-3}	.042	5
25	10^{-3}	.05	10.15	1.96	7.41×10^{-4}	.043	4
25	10^{-3}	.05	9.24	2.05	7.74×10^{-4}	.054	3
25	2×10^{-3}	.05	8.4	3.99	1.45×10^{-3}	.077	2
25	10^{-3}	.05	8.4	2.26	8.19×10^{-4}	0.075	1
25	7.05×10^{-4}	.002	5.22	1.60	6.11×10^{-4}	2.9	26
25	5×10^{-4}	.001	5.23	1.01	3.85×10^{-4}	2.1	27
25	5.05×10^{-4}	.002	5.22	1.19	4.54×10^{-4}	2.6	28
25	5×10^{-4}	.0075	5.20	1.28	4.88×10^{-4}	2.9	29

In the *p*H range listed in Table I the carboxyl group is in the ionized form and its constant need not be considered.

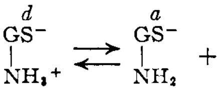
From the dissociation constants[6] of reduced glutathione ($pK_3'(\text{NH}_2^+) = 8.75$, $pK_4'(\text{SH}) = 9.65$ at 25°) the following values of the individual constants were obtained. Concentrations of the various species are denoted by letters above the symbols.



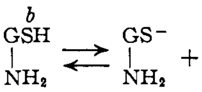
$$\overset{c}{\underset{\text{NH}_3^+}{\text{GSH}}} \rightleftarrows \overset{d}{\underset{\text{NH}_3^+}{\text{GS}^-}} + \text{H}^+;\quad pK_A' = 9.05 \text{ at 12 and } 25° \qquad (2)$$



$$\overset{c}{\underset{\text{NH}_3^+}{\text{GSH}}} \rightleftarrows \overset{b}{\underset{\text{NH}_2}{\text{GSH}}} + \text{H}^+;\quad pK_B' = \left\{\begin{matrix} 9.44 \text{ at } 12° \\ 9.05 \text{ at } 25° \end{matrix}\right. \qquad (3)$$



$$\overset{d}{\underset{\text{NH}_3^+}{\text{GS}^-}} \rightleftarrows \overset{a}{\underset{\text{NH}_2}{\text{GS}^-}} + \text{H}^+;\quad pK_C' = \left\{\begin{matrix} 9.74 \text{ at } 12° \\ 9.35 \text{ at } 25° \end{matrix}\right. \qquad (4)$$

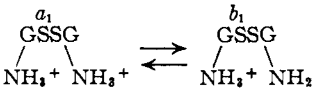


$$\overset{b}{\underset{\text{NH}_2}{\text{GSH}}} \rightleftarrows \underset{\text{NH}_2}{\text{GS}^-} + \text{H}^+;\quad pK_D' = 9.35 \text{ at 12 and } 25° \qquad (5)$$

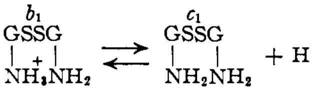
$$\Sigma\text{red. glutathione} = a + b + c + d = a\left\{1 + \frac{[\text{H}^+]}{K_4'} + \frac{[\text{H}^+]^2}{K_3'K_4'}\right\} \qquad (6)$$

where $a[\text{H}^+]/K_4' = b + d$ and $b = d$ at 25°. Equation 6 was used to calculate the concentration of each of the species of reduced glutathione at a given *p*H.

The dissociation constants of oxidized glutathione are given in equations 7 and 8



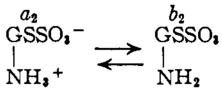
$$\overset{a_1}{\underset{\text{NH}_3^+\ \text{NH}_3^+}{\text{GSSG}}} \rightleftarrows \overset{b_1}{\underset{\text{NH}_3^+\ \text{NH}_2}{\text{GSSG}}} + \text{H}^+;\quad pK_5' \left\{\begin{matrix} 8.96 \text{ at } 12° \\ 8.57 \text{ at } 25° \end{matrix}\right. \qquad (7)$$



$$\overset{b_1}{\underset{\overset{+}{\text{NH}_3}\text{NH}_2}{\text{GSSG}}} \rightleftarrows \overset{c_1}{\underset{\text{NH}_2\text{NH}_2}{\text{GSSG}}} + \text{H}^+;\quad pK' \left\{\begin{matrix} 9.93 \text{ at } 12° \\ 9.54 \text{ at } 25° \end{matrix}\right. \qquad (8)$$

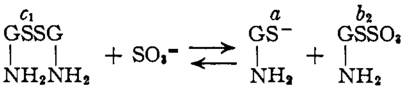
$$\Sigma\text{GSSG} = a_1 + b_1 + c_1 = b_1\left\{\frac{[\text{H}^+]}{K_5'} + 1 + \frac{K_6'}{[\text{H}^+]}\right\} \qquad (9)$$

The following dissociation of the glutathione sulfonate GSSO_3^- must also be considered in the *p*H range investigated.

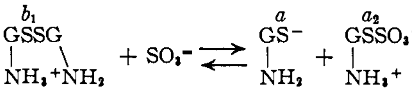


$$\overset{a_2}{\underset{\text{NH}_3^+}{\text{GSSO}_3^-}} \rightleftarrows \overset{b_2}{\underset{\text{NH}_2}{\text{GSSO}_3^-}} + \text{H}^+;\quad K_8 = \frac{b_2[\text{H}^+]}{a_2} \qquad (10)$$

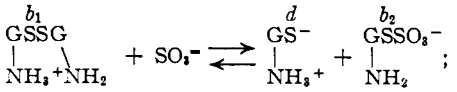
The equilibrium constants with reference to the various charge species of reduced and oxidized glutathione and of glutathione sulfonate can now be calculated.



$$\overset{c_1}{\underset{\text{NH}_2\text{NH}_2}{\text{GSSG}}} + \text{SO}_3^{=} \rightleftarrows \overset{a}{\underset{\text{NH}_2}{\text{GS}^-}} + \overset{b_2}{\underset{\text{NH}_2}{\text{GSSO}_3^-}};\quad K_1 = \frac{b_2 a}{c_1[\text{SO}_3^{=}]} \qquad (11)$$

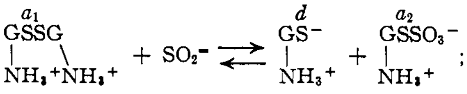


$$\overset{b_1}{\underset{\text{NH}_3^+\text{NH}_2}{\text{GSSG}}} + \text{SO}_3^{=} \rightleftarrows \overset{a}{\underset{\text{NH}_2}{\text{GS}^-}} + \overset{a_2}{\underset{\text{NH}_3^+}{\text{GSSO}_3^-}};\quad K_{11} = \frac{aa_2}{b_1[\text{SO}_3^{=}]} \qquad (12)$$



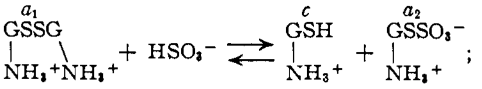
$$\overset{b_1}{\underset{\text{NH}_3^+\ \text{NH}_2}{\text{GSSG}}} + \text{SO}_3^= \rightleftarrows \overset{d}{\underset{\text{NH}_3^+}{\text{GS}^-}} + \overset{b_2}{\underset{\text{NH}_2}{\text{GSSO}_3^-}};$$

$$K_{III} = \frac{db_2}{b_1[\text{SO}_3^=]} \quad (13)$$



$$\overset{a_1}{\underset{\text{NH}_3^+\ \text{NH}_3^+}{\text{GSSG}}} + \text{SO}_2^= \rightleftarrows \overset{d}{\underset{\text{NH}_3^+}{\text{GS}^-}} + \overset{a_2}{\underset{\text{NH}_3^+}{\text{GSSO}_3^-}};$$

$$K_{IV} = \frac{da_2}{a_1[\text{SO}_3^-]} \quad (14)$$



$$\overset{a_1}{\underset{\text{NH}_3^+\ \text{NH}_3^+}{\text{GSSG}}} + \text{HSO}_3^- \rightleftarrows \overset{c}{\underset{\text{NH}_3^+}{\text{GSH}}} + \overset{a_2}{\underset{\text{NH}_3^+}{\text{GSSO}_3^-}};$$

$$K_V = \frac{ca_2}{a_1[\text{HSO}_3^-]} \quad (15)$$

By proper combination of the above equations we obtain the following relations

$$K_{II} = \frac{K'_6}{K_S} K_I \quad (16)$$

$$K_{III} = \frac{K'_6}{K'_C} K_I \quad (17)$$

$$K_{IV} = \frac{K'_5 K'_6}{K'_C K_S} K_I \quad (18)$$

$$K_V = \frac{K_{2(H_2SO_3)} K'_5 K'_6}{K_S K'_3 K'_4} K_I \quad (19)$$

where $K_{2(H_2SO_3)}$ is the second dissociation constant of sulfurous acid.

It is seen that the equilibrium constants involving the various charge species can be expressed in terms of K_I and K_S, of the known dissociation constants of reduced and oxidized glutathione and of the dissociation constant $K_{2(H_2SO_3)}$ of sulfurous acid.

The constants K_I and K_V can be found directly from experiments with solutions of sufficiently high and sufficiently low *p*H, respectively.

It is seen from Table I that the apparent constant has practically the same value over the *p*H range between 11.2 and 12.1. Solutions of this *p*H range contain almost exclusively the species with uncharged amino groups and K_{app} of these solutions is therefore practically equal to K_I. The two dissociation constants of sulfurous acid are $K_{1}(H_2SO_3) = 0.017$ and $K_{2(H_2SO_3)} = 6.24 \times 10^{-8}$ at 25°[8] and no corrections need be made for the presence of bisulfite at *p*H greater than 9. In the calculations of the apparent equilibrium constants at anionic strength of one concentrations have been written instead of activities. Activity coefficients of the various ionic species cannot be calculated with a fair accuracy at such a high ionic strength. All equilibrium constants referred to and determined in this paper are apparent constants with the exception of the two ionization constants of sulfurous acid.

The average values of K_I are found to be 0.039 and 0.019 at 25 and 12°, respectively.

The value of K_V can be obtained from experiments with solutions of *p*H 5 since at this *p*H the amino groups of oxidized and reduced glutathione are practically all present in the charged form while the sulfhydryl group is uncharged. Almost all of the sulfite is present as HSO_3^- ion at *p*H 5. The apparent constant at *p*H 5 can therefore be considered to be equal to K_V. The average value of K_V is 2.6 at 25°.

Knowing K_I, the constant K_S of equation 10 can be calculated from equations 6, 9 and 11 at a lower *p*H (8 to 9). In this calculation the accuracy of the values of K_S depends greatly on the value of the concentration a_2 of the glutathione sulfonate with the charged amino group. Since a_2 is determined as a small difference between two relatively large figures (for example: $a_2 = 7.74 \times 10^{-4}$ ($\Sigma GSSO_3^-$) $- 7.13 \times 10^{-4}$ (b_2) at *p*H 9.24, expt. 3) the accuracy of a_2 is not comparable to the accuracy of our measurements and values of K_S obtained by this calculation showed rather large variations (1.5×10^{-8} at *p*H 8.4 and 7.2×10^{-9} at *p*H 9.24).

It is more accurate and simpler to calculate K_S from both K_I and K_V with the aid of equation 19. The value of K_S of 1.8×10^{-9} thus obtained was used for the calculation of the equilibrium constants K_{II} and K_{IV}. Taking a value of 12,000 cal. for ΔH of the amino group,[1] K_S at 12° is found to be 7.4×10^{-10}. Assuming that ΔH for the amino groups of GSSG, GSH and $GSSO_3^-$ is the same and that $K_{2(H_2SO_3)}$ is practically constant between 12 and 25°, it is found that the heat of reaction of the five equilibrium reactions is the same. ΔH was estimated from the experimental values of K_I at 12 and 25° and found to be +9500 cal.

Since most of the calculations of the constants are quite similar to those reported in detail for the cystine–sulfite system[1] specific examples of these calculations are not given in the present paper. From equations 12, 13 and 14 the constants K_{II}, K_{III} and K_{IV} have been calculated. The values of these constants, the change in free energy ($\Delta F = RT \ln K$) and the heat of reaction ΔH for reactions I, II, III, IV and V are listed in Table II which, for comparison, also gives the data for the corresponding reactions of cystine and dithiodiglycolic acid. The value of K_V for the cystine–sulfite system which could not be determined experimentally and which was not given previously[1] was calculated from equation 19 using the corresponding data for cystine and cysteine.

An inspection of Table II shows that the values of K_I, K_{II}, K_{III} and K_{IV} are all smaller than one while K_V is larger than one. Comparing reactions III and II it is interesting to note that K_{III} is four to three times larger than K_{II} for both RSSR and GSSG. This is remarkable, considering the fact that the products on the left side of equations 12 and 13, presenting reactions II and III are identical. Expressing the equilibrium constant as the ratio of the rate constants of the forward (left to right) and backward reaction ($K = k_1/k_2$) the difference between K_{II} and K_{III} is explained by the difference in the rate of the reactions between the various charge species of reduced glutathione and glutathione sulfonate. The rate of the reaction between $\overset{\text{GS}^-}{\underset{\text{NH}_2}{|}}$ and $\overset{\text{GSSO}_3^-}{\underset{\text{NH}_2^+}{|}}$ is thus about 4 times as large as that of the reaction between $\overset{\text{GS}^-}{\underset{\text{NH}_3^+}{|}}$ and $\overset{\text{GSSO}_3^-}{\underset{\text{NH}_2}{|}}$, assum-

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(8) Norio Yui, *Bull. Inst. Phys. Chem. Research*, **19**, 1229 (1940); H. V. Tartar and H. H. Garretson, THIS JOURNAL, **63**, 808 (1941).

ing that the rate of the forward reaction is the same in both equilibria. The variation in these reaction rates is related quantitatively to the relation between the constants K_S and K'_C (see equations 16 and 17), the charged amino group of the sulfonate ($K_S = 1.8 \times 10^{-9}$) being a stronger acid than that of reduced glutathione ($K'_C = 4.5 \times 10^{-10}$). The larger reaction rate between $\overset{\text{GS}^-}{\underset{\text{NH}_2}{|}}$ and $\overset{\text{GSSO}_3^-}{\underset{\text{NH}_3^+}{|}}$ may possibly be explained by assuming that the reaction between these two ions is initiated by an exchange of protons between the amino group of the sulfonate and that of reduced glutathione.

Comparing the three disulfides GSSG, RSSR and TSST it is seen that the constant K_I is largest for GSSG and smallest for TSST. Thus in alkaline medium the equilibrium involving TSST lies farthest to the left. Experiments to be reported in a subsequent paper on the alkaline fission of the three disulfides in the presence of silver or mercury salts indicate that cystine reacts the slowest and dithiodiglycolic acid the fastest. It thus appears that the reactivity of the three disulfide compounds toward sulfite is not related to their fissility in alkaline medium. It is interesting to note that in contrast to K_I, the values of K_V for the sulfite–disulfide system in acid medium are of the same order of magnitude for the three disulfides studied. This is accounted for by the numerical values of the constants which determine K_V (see equation 19 for GSSG and RSSR and equation 20[1] for TSST).

TABLE II

EQUILIBRIUM CONSTANTS, CHANGE IN FREE ENERGY ΔF AND HEAT OF REACTIONS I, II, III, IV AND V FOR GLUTATHIONE (GSSG) AND CYSTINE (RSSR) AND OF REACTIONS I AND V FOR DITHIODIGLYCOLIC ACID (TSST)

	GSSG		RSSR		TSST
	12°	25°	12°	25°	25°
K_{I}	0.019	0.039	0.0060	0.010	0.00047
K_{II}	.0030	.0062	.00031	.00052	
K_{III}	.012	.025	.0095	.016	
K_{IV}	.018	.037	.086	.15	
K_{V}	1.3	2.6	2.5	4.1	1.4
ΔF_{I}, cal.	−2200	−1900	−2900	−2700	−4500
ΔF_{II} cal.	−3300	−3000	−4600	−4500	
ΔF_{III} cal.	−2500	−2200	−2600	−2500	
ΔF_{IV} cal.	−2300	−2000	−1400	−1100	
ΔF_{V} cal.	+140	+570	+290	+890	+200
ΔH, cal.	+9500		+7000		

$$K_{\mathrm{V(TSST)}} = \frac{K_{2(\mathrm{H_2SO_3})}K_1}{K_{(\mathrm{SH})}} \qquad (20)$$

where $K_{(\mathrm{SH})}$ is the dissociation constant of the sulfhydryl group of thioglycolic acid.

The numerical values of the ratios of the constant K_I and the dissociation constant of the sulfhydryl group (K_I/K'_4 for glutathione and cystine, $K_I/K_{(\mathrm{SH})}$ for dithiodiglycolic acid) are of the order of magnitude of 10^8 for the amino acids and 10^7 for TSST. The larger value (10^8) for GSSG and RSSR is multiplied by the term involving the dissociation constants of the NH_3^+ groups ($K'_5K'_6/K_SK'_3$ in equation 19) which is of the order of magnitude of 0.1, thus making the values of K_V of the three disulfides of the same order of magnitude. The numerical values of K_I and K_V of the three disulfides indicate that in alkaline medium equilibrium lies quite far to the left while in acid medium the equilibrium is more favored to the right side. The heat of reaction ΔH which has been determined only for glutathione and cystine is found to be of the same order of magnitude for the two acids.

Acknowledgment.—This investigation was supported by a research grant (C-721,C5) from the National Cancer Institute, U. S. Public Health Service.

MINNEAPOLIS, MINNESOTA

[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY OF THE UNIVERSITY OF MINNESOTA]

N,N'-Dihydroxyethylglycine and its Complexes with Iron[1,2]

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RECEIVED OCTOBER 16, 1954

At an ionic strength of 0.5 the acid dissociation constants K_1 and K_2 of N,N'-dihydroxyethylglycine, (DHEG), are $10^{-2.50}$ and $10^{-8.11}$, respectively. Ferric and ferrous iron and their mixtures in the presence of excess DHEG give reversible waves at the dropping mercury electrode; the standard potential of the system is −0.94 v. *vs.* the saturated calomel electrode. The electrode reaction in neutral and alkaline solution is $FeG(OH)_2 + G^- + e \rightleftarrows FeG_2 + 2OH^-$. Potentiometric measurements at the platinum electrode indicate that the electrode reaction in acid solution is probably $FeG(OH)_2 + e \rightleftarrows Fe^{++} + G^- + 2OH^-$ between *p*H 5 and *p*H 3, and $Fe^{+3} + e \rightleftarrows Fe^{++}$ in more acid solutions. The complex stability constants determined from electrometric data are $K_{\mathrm{III}} = 10^{30.1}$ and $K_{\mathrm{II}} = 10^{5.9}$ for $FeG(OH)_2$ and FeG_2, respectively.

N,N'-Dihydroxyethylglycine (DHEG) is an amino acid with the structure R_2NCH_2COOH, where $R = C_2H_4OH$. In common with glycine and other amino acids, it is likely that the uncharged form of DHEG exists as a dipole ion, $R_2H^+NCH_2COO^-$. DHEG is a white solid which forms colorless aqueous solutions. Both the acid and its salt are soluble in water. In the present paper we report on the determination of the dissociation constants of DHEG and the stability constants of its ferric and ferrous complexes. Equations of the electrode reactions involving the ferric–ferrous complexes of DHEG at the dropping mercury and at a platinum electrode have also been established.

Materials.—The DHEG was furnished by the Alrose Chemical Company. It was purified by dissolving the acid in a small amount of hot water and precipitating with ethanol. A brown color which was not completely removed

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[1] This work was carried out under the sponsorship of the Reconstruction Finance Corporation, Office of Synthetic Rubber, in connection with the Synthetic Rubber Program of the United States Government.

[2] From a Ph.D. Thesis submitted by P. E. Toren to the Graduate School of the University of Minnesota, December, 1953.