Oxidation of an equilibrium mixture of *n*- and *t*-butyl mercaptans did not give a product containing the disulfides in the equilibrium ratio. There is no reason to expect that they should, since the nature of the products must be determined by the relative rate of reaction. The fact that Birch, *et al.*,[7] obtained the same product by oxidation and base-catalyzed equilibration in two cases may well be a coincidence.

The propyl–*sec*-butyl system gives a value of K_1 essentially identical with that predicted on the basis of probability. This falls in line with the values found by other investigators for *n*- and isoalkyl compounds. The present work shows, furthermore, that K_2 and K_3 separately conform to the statistical values.

Substitution of diethylamino or hydroxo groups for hydrogen reduce K_1 and K_2 each by a factor of about two; *i.e.*, the substituted disulfides are slightly better oxidizing agents than the *n*-alkyl disulfides; K_1, however, conforms closely to the statistical value. Phenyl disulfide is a still better oxidizing agent, and, in addition, the mixed disulfide is relatively favored. All in all, the deviations from statistical behavior must be regarded as small, and, in view of the variety of compounds examined, it is concluded that the effect is a general one. In the absence of steric or extraneous chemical effects,[6] therefore, it may be expected that mercaptan–disulfide interchange reactions will conform closely to the distribution predicted on the basis of probability.

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Mercaptan–Disulfide Interchange Reactions.[1] III. Reaction of Cysteine with 4,4′-Dithiobis(benzenesulfonic acid)

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Sodium 4,4′-dithiobisbenzenesulfonate (Na_2BSSB) has been prepared from sulfanilic acid. The ultraviolet absorption spectra have been determined for BSSB, the mixed disulfide formed from it upon reaction with cysteine (BSSR), 4-mercaptobenzenesulfonate (BSH), and its mercaptide ion (BS^-). The ionization constant of BSH is $10^{-5.7}$. The following equilibrium constants have been measured: $[(BSSR)(BSH)]/[(BSSB)(RSH)] = 1.2$ and $[(RSSR)(BSH)]/[(BSSR)(RSH)] = 1.0$ at 25°. The ionization of BSH tends to drive the reaction of cysteine with BSSB toward completion at pH values above 6.5. Some measurements of reaction rate are reported.

Cysteine and some of its derivatives are involved in many important physiological processes: for example, enzymic catalysis,[2] protein aggregation,[3] and cell division.[4] A measure of protection against ionizing radiation is afforded by cysteine and a few congeners, and it has been suggested that the radioprotective action involves the formation of mixed disulfides with protein molecules.[5] Some measurements of equilibria have been made for mercaptan–disulfide interchange reactions involving these compounds; these data will be considered briefly in the Discussion section. The experimental results are limited and in some cases uncertain. The present investigation was undertaken because further study of the problem seemed desirable.

The reaction of cysteine with 4,4′-dithiobisbenzenesulfonate can be measured with comparative ease because the 4-mercaptobenzenesulfonate formed in the reaction ionizes to give a mercaptide anion that has a characteristic absorption peak in the ultraviolet and can be determined spectrophotometrically. The ionization of 4-mercaptobenzenesulfonate and the employment of a buffered medium introduce additional considerations, however. The interchange reactions may be represented by the equations

$$BSSB + RSH \rightleftharpoons BSSR + BS^- + H^+ \quad (1)$$

$$BSSR + RSH \rightleftharpoons RSSR + BS^- + H^+ \quad (2)$$

where BSSB is the doubly charged anion, 4,4′-dithiobisbenzenesulfonate; RSH is cysteine; BSH is 4-mercaptobenzenesulfonate; BS^- is the corresponding mercaptide anion, 4-sulfidobenzenesulfonate (since the sulfonic acid groups are completely ionized in all the conditions of interest, the charges resulting from their ionizations are not indicated).

The ionization constant expression for BSH

$$K_i = [(BS^-)(H^+)]/(BSH) \quad (3)$$

relates the equilibrium-constant expressions for eq. 1 and 2 to those given in paper II,[1] which are written for unionized compounds.

$$K_2' = [(BSSR)(BS^-)(H^+)]/[(BSSB)(RSH)] = K_2K_i \quad (4)$$

$$K_3' = [(RSSR)(BS^-)(H^+)]/[(BSSR)(RSH)] = K_3K_i \quad (5)$$

$$K_4 = K_2'K_3' = [(RSSR)(BS^-)^2(H^+)^2]/[(BSSB)(RSH)^2] = K_2K_3K_i^2 \quad (6)$$

Experimental

Materials.—Cysteine hydrochloride monohydrate was an "A" grade product of the California Corporation for Biochemical Research, Los Angeles 63, Calif. Cystine and oxidized glutathione were products of Schwarz Laboratories, Mt. Vernon, N. Y.;

(1) See preceding paper: G. Dalman, J. McDermed, and G. Gorin, *J. Org. Chem.*, **29**, 1480 (1964).

(2) P. D. Boyer, "The Enzymes," Vol. I, P. D. Boyer, H. Lardy, and K. Myrbäck, Ed., Academic Press, New York, N. Y., 1959, p. 511.

(3) E. V. Jensen, *Science*, **130**, 1319 (1959).

(4) D. Mazia, "Sulfur in Proteins," R. Benesch, *et al.*, Ed., Academic Press, Inc., New York, N. Y., 1959, p. 357.

(5) L. Eldjarn and A. Pihl, "Mechanisms in Radiobiology," Vol. II, M. Errera and A. Forssberg, Ed., Academic Press, Inc., New York, N. Y., 1960, p. 231.

glutathione of Matheson Coleman and Bell, Cincinnati 12, Ohio. Other chemicals were of ACS reagent grade. Phosphate buffers were mixed from sodium phosphates or from sodium hydroxide and phosphoric acid to give a total phosphate concentration of 0.02 *M*; 1 g. of ethylenedinitrilotetraacetic acid (EDTA) was added per liter of solution.

Preparation of Sodium 4,4′-Dithiobisbenzenesulfonate [Na_2-BSSB].—Dissolve sulfanilic acid, 47.5 g. (0.25 mole), and 13.25 g. of anhydrous sodium carbonate (0.13 mole) in 500 ml. of water by warming. Cool to 15°. Add 18.5 g. of sodium nitrite (0.27 mole) in 50 ml. of water and pour the mixture slowly with stirring into 52.5 ml. of concentrated hydrochloric acid (0.64 mole) and 300 g. of crushed ice. After 15 min., a test for nitrous acid with starch–iodide paper should be positive.

Dissolve 65 g. of crystalline sodium sulfide nonahydrate (0.27 mole) and 8.5 g. of powdered sulfur (0.27 mole) in 75 ml. of water at 100°; add 10 g. of sodium hydroxide (0.25 mole) in 100 ml. of water. Cool below 5° and add the diazo solution over a period of 20–30 min., along with 48 g. of ice to keep the temperature below 5°. Remove the ice bath and allow the mixture to come to room temperature; the evolution of nitrogen should cease in about 2 hr. Add concentrated hydrochloric acid to pH 2, filter to remove the sulfur, and heat the filtrate to drive out hydrogen sulfide. Neutralize to pH 7 with concentrated sodium hydroxide; filter if necessary. Collect 10–15 g. (25–35% yield) of the disulfide by evaporation of the solvent at 60–80° or under reduced pressure (by further evaporation, more material can be recovered, to about 70% yield, but its purification from contaminating sodium chloride and other impurities is not worthwhile).

Crystallize the product by dissolving in the minimum amount of 80% ethanol at the boiling point, filtering hot, and cooling to 0°. The purification is repeated until the absorbancy coefficient at 254 mμ reaches the value of 48.86 l. g.$^{-1}$ cm.$^{-1}$.

Anal. Calcd. for $C_{12}H_8Na_2O_6S_4 \cdot 2H_2O$: C, 31.44; H, 2.64; S, 27.98; H_2O, 7.86. Found: C, 31.43; H, 2.83; S, 28.25; H_2O (by drying at 110°), 7.59.

The bis(S-benzylthiuronium) salt melts at 167°.

Apparatus.—Spectra were determined at room temperature in 1-cm. silica cells using a Beckman DU spectrophotometer. Measurements of pH were done with a Beckman Model G pH meter.

Sodium 4-Mercaptobenzenesulfonate.—This substance was not isolated. It was prepared in solution by reduction of Na_2(BSSB) with (a) an excess of cysteine or (b) with zinc and hydrochloric acid.

(a) A solution of Na_2(BSSB), 3.05×10^{-5} *M*, and cysteine, 1.36×10^{-3} *M*, was adjusted to pH 9 with sodium hydroxide. The spectrum was determined against a blank containing the cysteine solution; the measurement was repeated after adjustment to pH 3.0.

(b) Na_2(BSSB), 0.668 g., was dissolved in 54 ml. of 0.5 *M* hydrochloric acid, and the zinc dust was added. After stirring for 30 min., the solution was filtered and its spectrum was determined; the spectrum was again determined after adjustment to pH 9.0 with sodium hydroxide.

Ionization Constant of Mercapto Group in 4-Mercaptobenzenesulfonate.—BSH, 2 mg., produced by either methods a or b of the preceding section, was added to 100 ml. of 0.2 *M* phosphate buffer containing 10^{-4} *M* ethylenedinitrilotetraacetic acid (EDTA). Hydrochloric acid, 1 *M*, and sodium hydroxide, 2 *M*, were added as needed to give various pH values between 4 and 9. Small aliquot portions were withdrawn and the absorbance was measured at 285 and 254 mμ. The blank contained buffer, EDTA, and the appropriate reducing agent. The concentrations of BSH and BS^- were calculated from the absorbances at the two wave lengths by solving the simultaneous equations (see Table I): $A_{285} = 1200(BSH) + 17{,}300(BS^-)$; $A_{254} = 15{,}400 \times (BSH) + 4950(BS^-)$.

TABLE I

SPECTRAL PROPERTIES OF MERCAPTANS AND DISULFIDES

Compound	λ_{max}, mμ	A_m at 254 mμ	A_m at 285 mμ
BSSB	254	22,400	4,800
BS^-	285	4,950	17,300
BSH	254	15,400	1,200
BSSR	248[a]	9,400	(2,400)[b]

[a] A_m = 10,300. [b] Assumed value, see text.

Allowance was made for dilution by the added reagents. The total concentration of BSH + BS^- could be estimated from the absorbances at pH 9 or 3, where (BSH) or (BS^-), respectively, could be set equal to 0. As a check, the sum of (BSH) and (BS^-) was calculated for each set of measurements; the sum was constant within 1% over virtually the entire pH range. Identical results were obtained with BSH produced by method a or method b. For BSH produced by method a, the blank absorbance at 254 mμ became appreciable above pH 7, and no measurements were made beyond this point at this wave length.

Procedure for Equilibrium Measurements.—The experiments were conducted in 0.02 *M* phosphate buffers of pH 5.9 to 7.6 containing EDTA. Solutions of BSSB and RSH were prepared in the appropriate buffer and mixed to give the desired compositions; all solutions were freshly prepared, that of RSH immediately before use. Absorbances were determined at 254 and 285 mμ until constant values were reached; the blank contained all reagents except BSSB. Solutions of BSSB in buffer showed no alterations in the absorbance over periods of time much longer than those required for the equilibrium measurements, but BSSB was slowly decomposed in alkaline solutions.

Procedure for Rate Measurements.—The experiments were conducted in 0.02 *M* phosphate buffers of pH 6.0, 6.5, and 7.0, containing EDTA and 0.2 *M* sodium chloride. The freshly prepared solutions were mixed and the absorbance at 285 mμ was recorded as a function of time until a constant value was reached. Corrections for the absorbances of RSH and BSSB were made in the calculations.

Results

Sodium 4,4′-dithiobisbenzenesulfonate [Na_2(BSSB)] has been prepared from sulfanilic acid by diazotization followed by coupling with sodium disulfide. Table I lists some of its spectral characteristics.

Treatment of BSSB with a large excess of cysteine at pH 9, or reduction of BSSB with zinc and acid followed by adjustment to pH 9, resulted in the disappearance of the maximum at 254 mμ and the development of a new maximum at 285 mμ; this peak can be ascribed to the BS^- ion. Lowering the pH to 3 resulted in the disappearance of the 285-mμ peak and development of a maximum at 254 mμ, which had, however, a lower intensity than that of BSSB. Titration of BS^- in the presence of buffer salts and analysis of the absorbances found at 285 and 254 mμ as a function of pH corresponded to the conversion of BS^- to BSH; Table I lists their spectral properties. The ionization constant of the mercapto group in BSH, eq. 3, was found to be $10^{-5.73}$.

Mixtures originally containing more nearly equivalent amounts of BSSB and RSH at pH 5–7 also showed the development of a 285-mμ peak. Typically, the absorbance rose quite rapidly to a maximum value and then decreased slowly. It was suspected that the slow decrease might be due to oxidation of BSH by dissolved oxygen, and it was sought to prevent this by adding some ethylenedinitrilotetraacetic acid (EDTA). In the presence of EDTA, stable values of the absorbance at 285 mμ were in fact obtained. Since BS^- is the principal absorbing species at this wave length, its concentration could be easily approximated and the amount of BSH calculated from eq. 3. It could thus be ascertained that the conversion of BSSB to (BSH + BS^-) was not complete in many conditions.

The information did not, however, suffice for calculating constants K_2' and K_3'. This can be seen from the following considerations. The experimentally determined or determinable quantities are the total amounts of mercaptan (*M*), of disulfide (*D*), and of BS residues (*B*) taken in any experiment, the molar ab-

TABLE II

REPRESENTATIVE EQUILIBRIUM CONCENTRATIONS

	Initial concn. × 10^5			Equilibrium concn. × 10^5					A_{254}^{BSSR}	
pH	BSSB	RSH	RSSR	RSH	BSH	BS^-	RSSR	BSSR	× 10^{-4}	K_3' × 10^6
6.58	1.36	2.83	20.8	1.79	0.12	0.92	20.5	1.68	1.00	1.66
6.81	1.36	2.83	29.2	1.63	0.10	1.10	29.0	1.53	1.00	1.97
7.35	1.36	2.83	29.2	1.12	0.07	1.64	29.5	1.01	0.85	1.91
7.69	1.36	2.83	29.2	0.85	0.04	1.94	29.7	0.74	0.72	1.87
6.51	1.36	2.97	4.11	1.20	0.23	1.54	4.52	0.95	1.00	1.89
6.72	1.36	2.97	12.4	1.38	0.14	1.46	12.6	1.12	0.98	2.27
7.63	1.36	2.97	28.8	0.94	0.04	1.99	29.5	0.69	0.74	2.12
							Average[a]		0.90	1.96
							Average deviations		±0.11	±0.14

[a] Average of 22 determinations 1.88 ± 0.10 × 10^{-6}.

TABLE III

REPRESENTATIVE EQUILIBRIUM CONCENTRATIONS

	Initial concn. × 10^5			Equilibrium concn. × 10^5						
pH	BSSB	RSH	RSSR	RSH	BSH	BS^-	RSSR	BSSR	BSSB	K_2' × 10^6
6.10	3.08	3.31	1.66	0.89	0.60	1.82	2.01	1.71	1.02	2.72
6.10	3.08	4.96	1.66	1.82	0.78	2.36	2.55	1.37	0.83	1.70
6.36	3.08	3.31	1.66	0.69	0.42	2.20	2.17	1.58	0.99	2.22
6.41	3.08	3.31	3.32	0.82	0.37	2.12	3.61	1.90	0.90	2.13
6.41	3.08	4.96	3.32	1.57	0.50	2.89	4.23	1.58	0.61	1.86
6.58	3.08	3.31	4.14	0.56	0.31	2.44	4.25	2.55	0.44	2.07
6.66	3.08	4.96	6.64	1.40	0.36	3.20	7.44	1.95	0.34	2.88
								Average		2.23
								Average deviation		±0.33

sorbancy coefficients (A_m) of the several species at the wave length chosen for measurement, excepting that of the mixed disulfide (A_m^{BSSR}), and the absorbances experimentally measured. The following equations can be written

$$M = (BSH) + (BS^-) + (RSH) \quad (7)$$

$$D = (BSSB) + (BSSR) + (RSSR) \quad (8)$$

as well as an equation for the total concentration of BS residues

$$B = 2(BSSB) + (BSSR) + (BSH) + (BS^-) \quad (9)$$

(inclusion of a similar equation for RS residues results in a dependent system of equations). For the absorbances at 285 and 254 mμ one can write

$$A_{285} = 17{,}300(BS^-) + 1200(BSH) + 4800(BSSB) + A_{m,285}^{BSSR}(BSSR) \quad (10)$$

$$A_{254} = 4950(BS^-) + 15{,}400(BSH) + 22{,}400(BSSB) + A_{m,254}^{BSSR}(BSSR) \quad (11)$$

since the absorbances of RSH and RSSR are negligible (*cf.* Table I). Finally, one has eq. 3, with $K_i = 10^{-5.73}$. In these six equations there are eight unknowns, to wit, (BSH), (BS^-), (RSH), (BSSB), (BSSR), (RSSR), $A_{m,285}^{BSSR}$, and $A_{m,254}^{BSSR}$, and it follows that the system of equations cannot be solved.

The first approach to an approximate solut.on was made by assuming that the concentration of the mixed disulfide (BSSR) is negligible, *i.e.*, that the ratio K_2/K_3 is very small. This assumption eliminates the terms A_m^{BSSR} as well, and all other concentrations can then be calculated. However, substitution of the values obtained into eq. 6 does not give consistent values of K_4'. This is not surprising, since what is known about other mercaptan–disulfide interchange reactions leads one to expect that $K_2 \approx K_3 \approx 1$. If this is the case, however, reaction conditions can be chosen which make (BSSB) negligible, while all other species are present in substantial amounts; in general, these conditions entail a comparatively high pH, which tends to drive both reactions 1 and 2 to the right, and a large excess of RSSR, which counteracts the effect of pH in the case of eq. 2.

Table II lists a set of experiments which were designed to give minimal concentrations of BSSB at equilibrium. Attainment of equilibrium was indicated by a constant value of A_{285}. Equations 7–11 were solved on the assumptions that (BSSB) was zero and that $A_{m,285}^{BSSR}$ was 2400, one-half the value for BSSB. Since BSSR provides only a minor fraction of the total absorbance at 285 mμ (BS^- predominates above pH 6), an error in A_m^{BSSR} has little effect on the calculation. One can then estimate a value for A_m^{BSSR} at 254 mμ, which is listed in Table I, and determine other spectral characteristics. The results are consistent with the findings of Parker and Kharasch,[6] who found that in a series of unsymmetrical aromatic–aliphatic disulfides the absorption maxima of the symmetrical and unsymmetrical compounds were only a few millimicrons apart and that the absorbancy coefficients of the latter were about half those of the former. The values of K_3' calculated on this basis are quite consistent.

K_2' cannot be obtained from the experiments listed in Table II because in these cases (BSSB) was deliberately made negligible. Experiments in which substantial amounts of all participating species were present at equilibrium are reported in Table III. Since K_3' was now known, eq. 5 was available to make seven equations in six unknowns. It was decided to discard eq. 11 and calculate K_2' on the basis of the others. The values of K_2' calculated from the results are not as consistent as those of K_3', but the consistency must be considered adequate in view of the experimental and

(6) A. J. Parker and N. Kharasch, *J. Am. Chem. Soc.*, **82**, 3071 (1960).

TABLE IV

EQUILIBRIUM CONSTANTS FOR MERCAPTAN–DISULFIDE REACTIONS

Mercaptan	pH (temp., °C.)	Method	K_1	K_2	K_3	K_4	Ref.
		Disulfide = Cystine					
$HSCH_2COOH$	6 (30)	Polarimetry			1.0		*a*
		Solubility	0.8	4.1	(3.3)[b]	5.1	*c*
		Reaction velocity	7.9	1.29	(10.2)[b]	6.1	*d*
Glutathione	(25)	Solubility	2.8	1.0	(2.8)[b]	3.0	*c*
	7.4 (37)	Chromatography	12.4	0.17	(2.11)[b]	(73)[b]	*f*
$H_2NCH_2CH_2SH$	7.4 (37)	Chromatography	4.76	0.75			*e*
$MeCONHCH_2CH_2SH$	7.4 (37)	Chromatography	5.00	0.62			*e*
$Me_3NHCH_2CH_2SH$	7.4 (37)	Chromatography	2.78	55.6			*f*
$HOCH_2CH_2SH$	7.4 (37)	Chromatography	1.39	0.66			*f*
Eight other mercaptans[g]	7.4 (37)	Chromatography	2.04–2.94	0.31–0.66			*e, f*
		Disulfide = Oxidized Glutathione					
$H_2NCH_2CH_2SH$	7.4 (37)	Chromatography	5.0	0.34			*e*
$MeCOHNCH_2CH_2SH$	7.4 (37)	Chromatography	2.86	0.28			*e*
Three other mercaptans[g]	7.4 (37)	Chromatography	1.56–1.92	0.25–0.32			*e*

[a] T. Bersin and J. Steudel, *Ber.*, **71B**, 1015 (1938); [b] Calculated value. [c] I. M. Kolthoff, W. Stricks, and R. C. Kapoor, *J. Am. Chem. Soc.*, **77**, 4733 (1955); [d] H. Lamfrom and S. O. Nielsen, *Compt. rend. trav. lab. Carlsberg*, **30**, 349 (1957); [e] L. Eldjarn and A. Pihl, *J. Am. Chem. Soc.*, **79**, 4589 (1957); [f] A. Pihl and L. Eldjarn, Fourth International Congress of Biochemistry, 1958, Vienna, Vol. XIII, Colloquia, Pergamon Press, London, 1959, p. 43. [g] The following mercaptans were exchanged both with cystine and with oxidized glutathione: $Me_2NCH_2CH_2SH$, $Et_2NCH_2CH_2SH$, $C_5H_{10}NHCH_2CH_2SH$. The following mercaptans were treated with cystine: $MeNHCH_2CH_2SH$, $O(CH_2CH_2)_2NHCH_2CH_2SH$, $H_2N(C{:}NH)NHCH_2CH_2SH$, $PhCH_2NHCH_2CH_2SH$, Aletheine.

computational difficulties involved. The corresponding values of K_2 and K_3 from eq. 4 and 5 are 1.2 and 1.0, respectively.

Since two consecutive, reversible reactions are involved, the kinetics of the interchange reaction must be complicated. Only a restricted study has been made so far. Figure 1 shows some representative values obtained at pH 6.0. The initial BSSB concentration was 2.5×10^{-5} *M* and the initial cysteine concentration in one case was 5×10^{-4} *M*, in the other 1×10^{-4} *M*. In the former case, the value of A_{285} finally attained must correspond to essentially complete conversion of the BSSB to (BSH + BS^-), and the reaction takes place in about 10 min. In the latter case, the final value corresponds to 85% reaction, which accords with the point of equilibrium estimated by means of the constants given above; the time required for releasing one-half the stoichiometric amount of (BSH + BS^-) is 5.5 min. At pH 6.5 the half-time is 1.5 min., and at 7.0 <0.5 min. Clearly, the reaction is quite rapid in all cases, and the rate increases with pH; only qualitative significance should be attached to these results since the rates are too fast for accurate determination in the conditions.

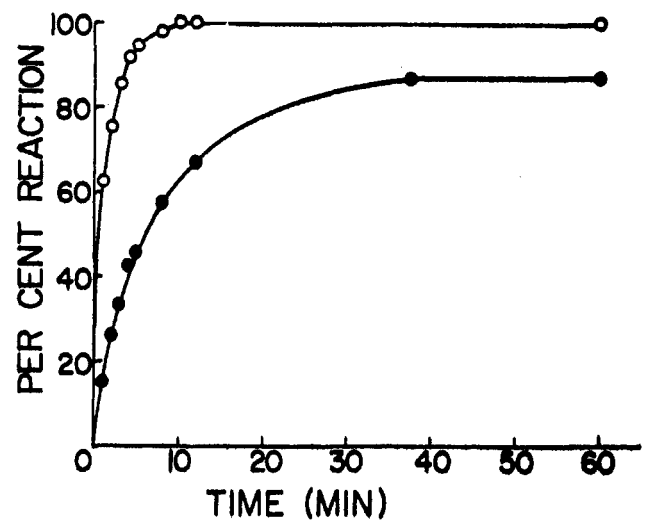

Fig. 1.—Reaction of 2.5×10^{-5} *M* BSSB with excess cysteine, at pH 6.0 and 25°: empty circles, 20:1 excess; full circles, 4:1 excess.

Discussion

Table IV summarizes the results obtained by other investigators for the equilibrium constants of mercaptan–disulfide interchange reactions that were conducted in aqueous buffered medium (for other cases, see paper II[1]). It can be seen that, unfortunately, there is not good agreement between the results of different investigators.

In the pioneering work of Bersin and Steudel on the cystine–mercaptoacetic acid system it was assumed that no appreciable amount of mixed disulfide was formed; since it is now quite certain that this cannot be the case, their value of $K_4 = 1$ must be rejected. Reasons for the other discrepancies are not so clear, however. The extensive investigation of Eldjarn and Pihl was effected with the aid of radioactively labeled compounds which were separated by paper electrophoresis and analyzed. This method probably did not give results of high accuracy, and at least one figure in the quoted values of K is of questionable significance; on the other hand, the method is more direct than those based on the measurement of a physical property. For five of the mercaptans examined by Eldjarn and Pihl, the equilibrium constants obtained in the interchange reactions with cystine and with oxidized glutathione were shown to be internally consistent; for details, the reader is referred to the original references.

Clearly, the data should be regarded with some skepticism, and confirmatory investigations are desirable. On the other hand, it is not likely that the majority of results would be grossly in error, and therefore it can be concluded that structural changes in the groups attached to sulfur have no pronounced influence on the equilibrium constants for the interchange reactions, except when steric interferences may be involved. The

results obtained in this work for cysteine–BSSB differ by a factor of about two from the values expected on the basis of probability and are in agreement with this conclusion.

Despite the fact that the constants are by no means unusual, the behavior of cysteine–BSSB is different from that of systems previously studied, and this serves to point out the important role which may be played by the buffer medium. At pH 7.0, for instance, BSH is almost completely ionized, while RSH is not. As a result, BSSB is almost completely reduced by an equivalent amount of RSH. Qualitative observations of similar import had been made by Eldjarn and Pihl, who reported that cystine was not reduced at pH 7.4 by aromatic mercaptans. It is important to realize this is not due to significant differences in the stabilities of the disulfide bonds involved, at least in the case of BSSB and RSSR, but to the fact that the acid–base reactions taking place with the buffer components provide the driving force for the reaction producing BS^-.

The case of BSH is of course an extreme one. With aliphatic mercaptans, little or no ionization will take place below pH 7, and hence there will be no effect on the interchange reaction. Above pH 7, *e.g.*, in the work of Eldjarn and Pihl that was conducted at pH 7.4, ionizations would certainly be appreciable. Their effect on the point of equilibrium is difficult to evaluate, however, since this depends on the difference between the ionization characteristics of the reactants and products, which are not known for the most part.

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Mercaptan–Disulfide Interchange Reactions. IV. Cysteine and Related Compounds with 3,5-Diimino-1,2,4-dithiazoline[1]

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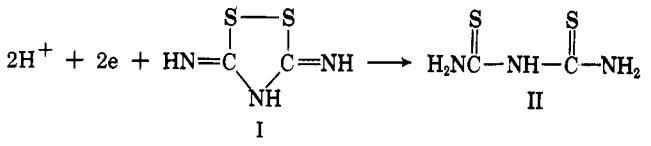
The reaction of 3,5-diimino-1,2,4-dithiazoline (DS_2) with cysteine, glutathione, and 2-aminoethanethiol is first order in DS_2 and second order in mercaptan; the specific rate constants are 9.4, 6.9, and 17.0 × 10^3 moles^2 l.$^{-2}$ min.$^{-1}$, respectively, at pH 4.60 and 30°. At pH 4.6–3.5, the rate is approximately proportional to the reciprocal of hydrogen ion activity. A mechanism is proposed.

This paper is part of a series[2] dealing with the interaction of disulfide and mercapto compounds, particularly those of importance in biochemistry. 3,5-Diimino-1,2,4-dithiazoline (DS_2, I) is a cyclic disulfide of unusual constitution, and a study of its reaction with cysteine and some cysteine derivatives was undertaken in the hope of finding some novel and useful results. DS_2 and dithiobiuret (II) possess the important property of forming a thermodynamically reversible half-cell (E_0 = +0.251 v. at pH 0).[3] The potential of other mercaptan–disulfide systems, which do not give reversible half-cells and are still to some extent uncertain,[4]



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might be determined if an equilibrium between them and DS_2–dithiobiuret could be established and measured.

This possibility has not yet been realized, principally for the reason that the reaction is very slow at the pH and concentrations that might otherwise be suitable. The present paper reports measurements of the reaction rates, and some deductions concerning the reaction mechanism.

Experimental

Materials.—Dithiobiuret, from the American Cyanamid Co., New York, N. Y., was recrystallized three times from hot 0.01 *M* hydrochloric acid, washed with ethanol, and dried *in vacuo*. 3,5-Diimino-1,2,4-dithiazoline hydrochloride was prepared as described by Preisler and Bateman.[3] 2-Aminoethanethiol (β-mercaptoethylamine), from Evans Chemical Co., New York 17, N. Y., was recrystallized from methanol and dried *in vacuo*. L-Cysteine hydrochloride hydrate, from California Corporation for Biochemical Research, Los Angeles 63, Calif., and glutathione, from Schwarz Laboratories, Mt. Vernon, N. Y., were used as obtained. All other chemicals were of reagent grade. The water used in the preparation of all solutions was distilled, passed through a column of Amberlite MB-1 ion-exchange resin, boiled for 20–25 min., cooled with a stream of nitrogen passing through, and stored under nitrogen for no longer than 2 days.

The buffers of pH 4.6–3.8 contained acetic acid–sodium acetate of total concentration 0.1 *M*; the buffers of pH 3.7 – 3.5 were prepared similarly from chloroacetic acid.

Apparatus.—Spectral measurements were done with a Beckman DU spectrophotometer, in 1-cm. silica cells. Measurements of pH were done with a Beckman Model GS pH meter, standardized with commercial buffers.

Kinetic Measurements.—Solutions of DS_2 and mercaptan were prepared in air-free buffer shortly before the measurements; in many but not all cases, the solutions were also made 10^{-3} *M* in ethylenedinitrilotetraacetic acid. Appropriate amounts were mixed to give about 5 × 10^{-5} *M* DS_2 and the desired mercaptan ratio, and an aliquot portion was transferred to a spectrophotometer cell. The absorbance at 246 and 282 mμ was then measured at intervals, against a blank solution containing mercaptan and buffer. The temperature was maintained at 30 ± 0.5°

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(2) See preceding papers: *J. Org. Chem.*, **29**, 1480, 1484 (1964).

(3) P. W. Preisler and M. M. Bateman, *J. Am. Chem. Soc.*, **69**, 2632 (1947).

(4) W. M. Clark, "Oxidation-Reduction Potentials of Organic Systems," Williams and Wilkins, Baltimore, Md., 1960, pp. 471–487.