LXXXIX. THE REDUCTION POTENTIALS OF CYSTEINE, GLUTATHIONE AND GLYCYLCYSTEINE.

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Introduction.

Dixon and Quastel [1923] investigated the potentials of cysteine-cystine and glutathione-oxidised glutathione mixtures. They observed that these two systems presented peculiar difficulties, in that the potentials exerted at noble metal electrodes were drifting and difficult to reproduce. The platinum-calomel cell usually employed for the determination of the reduction potentials of the type quinol-quinone was found to be unsuitable for work on the RSH—RSSR type, but with the use of the gold-calomel cell satisfactory results were obtained. Unlike the potentials of typically reversible systems, both the cysteine and glutathione potentials depended only upon the p_H and the logarithm of the concentration of reduced component. The oxidants in both cases did not affect the electrode. The relationship of the variables in the electrode equation was shown to be the following:

$$E_h = E_0 - \frac{RT}{F} p_H - \frac{RT}{F} \log RSH.$$

Michaelis and Flexner [1928] showed the above equation to hold for cysteine solutions, regardless of the nature of the electrode employed with the exception of the solid gold electrode. By carefully excluding all traces of oxygen from their cell, they obtained identical potentials at electrodes of blank platinum, gold-plated platinum and mercury, reproducible to within 5 millivolts. The most rapid and consistent results came with the use of the mercury electrode. An E_0 of $-0{\cdot}001$ volt was assigned to the electrode equation.

Kendall and Nord [1926] claimed that if an activating agent like indigo carmine was added to a mixture of cysteine and cystine, the reduction potential was determined by the ratio of RSH to RSSR. Their evidence, however, is fragmentary and has been disputed by Dixon and Tunnicliffe [1923] and by Michaelis [1929]. Williams and Drissen [1930] oxidised cysteine to cystine by oxidising agents (I, KIO_3, $K_2Cr_2O_7$, *etc.*) and attempts were made to locate the redox potential by electrometric titration. When so oxidised by titration methods, the cysteine system showed a variation of potential as the oxidation proceeded, which resembled that of the ordinary reversible oxidation-reduction system. However, Ghosh *et al.* [1932] pointed out that what was actually measured, for example in the case of iodine as oxidising agent, was the redox potential of the system $I_2 \rightleftharpoons 2I$. This criticism was substantiated by the experimental result that the E_0 depended upon the nature of the oxidising agent.

Ghosh *et al.* [1932] presented evidence that by the electrolytic reduction of cystine to cysteine at a mercury electrode a reversible system was set up, the

potential of which was determined by the concentration of both the reduced and oxidised forms. The potentials were found to agree with the following equation:

$$E_0 = 0{\cdot}078 - \frac{RT}{F} p_H - \frac{RT}{F} \log \frac{\text{cysteine}}{\sqrt{\text{cystine}}}.$$

These observations of Ghosh *et al.* presented a curious dilemma. When a mixture of cysteine and cystine is prepared by electrolytic reduction, the potential is a function of both the SH and SS concentration, and the E_0 is + 0·078 volt. But when the same mixture of cysteine and cystine is prepared by solution of the crystalline substances, the potential is determined by the concentration of SH only, while the E_0 is − 0·001 volt.

The obvious element of difference between the two mixtures is in the mode of formation of the cysteine. In the one case, cysteine is prepared *in situ* by the electrolytic reduction of a solution of cystine: in the other case, crystalline cysteine prepared by the reduction of cystine with tin and hydrochloric acid is dissolved in the mixture fluid.

Various possibilities presented themselves.

(1) The electrolytic reduction of cystine results in an active form of cysteine, which is in equilibrium with the disulphide.

(2) The electrolytic reduction of cystine involves formations other than that of cysteine, and these formations determine the reversible state.

(3) Electrolytic reduction of cystine may convert it into a form which is in equilibrium with the cysteine produced.

In this report, an experimental analysis is made of the reversible state of the cysteine-cystine system induced by the electrolytic reduction of cystine at a mercury electrode. In addition potential measurements on both glutathione and glycylcysteine are described.

Apparatus.

The nitrogen gas was dispersed into very minute bubbles by means of a Jena filter, and passed through a tower of alkaline pyrogallol (Fig. 1). The

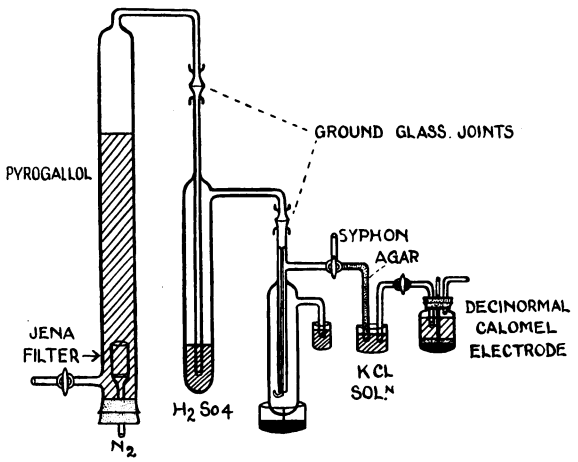

Fig. 1.

complete absorption of all traces of oxygen was attested by the failure of the issuing gas to develop any colour in an alkaline pyrogallol solution. Alkaline hydrosulphite solution was first employed for the removal of oxygen, but was

discarded when examination of the issuing gas disclosed volatile sulphur compounds which affect the electrode considerably.

The cell was devised to avoid the use of either rubber-tubing or bungs. Air leakage, which is the most serious source of error in potential measurements of sulphydryl compounds, is eliminated by the ground-glass joint connecting the cell with the rest of the apparatus. The inner portion of the cell consists of two tubes, one within the other. The inner tube is the gas inlet, and the outer tube the syphon for contact with the calomel electrode. Filling the syphon completely with fluid from the cell proved unsatisfactory in that the pressure in the cell forced liquid through the syphon into the beaker of KCl. This difficulty was overcome by filling the outer limb of the syphon with agar saturated with KCl. Contact of the agar with the fluid in the cell was made in the stopcock. The syphon served also for the withdrawal of samples of the contents of the cell for purposes of analysis. A burette was attached by means of rubber tubing to the limb of the syphon above the stopcock. Since the fluid within the syphon is not comparable in composition to the fluid in the cell proper, the first 3 cc. withdrawn were discarded. A platinum wire fused into the bottom of the vessel made electrical contact with the mercury electrode.

The apparatus is unique in its elimination of all but glass connections from the point of entry of the gas into the tower of pyrogallol to the point of exit from the cell. The cell permits of great ease and rapidity of manipulation.

For electrolysis a voltage of 200 volts was applied. The resistance of the circuit was sufficiently high to cut the amperage down to 5–20 milliamperes as shown by a milliammeter. By adjusting the position of the stopcock in the syphon, the amperage could be set to and maintained at any value in the above range. The cell with the mercury electrode served as the cathode while a beaker of saturated KCl with mercury at the bottom served as the anode.

Methods.

Cystine, prepared from the hydrolysis of hair, was recrystallised three times by dissolving in dilute NaOH and precipitating with dilute acetic acid. Cysteine hydrochloride, prepared by the reduction of cystine with tin and hydrochloric acid, was purified by three recrystallisations from concentrated HCl. This procedure has been shown by Elvehjem [1930] to render the preparation metal-free.

The iodimetric method of Okuda [1925] was employed for the estimation of cysteine. Where the buffer power of the solutions estimated interfered with the conditions of p_H required for correct titration, new factors had to be determined.

The p_H values of the solutions were determined by the hydrogen and quinhydrone electrodes. In the more alkaline regions, only the hydrogen electrode could be relied upon.

The mercury used as electrode was purified by distillation *in vacuo* and cleaned successively with dilute nitric acid and distilled water.

A decinormal calomel half cell was used as the lead off electrode. The potential in the range of temperature between 17° and 20° was taken as + 0·338 volt with reference to the hydrogen electrode. The calomel electrode was calibrated against a quinhydrone-acetate half cell.

The instability of sulphydryl potentials makes it useless to measure with an accuracy greater than 0·5 millivolt. The Cambridge unipivot potentiometer box was found to be sufficiently sensitive for sulphydryl potentials. The accuracy is of the order of magnitude of 0·5 millivolt.

Experimental.

Cysteine potentials.

An attempt was made to repeat the respective measurements of Michaelis and Flexner, and of Ghosh *et al.*

An E_0 value of + 0·030 volt characterises the potentials of cysteine over a wide range of concentration and p_H (Table I). Michaelis and Flexner [1928] reported an E_0 of − 0·001 volt, but in some later experiments by Barron, Flexner and Michaelis [1929], in which the technique was slightly modified, an E_0 more positive by 20–30 millivolts was observed.

Table I.

Cysteine obtained by reduction with tin.

Molarity of cysteine	p_H	E_0 in volts
0·001	8·34	+0·029
0·010	7·17	+0·028
0·010	4·63	+0·031
0·010	6·11	+0·028
0·010	8·08	+0·035
0·010	7·35	+0·029
0·004	8·50	+0·030

Mean E_0: +0·030 volt.
Michaelis E_0: −0·001 volt.

There is fairly good agreement between the E_0 value in Table II and that of Ghosh *et al.* From the dependence of the potential on the ratio of reduced to oxidised component, it follows that a reversible system is set up as the result of the cathodic reduction of cystine at a mercury electrode.

Table II.

Mixtures obtained by cystine electrolysis.

Molarity of cysteine	Molarity of cystine	p_H	E_0 in volts
0·0023	0·0088	9·2	+0·077
0·0038	0·0001	8·5	+0·077
0·0038	0·0001	8·2	+0·077
0·0050	0·0010	8·5	+0·078
0·0038	0·0001	8·5	+0·080
0·0030	0·0005	8·5	+0·073

Mean E_0: +0·077.
Ghosh E_0: +0·079.

The question arose whether the reversibility depended upon some alteration of the mercury electrode rather than upon a true state of equilibrium between cysteine and cystine in the body of the liquid. The following experiments were performed to determine this point.

(1) 0·002 *M* solution of cystine in a borate buffer of p_H 8·50 was electrolysed for 1 hour at 5 milliamperes. The equilibrium value was − 0·372 volt. The final potential was usually reached in about 2 hours. The criterion for equilibrium was constancy of the potential to within 2 millivolts over a period of 1 hour. Samples of the fluid in the cell were removed, and from the concentration of cysteine determined by titration, the theoretical potential demanded by the Ghosh equation was calculated. The agreement was to a millivolt.

The cell was then disconnected at the glass joint and the contents removed. The fluid with fresh mercury was poured back into the cell, and the cell connected again with the rest of the apparatus. The equilibrium potential turned out to be − 0·324 volt, which is precisely the potential demanded by the Dixon equation using the E_0 value of + 0·030 volt.

It is quite clear from this experiment, which was repeated many times, that by electrolysis an equilibrium system is set up at the mercury electrode, but that by renewal of the mercury electrode the condition of irreversibility is restored.

(2) 0·1 *M* cysteine hydrochloride was made up in a phosphate buffer of p_H 7·35. At equilibrium, a potential of − 0·281 volt was reached. A current of 5 milliamperes was allowed to pass through the solution for 1 hour. After this treatment, the equilibrium potential amounted to − 0·305 volt.

Since the solution contained the reduced form initially, the increase in negativity of the potential cannot be due to any increase in SH concentration. The only plausible explanation is that the mercury electrode itself has suffered some change. If this conclusion is correct, then if two mercury electrodes are used, one for electrolysis and the other for comparison, a serious discrepancy should be manifest in the equilibrium potentials recorded by the pair of electrodes.

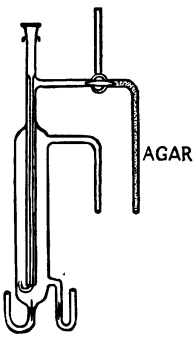

Fig. 2.

(3) This experiment was carried out with the cell shown in Fig. 2. A discrepancy of 60 millivolts was observed in the equilibrium potentials for the two mercury electrodes. The one through which the current passed recorded an E_h of − 0·398 volt which agreed with the Ghosh equation, whereas the control recorded an E_h of − 0·338 volt which agreed with the Dixon equation.

The nature of this alteration at the surface of the mercury electrode during electrolysis was investigated. The following experiments of a rough qualitative nature were carried out.

(1) Mercury was placed in a series of test-tubes containing solutions of cysteine, cystine and reduced and oxidised glutathione in phosphate buffer. The tubes were shaken vigorously for 3 minutes. The supernatant liquid was decanted and tested with H_2S. In all cases, a precipitate of mercuric sulphide resulted. If instead of shaking with air, nitrogen gas from a tank was bubbled through, hardly any precipitate formed. Purified nitrogen bubbled through the mercury for 30 minutes did not effect any observable solution of mercury.

It is quite clear that complex formation of mercury with either SH or SS compounds takes place rapidly in the presence of oxygen. In the complete absence of oxygen, no complex formation ensues from shaking mercury with sulphydryl solutions.

Barron, Flexner and Michaelis [1929] have shown that complex formation in the complete absence of oxygen can take place if a galvanic cell arrangement is set up. Cysteine in contact with mercury forms one half cell and a calomel electrode the other. The potential difference between the cysteine solution and the calomel directs a flow of current from the calomel to the cysteine half cell. If current is allowed to pass for several hours, a distinct coloration of the solution with H_2S is obtained, even though purified nitrogen has removed all traces of oxygen from the cysteine half cell.

This experiment was repeated with cysteine and reduced glutathione. In both cases, the formation of a mercury complex was confirmed. It is noteworthy that in electrolysis the flow of current is opposite to the flow in the

galvanic cell. The observation of Ghosh *et al.* that after electrolysis of cystine H_2S does not form a precipitate was confirmed. But their conclusion from this negative test that no complex formation took place was hardly justified. If mercury goes into solution in a galvanic cell, it may not be unreasonable to expect that with the current flowing in the opposite direction as in electrolysis, the cysteine will go into the surface of the mercury, forming some complex which is confined to the mercury surface. If that were the case, renewal of the surface should eliminate the complex. The experiments described above confirm this prediction.

It may be concluded from these observations that during electrolysis some complex of mercury and cysteine is formed. This complex determines the reversibility of the system. It is conceivable either that this complex is in equilibrium with cysteine and cystine, or that the complex acts as a catalyst in promoting a state of equilibrium between the cysteine and the cystine. By removing the mercury used for electrolysis, the complex is likewise removed and the new mercury electrode attains a potential which depends only upon the concentration of SH.

Colorimetric measurements.

The other experiments on the cysteine potential are concerned with the agreement between the electrode potentials and the potentials determined by the reduction of reversible indicators. It is of great importance in the proper analysis of the cysteine potential to know whether cysteine can reduce dyestuffs to the degree demanded by the formula.

The experiments were carried out in Thunberg tubes. To insure the complete removal of all traces of oxygen, the tubes were three times exhausted and filled with purified nitrogen.

The data in Tables III and IV show that the reducing power as measured by indicators is less than that predicted by the electrode equation. However, the increase in reducing power with increase in concentration seems closely parallel with the electrode phenomena.

Table III.

Cysteine HCl; p_H 7·0; 30°

Indicator	r_H	0·001 *M*	0·010 *M*	0·100 *M*	0·500 *M*
O-Cresolindophenol	20·5	Reduced	Reduced	Reduced	Reduced
Cresyl blue	15·1	,,	,,	,,	,,
Methylene blue	14·4	,,	,,	,,	,,
Janus green A	—	,,	,,	,,	,,
Indigo carmine	9·9	Partial reduction	,,	,,	,,
Nile blue	9·2	No reduction	Partial reduction	,,	,,
Cresyl violet	8·3	,,	,,	,,	,,
Phenosafranine	5·7	,,	No reduction	No reduction	,,
Janus green B	5·2	,,	,,	,,	,,
Neutral red	2·3	,,	,,	,,	No reduction

Table IV.

Cysteine HCl; p_H 7·0; 30°

Molarity	Calculated r_H	Observed r_H
0·001	7·0	9·9
0·010	5·0	8·5
0·100	3·0	6·6
0·500	1·6	3·5

$$r_H = \frac{E_h + 0{\cdot}060\, p_H}{0{\cdot}030}$$

$$E_h = 0{\cdot}030 - 0{\cdot}060\, p_H - 0{\cdot}060 \log \mathrm{CySH}.$$

GLUTATHIONE POTENTIALS.

Dixon and Quastel [1923] studied the properties of impure glutathione.

In Table V are recorded the potentials exerted by solutions of pure crystalline glutathione [Hopkins, 1929] of various concentrations and of various degrees of

Table V.

Glutathione

Molarity	E_h observed in volts	E_h calculated in volts	p_H	E_0 in volts
0·001	−0·0345	−0·0320	4·63	0·0596
0·001	−0·1220	−0·1228	6·22	0·0630
0·001	−0·1685	−0·1675	6·90	0·0628
0·001	−0·1740	−0·1742	7·09	0·0624
0·001	−0·2162	−0·2168	7·96	0·0620
0·001	−0·1725	−0·1711	7·00	0·0636
0·010	−0·2328	−0·2326	7·09	0·0624
0·005	−0·2123	−0·2110	7·09	0·0635
0·005	−0·1645	−0·1644	7·09	0·0623
0·020	−0·2510	−0·2522	7·09	0·0610

Mean $E_h = +0·0622$ volt.

acidity and alkalinity at a mercury electrode. The E_0 value is 30 millivolts more positive than that of cysteine. The potentials agree closely with the following equation:

$$E_h = 0·0622 - \frac{RT}{F} p_H - \frac{RT}{F} \log GSH.$$

The following experiment was performed to prove the inactivity of the oxidised form at the mercury electrode. The cell was filled with 0·001 *M* reduced glutathione in phosphate buffer of p_H 7·09. The equilibrium potential was − 0·174 volt. The cell was disconnected and 62 mg. of oxidised glutathione were added to the contents through the gas inlet tube, making the solution 0·001 *M* with respect to the oxidised form. The equilibrium potential remained at its original value of − 0·174 volt.

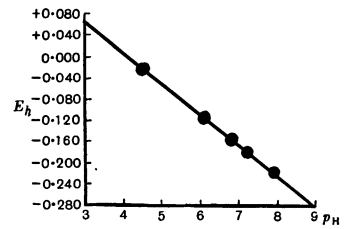

Fig. 3. Glutathione, *M* 0·001; 18°; $E_h = -0·058\ p_H + 0·2354$.

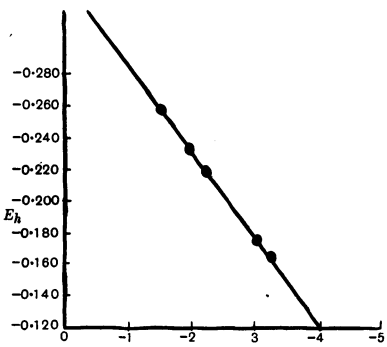

Fig. 4. Glutathione, p_H 7·09; 18°; $E_h = -0·3470 - 0·058 \log GSH$.

Fig. 4 shows graphically the variation in the potential with changes in the molar concentration of glutathione at p_H 7·09. Fig. 3 shows the variation in the potential with changes in the p_H of 0·001 *M* glutathione. The linear relations observed agree closely with the electrode equation.

Colorimetric measurements.

A comparison of the electromotive activity and the reducing power as measured by indicators was also made. The technique was the same as that described for the study with cysteine.

Table VI.

Glutathione; p_H 7·0; 30°

Indicator	E_0 at p_H 7·0	r_H	0·01 *M*	0·10 *M*
O-Cresolindophenol	+0·195	20·5	Reduced	Reduced
Cresyl blue	+0·032	15·1	,,	,,
Methylene blue	+0·011	14·4	,,	,,
Janus green A	—	—	,,	,,
Indigo carmine	−0·125	9·9	,,	,,
Nile blue	−0·142	9·2	No reduction	,,
Cresyl violet	−0·167	8·3	,,	,,
Phenosafranine	−0·242	5·7	,,	No reduction
Janus green B	−0·258	5·2	,,	,,
Neutral red	−0·340	2·3	,,	,,

Table VII.

Glutathione; p_H 7·0; 30°

Molarity	Calculated r_H	Observed r_H
0·0001	10·0	—
0·0010	8·0	—
0·0100	6·0	9·3
0·1000	4·0	6·6

$$r_H = \frac{E_h + 0{\cdot}060\, p_H}{0{\cdot}030}.$$

$$E_h = 0{\cdot}0622 - 0{\cdot}420 - 0{\cdot}060 \log \mathrm{GSH}.$$

It is clear from Tables VI and VII that there is some discrepancy between the potentiometric and colorimetric data. Several indicators which should be completely reduced by 0·01 *M* glutathione, for example Nile blue, are not even partially reduced. However there is an increase in reducing power with increase in the molarity of glutathione as demanded by the electrode formula. Qualitatively colorimetric data compare favourably with potentiometric data, but quantitatively the data are hardly concordant.

GLYCYLCYSTEINE POTENTIALS.

The difference in E_0 values for cysteine and glutathione raised the interesting question of the effect of the number of amide linkages and of the nature of the linkage upon the potentials of cysteine peptides. Cysteinylglycine, which is intermediary between cysteine and glutathione, has never been successfully prepared. Glycylcysteine has been prepared in fairly pure state by Pirie [1931]. This peptide involves an amide linkage between the amino-group of cysteine and the carboxyl group of glycine, whereas in glutathione the amino-group of glycine is coupled with the carboxyl group of cysteine.

In Table VIII are recorded the potential measurements of solutions of glycylcysteine. Surprising indeed is the fact that the E_0 value is even more negative than that of cysteine. Apparently the number of peptide linkages is not a determining factor of the reducing power of cystine derivatives. What is more likely to be the controlling factor is the nature of the peptide linkage.

Table VIII.

Glycylcysteine.

Molarity	E_h observed in volts	E_h calculated in volts	p_H	E_0 in volts
0·0013	−0·2170	−0·2168	7·09	0·0250
0·0100	−0·2670	−0·2673	7·09	0·0255
0·0500	−0·3085	−0·3088	7·09	0·0255
0·0100	−0·1253	−0·1251	4·63	0·0250
0·0100	−0·3950	−0·3948	9·28	0·0250
0·0100	−0·3211	−0·3211	8·00	0·0252
0·1000	−0·3263	−0·3264	7·09	0·0253

Mean $E_0 = +0·0252$ volt.

The typical irreversibility of sulphydryl compounds is also shown by glycylcysteine. In Figs. 5 and 6 are shown the relationship of the variables in the electrode formula

$$E_h = 0·025 - \frac{RT}{F} p_H - \frac{RT}{F} \log RSH.$$

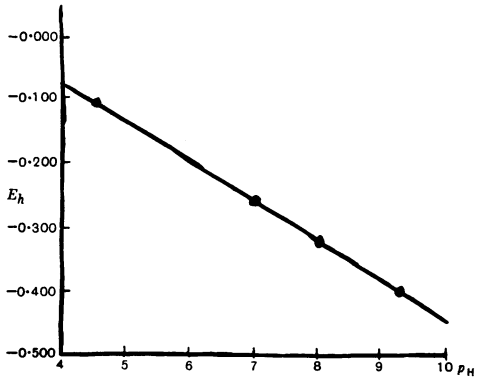

Fig. 5. Glycylcysteine, 0·01 *M*; 18°; $E_h = 0·1407 - 0·058\ p_H$.

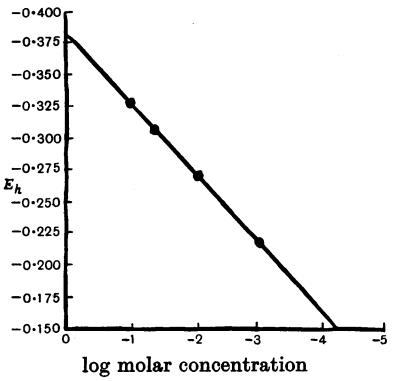

Fig. 6. Glycylcysteine, p_H 7·09; 18°; $E_h = -0·3870 - 0·058$ log RSH.

Discussion.

Dixon and Quastel [1923] in an attempt to explain the peculiarities of the cysteine potential, assumed that the oxidation of cysteine to cystine took place in two distinct steps.

(1) $RS^- \rightleftharpoons RS^* + E.$

(2) $2RS^* \longrightarrow RSSR.$

The first step, involving the oxidation of the anion of cysteine to the free radical, is reversible. The second step, involving the condensation of the free radical to cystine, is irreversible. Thus it follows from the equation that for a constant p_H the potential is determined solely by the concentrations of cysteine and its primary oxidation product, *viz.* RS*. To account for the experimental result that cysteine alone determined the potential, Dixon and Quastel [1923] further assumed that the concentration of the primary oxidation product is both small and constant. The solution will then always be saturated with primary oxidation product.

Michaelis has objected to this theory on the following grounds. (1) If the irreversibility of the second reaction is absolute, then at equilibrium RS* is zero and the potential should be *minus* infinity. (2) If the irreversibility is relative, changes in the concentration of cystine should affect the potential. The experimental results are not in accordance with either of these predictions from the theory.

Dixon [1927] later offered another theory which is based on his observations that the potential of a cysteine solution is approximately 200 millivolts more negative against mercury than against an electrode of pure gold. It is well known that gold and mercury differ strikingly in their capacity for hydrogen over-voltage, and Dixon attempted to relate the differences in the cysteine potentials at these metals to the differences in this property.

Michaelis objected to this theory also on the following grounds. (1) The potentials reached by all electrodes except gold are identical provided all traces of oxygen are removed. (2) If the potential depends on hydrogen over-voltage, then substituting hydrogen gas for nitrogen should lower the potential to a more negative value. In point of fact, the potential at mercury is exactly the same whether a current of nitrogen or hydrogen be passed through the solution. (3) If the potential is determined by equilibrium between the rate at which cystine charges the electrode with hydrogen atoms and the rate at which hydrogen escapes from the electrode as gas, it should be possible to vary the potential by addition of a catalyst increasing the reduction velocity of cysteine. Harrison and Quastel [1928] have shown, however, that additions of small amounts of iron and copper do not appreciably alter the potential of a metal-free cysteine solution.

Michaelis [1929] has offered his own theory to explain the anomalies of the cysteine potential. He postulates that the potentials of cysteine solutions in the presence of an electrode are due to metal complexes of cysteine and not to cysteine alone. Barron, Flexner and Michaelis [1929] have amassed a great deal of evidence for the formation of complexes of cysteine with mercury, platinum and other metals and consider the evidence as sufficient proof of the theory.

The theory, however, has many serious drawbacks. (1) It seems very improbable that if the potential of cysteine solutions involved metal complexes, the same potential should be reached whether mercury, platinum or gold be used as the electrode. The chances that complexes of such different metals should be equivalent electromotively are very slight indeed. (2) The aberrations of the cysteine potential at a p_H of 9 and higher have been explained as due to the dissociations of cysteine. Michaelis and Flexner [1928] have observed this irregular behaviour of the potential in the more alkaline regions, regardless of the type of electrode used. It is certainly difficult to believe that complexes of cysteine with a variety of electrode metals should all possess approximately the same dissociation constants. The variation of the potential with the p_H would be expected to depend upon the nature of the electrode used, which it does not. (3) The evidence is hardly conclusive that complex formation takes place under the conditions of the experiments in which potential measurements are made. That complexes are formed as a result of the passage of a current or as the result of shaking cysteine and mercury in the presence of oxygen does not necessarily imply complex formation in the absence of oxygen and the absence of any current. (4) If the potential of cysteine solutions in the presence of mercury depends on the ratio $\frac{[RSH]}{[HgS_2R_2]}$, the addition of a saturated solution of the mercury complex should change the potential enormously. In point of

fact, Barron, Flexner and Michaelis [1929] failed to observe a change of more than a few millivolts. (5) All known oxidation-reduction systems which are reversible have a span of potentials from the 95 % reduced to the 95 % oxidised state of approximately 70 millivolts. The cysteine potentials, however, can be made to spread over 300–400 millivolts by progressive increases in concentration. It is quite clear that the ordinary type of reversible system cannot explain the cysteine potential. If as Barron, Flexner and Michaelis postulate, the electrode reaction is as follows

$$2RSH + Hg \rightleftharpoons (RS)_2Hg + 2H$$

it is difficult to see why this reversible system should have a span of potentials many times that of the methylene blue system, for example. In going from a state of 95 to 100 % oxidation, the potentials of the methylene blue system vary by a matter of 10 millivolts. Since the concentration of $(RS)_2Hg$ must be extremely small compared to the concentration of cysteine, the Michaelis theory virtually attributes a span of 300–400 millivolts to the change from about 99·9 to 100 %. This of course makes the theory very difficult to accept.

In the usual type of reversible system, two processes are at work at the electrode; the reductant charging the electrode with electrons, and the oxidant discharging the electrode. That is to say there are two mutually compensating processes at the electrode. In practice, it is almost impossible to get either pure oxidant or pure reductant—which means that there are limits to either the positivity or negativity which the system can attain.

Sulphydryl systems lack that simple condition. In terms of electrons, the inactivity of cystine at the electrode means that it cannot discharge the electrons released by cysteine. From electrolysis experiments it is known that only when the electron pressure is comparatively enormous does cystine accept the electrons at the mercury surface. Therefore cysteine builds up an electron pressure, so to speak, which is not in equilibrium with cystine. However, there must be some compensating process, for otherwise the potential would be infinitely negative. Also this compensating process must be constant whatever the concentration of cysteine, for otherwise it would be impossible to get increasingly negative potentials with increases in the concentration of cysteine. What remains to be ascertained is the nature of this compensating process at the electrode. It is noteworthy that the theory of Dixon [1927] is the only one yet proposed which provides a compensation mechanism for the cysteine potential. The theory of Michaelis [1929] completely overlooks this special aspect of the cysteine potential.

The colorimetric measurements with glutathione suggest that the potentials of animal tissues may very well be determined by this sulphydryl compound. A 0·01 *M* solution of glutathione at p_H 7·0 has an r_H of 9·3 which is approximately the anaerobic r_H of tissues.

Summary.

1. The reversibility claimed by Ghosh *et al.* for the cysteine-cystine system prepared by electrolytic reduction has been subjected to experimental analysis. It has been shown:

(*a*) That the mercury electrode employed for the electrolytic reduction undergoes some alteration.

(*b*) That with renewal of the mercury electrode, the potentials do not conform with the equation for the reversible state.

(*c*) That after passage of current through a mercury electrode in contact with a solution of cysteine, the equilibrium potentials of the mercury electrode are shifted to more negative values.

(*d*) That in a solution containing a mixture of cysteine and cystine prepared by electrolytic reduction, the potential at the mercury electrode through which the current has passed agrees with the equation of Ghosh *et al.*, whereas the control mercury electrode agrees with the Dixon and Quastel equation.

2. The colorimetric and potentiometric values for the cysteine potential are not in agreement. Potentiometric values are more negative by approximately 90 millivolts or 3 r_H units. However, the increase in the reducing power of cysteine with increase in the molar concentration, parallels the electrode phenomena closely.

3. Potential measurements on both glutathione and glycylcysteine are recorded. These two sulphydryl compounds exert negative potentials which depend only upon the concentration of reduced component. The E_0 of glutathione is + 0·062, and of glycylcysteine + 0·025.

4. The colorimetric and potentiometric values for the glutathione potential are not in agreement. The potentiometric values are more negative by approximatively 60 millivolts or 2 r_H units. Qualitatively, however, colorimetric and potentiometric data are comparable.

5. The various theories proposed to explain the anomalies of sulphydryl potentials have been reviewed. A suggestion has been made as to the lines of approach which a complete analysis must follow.

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