LXXXIII. THE REDUCTION POTENTIAL OF CYSTEINE.

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(Received April 25th, 1928.)

It has been shown by Dixon and Quastel [1923] that the reduction potential of cysteine-cystine mixtures depends entirely on the concentration of the reduced form, cysteine. Cystine seems to exert no potential under the experimental conditions employed. The suggestion made to explain this anomalous behaviour was contained in the following words: "It seems almost necessary, therefore, to postulate the possible presence of an intermediate compound which would be the "true" oxidised form of cysteine and which is transformed irreversibly into cystine. In such a case the concentration of this intermediate oxidised substance would appear to be maintained at a constant value, as though the solution in presence of cysteine must be "saturated" with respect to this particular compound."

Dixon [1927] has advanced another interpretation of this phenomenon based upon an extension of the Wieland theory of activation of hydrogen. According to Dixon, when an electrode is placed in a solution containing a substance AH_2 which gives a reduction potential, the electrode surface becomes "more or less completely covered with hydrogen atoms loosely attached to the surface." This hydrogen "which corresponds to the 'active hydrogen' of Wieland must not be regarded as atomic hydrogen since it is in combination with the platinum. The attachment is, however, only loose so that it does actually approximate to atomic hydrogen in behaviour." The hydrogen in this state is in equilibrium with hydrogen molecules, with AH_2 molecules and with hydrogen ions, and the potential is determined by the pressure of hydrogen at the electrode and the concentration of hydrogen ions. Now in interpreting the potential of cysteine-cystine mixtures on this view it is necessary to conclude "that hydrogen atoms can be transferred from the sulphhydryl group to the electrode, but that the disulphide form is quite incapable of taking hydrogen back again from the electrode," the system being essentially an irreversible one. But, as Dixon points out, if the hydrogen is transferred irreversibly to the electrode it is to be expected that the pressure of hydrogen will rise to high values, the rate at which the rise occurs being dependent upon the rate of transference of hydrogen from the cysteine to the

electrode. Thus the reduction potential should become more and more negative. Experiment has shown, however, that a steady potential is produced by a given quantity of cysteine, and to explain this Dixon has sought a compensating process, which he has found in the diffusion of hydrogen gas from the electrode. The potential of cysteine is secured, he maintains, by the attainment of a steady state at the electrode which occurs when the velocity of transference of hydrogen atoms to the electrode from the cysteine is balanced by the velocity of diffusion of hydrogen gas from the electrode.

The two processes are represented by the equations:

The velocity v_1 of reaction (1) is $K_1 [\text{R.SH}]^2$; the velocity v_2 of reaction (2) is $K_2 [\text{H}]^2$ where K_1 and K_2 are constants. The steady state occurs when $v_1 = v_2$.

So that

$$K_2 [\text{H}]^2 = K_1 [\text{R.SH}]^2$$

or

$$[\text{H}] = \sqrt{\frac{K_1}{K_2}} \cdot [\text{R.SH}].$$

The value of $[\text{H}]$, *i.e.* of K_1 , K_2 and $[\text{R.SH}]$, determines the potential secured.

Therefore, it is clear that if the velocity constants K_1 or K_2 can be altered, there will be a change in the steady state at the electrode and hence in the magnitude of the reduction potential. The H in equations (1) and (2) corresponds, as Dixon says, to the "active hydrogen" of Wieland. It is the hydrogen which is taken up by "acceptors" such as quinone or methylene blue. The reduction of methylene blue by cysteine, on the Wieland view, must be regarded as due to the transference of active hydrogen from one molecule to the other, the hydrogen in the *reactive* cysteine molecules being considered in an active condition. Now the rate of reduction must be dependent upon the rate of formation of *reactive* cysteine molecules and therefore it is dependent upon the rate of formation of "active hydrogen."

If, then, keeping the concentrations of cysteine and methylene blue constant, the rate of reduction is increased by the addition of a catalyst, it must be concluded that the effect of the catalyst is, virtually, to increase the rate of formation of active hydrogen from the cysteine (*i.e.* to increase the number of fruitful collisions by increasing the lability of the hydrogen of the sulphhydryl group). The effect of a catalyst, therefore, is to increase the velocity constant K_1 of equation (1) to a new constant K_3 .

Let us now consider an electrode present in a solution of cysteine. A steady state is reached when

$$[\text{H}] = [\text{R.SH}] \sqrt{\frac{K_1}{K_2}}.$$

Let a small quantity of a catalyst, *e.g.* a trace of cupric or ferric ion be added

to the solution. Then since K_1 is increased to K_3 , a new steady rate will be reached given by

$$[H] = [R.SH] \sqrt{\frac{K_3}{K_2}}.$$

It cannot be maintained, for the purposes of this argument, that the electrode has properties so essentially different from methylene blue that whereas the effect of an added catalyst occurs in the latter case it does not in the former; for if the effect of a catalyst (assumed to be specific for the hydrogen donor) is to render more labile the bonds between the hydrogen atoms and the rest of the molecule, with resulting increased fruitful collisions with a hydrogen acceptor (*i.e.* an increased rate of transference of active hydrogen), such an increase in fruitful collisions must be observed with any hydrogen acceptor including the electrode; for this, according to Dixon, must be a hydrogen acceptor, or otherwise a potential would not be secured.

In the change of steady state from $[R.SH] \sqrt{\frac{K_1}{K_2}}$ to $[R.SH] \sqrt{\frac{K_3}{K_2}}$ induced by the catalyst the assumption has been made that K_2 is unaltered. This appears to us a reasonable assumption as there is no evidence to suppose that the catalyst in question can affect the velocity of diffusion of hydrogen gas from the electrode.

Hence it is to be expected on Dixon's view that the (hypothetical) steady state at the electrode should be changed on the addition of a catalyst with a resulting increase in the negative reduction potential.

Experiments described in this paper show that purified metal-free cysteine, which reduces methylene blue very slowly, gives a high negative reduction potential in presence of an electrode which is itself not catalytically active. This potential is *not* increased by the addition of a catalyst (Cu^{++} or Fe^{+++}) although under the same conditions the rate of reduction of methylene blue is increased tenfold.

It would seem, therefore, that Dixon's view of the mechanism by which the potential of the sulphhydryl group is obtained at the electrode, is untenable. It seems more likely that the interpretation already suggested (Dixon and Quastel) is correct. Let us suppose that the hypothetical intermediate substance which is the "true" oxidised form of R.SH is the radical R.S and that this is transformed irreversibly into R.SS.R. Then the equations which account for the facts would be as follows:

the presence of a catalyst being without effect on the equilibrium point of the system, and hence on the reduction potential observed. We have, however, no definite evidence yet for the existence of R.S, our suggestion of its presence being purely tentative.

EXPERIMENTAL.

Reduction of methylene blue by metal-free cysteine in presence of the gold electrode.

Since it was desirable that an electrode should be used which was free from catalytic action on the reduction of methylene blue, preliminary tests were made to determine whether the electrode used in our experiments was inert in this respect. Experiments showed that the rate of reduction of methylene blue by metal-free cysteine was not appreciably affected by the presence of the gold electrode. The metal-free cysteine was prepared by the method of Harrison [1924] and the reduction was carried out by the technique described by him [1927]. The pure gold electrode was a plate 1 cm. by 2.5 cm. and 0.5 cm. thick, joined to a pure gold wire. Reductions were carried out *in vacuo* at p_H 7.4 (phosphate buffer) and 37°.

Reduction potentials of cysteine in presence of the gold electrode.

The electrode vessel consisted of a closed tube of Pyrex glass, through which a stream of pure nitrogen was passed by means of a tube dipping in the solution. The nitrogen was purified by passing it through a series of five alkaline pyrogallol solutions and two solutions of alkaline sodium hydro-sulphite and finally through a dilute solution of sodium hydroxide. The bubbling was continued while observations were taken, this being quite an efficient method of stirring. A calomel cell (containing saturated potassium chloride solution) was connected by means of an agar-salt bridge to the solution in the vessel.

In taking readings the procedure was to pipette a definite volume of a phosphate buffer solution (p_H 7.4) into the electrode vessel (thoroughly cleaned with acids), and to bubble nitrogen through until the gold-calomel potential had reached a steady value. The gold electrode was washed by immersing it a number of times in boiling water, after an initial treatment with hot acids. After this washing the electrode gave readings which varied between + 0.07 v. and + 0.09 v. If such an initial value were not obtained, the electrode was re-washed and the contents of the vessel rejected. A known volume of the pure cysteine solution was then introduced into the vessel and the potential of the solution, when steady, was determined by the potentiometer in the usual way. The temperature of the electrode vessel and calomel cell was maintained constant throughout each experiment by immersion in a thermostat.

The metal-free cysteine solution, which was prepared freshly in each experiment with quartz-distilled water and made up to the correct p_H with pure sodium hydroxide, was kept in a well evacuated tube. All tubes containing solutions used in the experiments were evacuated, filled with nitrogen and re-evacuated.

The following is a typical experiment. In the electrode vessel were placed 17.2 cc. of quartz-distilled water and 2.5 cc. of phosphate buffer (Clark and Lubs), p_H 7.4. Nitrogen was run through the solution and the steady potential obtained was + 0.072 v., the temperature during the experiment being 19°. 0.3 cc. of cysteine solution (containing 2.42 mg. pure cysteine) was then added to the electrode vessel, making the final concentration of cysteine $N/1000$. The potential quickly became negative and gave a steady reading in an hour's time at - 0.334 v. 1 cc. of this solution was withdrawn with a clean pipette, taking precautions not to admit oxygen, and was placed in a vacuum tube (specially cleaned as described by Harrison [1927]), together with 0.1 cc. of 1/5000 solution of methylene blue. The reduction time *in vacuo* at 25° was then determined. It was found to be 50 minutes. To the solution remaining in the electrode vessel was added 0.025 cc. of copper sulphate solution, containing 1/200 mg. copper, making the final concentration of copper in solution approximately 1/4000 mg. per cc. The reduction potential became slightly more positive and in half an hour's time was steady at - 0.318 v. 1 cc. of this solution was then withdrawn, placed as before in a specially cleaned vacuum tube, with 0.1 cc. 1/5000 methylene blue solution and the reduction time was again determined. This was found to be $3\frac{1}{2}$ minutes.

Thus the trace of copper decreased the reduction time from 50 to $3\frac{1}{2}$ minutes without effecting any increase in the negative reduction potential of the cysteine solution.

This experiment has been repeated a number of times, always with a similar result.

The effect of the ferric ion is similar to that of the cupric ion, *i.e.* a slightly more positive potential is obtained at equilibrium, whilst a catalytic action is observed. The catalytic effect of iron is much less marked than that of copper, this having already been observed by Harrison [1927].

Ferrous ions have a reverse effect to ferric and cupric ions, a slightly more negative reduction potential being observed on their addition. Since the object of these experiments was to determine whether the catalytic effect of traces of metals was necessarily connected with any change in reduction potential brought about by the metals, a mixture of ferrous ions and cupric ions was added to the metal-free cysteine solution in the electrode vessel. Cupric ions were used in preference to ferric ions because of their powerful catalytic action. It was found that a mixture of ferrous and cupric ions, consisting of 1/100 mg. iron (as ferrous ammonium sulphate) and 1/150 mg. copper (as copper sulphate) effected no change, within experimental limits of error, in the reduction potential of cysteine. For example, in one experiment the initial reading (with $N/1000$ cysteine solution at 18°) was - 0.303 v. and after addition of the mixture of metal ions given above, the steady reading in an hour's time was - 0.301 v. As before, when the steady potentials were obtained, 1 cc. of the solution was withdrawn, before and after the addition of the metals, and the reduction times of each solution were determined. The

reduction time of 0.1 cc. 1/5000 methylene blue solution before metal addition was 22 mins. at 19° and after metal addition 3 mins. at 19°.

The reduction experiments were performed at the same time, the first solution being stored in pure nitrogen until the second solution was withdrawn.

In another experiment the initial steady potential at 18° of *N*/1000 cysteine solution previous to the addition of a mixture of ferrous and cupric ions was - 0.294 v. and, after addition of the metal mixture, the steady potential was also - 0.294 v. (The addition to this solution of a further quantity of cysteine increased the negative reduction potential by an amount equal to that required by the equation obtained by Dixon and Quastel [1923], connecting the potential with the concentration of cysteine.)

The experiments we have described indicate that traces of metals, which bring about a marked catalytic effect with cysteine, do not bring about the increase in negative reduction potential which is demanded on Dixon's hypothesis.

SUMMARY.

Metal-free cysteine gives at a gold electrode a high negative reduction potential, which is not increased by the addition of traces of catalysts such as ferric or cupric ions. This is contrary to the result which would be expected on Dixon's hypothesis of the mechanism by which the reduction potential of cysteine is secured at the electrode.

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