

THE ELECTROCHEMICAL FORMULATION OF THE IRREVERSIBLE REDUCTION AND OXIDA- TION OF ORGANIC COMPOUNDS

JAMES B. CONANT

From the Chemical Laboratory of Harvard University, Cambridge, Massachusetts

The electrochemical study of reversible oxidation-reduction systems composed of organic compounds has been a fruitful field of inquiry during the last five years. These investigations were ably reviewed by Wm. Mansfield Clark (4) in this JOURNAL a little more than a year ago. The present paper is in the nature of a companion article dealing with some of those many oxidation or reduction reactions which proceed readily in solution but which are not strictly reversible. Recent work has indicated that certain of these may be profitably considered from the electrochemical standpoint although, of course, the usual electrochemical equations do not apply. It is the purpose of this review to summarize these recent efforts and to discuss their extension to other examples of irreversible oxidation or reduction reactions, pointing out certain probable limitations. Since the object of this paper is to consider only how far the electrochemical concepts of "potential" may be useful in interpreting such reactions, I shall make no attempt to review all the recent work in the field of oxidation and reduction and shall omit a detailed consideration of the subject of electrolytic reactions. I shall also have occasion to consider a few only of the many important results connected with oxidation which have been obtained recently in the field of biochemistry. When one considers the bewildering mass of information which falls under the head of irreversible oxidation or reduction, it is obvious that in a single article one could only hope either to deal with a small subdivision of this field or to restrict oneself to viewing the whole from a particular angle. I have chosen the latter course; to what

extent this viewpoint is of value is a problem for the future. The beginnings are as yet so small that one can only suggest the general lines along which the next advances will be made.

GENERAL INTRODUCTION

At the outset of this discussion it is important to have in mind clearly the distinction between the two different types of reduction or oxidation processes which are commonly designated by the terms reversible and irreversible. We have certain rather peculiar compounds such as the quinones, the indophenols, and sulfonated indigos, which in solution with their reduction products (hydroquinone, leuco indigo sulfonate, etc.) form systems¹ which are strictly reversible under ordinary conditions. An inert electrode immersed in a solution containing the two components has a definite and reproducible potential which is a logarithmic function of the relative concentrations of the oxidized and reduced substances (4, 6I). Since these potentials are generally a function of the hydrogen ion concentration one must be careful to employ buffered solutions of known pH. If the components of two such reversible systems are mixed, a rapid reaction takes place which comes to a definite equilibrium; the extent of this reaction can be predicted from the oxidation-reduction potentials of the two systems. For example, a solution of quinone in dilute acid is rapidly reduced by titanous chloride or a dilute solution of hydroquinone is oxidized by potassium dichromate; the difference in potential in both instances is so great that the reactions go to practical completion although a definite and calculable amount of quinone and hydroquinone is present in the equilibrium mixture. On the other hand, the action of monochloroquinone on a solution of hydroquinone is incomplete, since the normal potentials of the quinone-hydroquinone and chloroquinone-chlorohydroquinone systems are very near each other. The potentials which determine the extent of such interactions of reversible systems are the normal poten-

¹A mixture of the oxidized and reduced forms of an organic compound (in solution) will be referred to as a *system* throughout this paper.

tials; one can not define or measure the potential of a single substance such as quinone.

A very different picture is presented by the behavior of such substances as unsaturated 1,4 diketones, certain azo dyes and nitro compounds. No significant potentials can be measured in a system composed of dibenzoyl ethylene ($C_6H_5COCH = CHCOC_6H_5$) and dibenzoyl ethane ($C_6H_5COCH_2CH_2COC_6H_5$) or in a mixture of an azo dye and its final reduction products which are the result of a cleavage of the molecule. Although the addition of titanous chloride to a solution of dibenzoyl ethylene rapidly reduces this substance to dibenzoyl ethane, it is impossible to reoxidize dibenzoyl ethane by the addition of even such strong oxidizing agents as potassium dichromate. Similarly, we know of no procedure by which it is possible to go from the cleavage products of an azo dye back to the dyestuff itself.

Still another example is afforded by aliphatic aldehydes which are readily oxidized in alkaline solution to acids, but the reaction can not be reversed by the action of even the most powerful reducing agents available in aqueous solution. Cases of oxidation or reduction in which the reaction proceeds readily in only one direction have been designated as irreversible, meaning thereby irreversible under the particular conditions employed in the experiment.

It is, of course, entirely conceivable that in certain solvents or in the presence of a suitable catalyst such a pair of substances as dibenzoyl ethylene and dibenzoyl ethane might behave like quinone and hydroquinone. As a practical matter no such catalysts have as yet been found² (the usual platinum catalysts seem to be without effect) and the behavior of a variety of substances seems to be the same in water, alcohol and acetone.

In the last analysis the distinction between reversible and irreversible processes is perhaps only one of rates. Quinone and the dyestuffs react very rapidly with soluble reducing agents and their reduction products also react very rapidly with soluble oxidizing agents. Equilibrium is therefore established in solu-

²Certain biochemical catalysts which may constitute an exception to this statement are discussed in a later section of this paper.

tion at room temperature in the course of a few seconds or few minutes when we are dealing with these compounds. On the other hand, while the reduction of dibenzoyl ethylene proceeds very rapidly, reoxidation of dibenzoyl ethane solutions is so slow that it has never been experimentally observed. Given infinite time dibenzoyl ethane and a soluble oxidizing agent might come to just as definite an equilibrium as hydroquinone and an oxidizing agent, but since in the laboratory we are concerned with relatively short intervals of time the distinction between hydroquinone and dibenzoyl ethane is significant. This distinction between reversible and irreversible changes is of the utmost importance, and will be emphasized throughout this review; certain experimental criteria for differentiating the two types of reactions will be suggested later. *The term oxidation-reduction potential has meaning only when applied to systems which are strictly reversible.* Unless the potential of an inert electrode is a logarithmic function of the concentration of both components of the system (the oxidized and reduced compounds) the presumption must be that the system is irreversible. The reduction or oxidation of one component of such irreversible systems can not be formulated in terms of a true oxidation-reduction potential.

If it be granted that there is no direct method of measuring oxidation-reduction potentials of irreversible systems, the question arises as to the possibility of studying such changes quantitatively. If we have a certain substance, for example an azo dye, which is irreversibly reduced by soluble reducing agents (with cleavage of the molecule) the general experience of organic chemists would lead us to believe that this process is in some way conditioned by the oxidation-reduction potential of the reducing agent. Qualitatively the idea of "powerful" reducing agents and "mild" reducing agents has long been in vogue. Is this concept sound? The problem is really this: is the rate of an irreversible process governed by the free energy change involved? In other words, will the speed of reduction of a substance be a function of the oxidation-reduction potential of the reagent employed? Before considering the experimental evidence which

has been collected in the last few years to answer this question, it will be well to outline some of the general principles involved.

It is now usually recognized (in spite of statements to the contrary in the older textbooks of physical chemistry) that there is, in general, no necessary relationship between the speed and the free energy change of a reaction. Thus, if two different reducing agents with the same potential react with the oxidized compound A which is *reversibly* reduced in solution, the speed at which equilibrium is attained may be very different, though the final composition of the equilibrium in regard to A and its reduction product will be identical in both cases. There are many facts which can be quoted to support the contention that in a great number of instances free energy changes and rates of reaction do not run parallel. I am indebted to Professor G. N. Lewis for the apt illustration of this point which a consideration of the process of racemization supplies. Here the free energy change from one pure antipode to another is zero; yet with many compounds racemization is very rapid, while with others it is very slow.

There is obviously one set of conditions under which the speed of a reaction must be governed by a free energy change,—that is, when some equilibrium process controls the amount of material undergoing a subsequent irreversible transformation. This can be expressed in general terms for the irreversible transfer of two hydrogen atoms as follows:



In the above scheme the total process with which we are concerned may be represented as an irreversible transformation of A into P. If this proceeds through the formation of AH_2 in a reaction which reaches equilibrium under the conditions of the experiment, the rate of the irreversible step will be a function of the amount of AH_2 in solution. This in turn will be different according as we use BH_2 (eq. 1) or another substance of different

potential, $B'H_2$ (eq. 2), as our reducing agent. (An algebraic formulation of this is given on page 17). It will be shown that similar mechanisms can be suggested and in some instances fairly well established for those cases in which processes of oxidation and reduction are dependent on the potential of the oxidizing or reducing agent employed. Where no such reversible step controls the rate, the potential of the reagent is probably of no significance.

Usually it is necessary to decide by a series of experiments whether or not a given process of reduction or oxidation is governed by the potential of the reagent employed. Such experiments will now be considered; where the potential of the reagent is the governing factor, they lead to quantitative methods of formulating irreversible reductions or oxidations which are more definite and satisfactory than those implied by the use of such terms as "powerful reducing agent" and "easily reduced substances."

IRREVERSIBLE REDUCTIONS

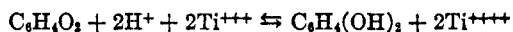
In order to study the relation between the speed of an irreversible reduction and the potential of the reducing agent, it is necessary to perform a number of experiments in which a graded series of reagents of known potential are employed. To determine the rate, one could obviously analyze the solution from time to time but this process is cumbersome and the reactions are often very rapid. A much more convenient method is that based on the observation of the color change of the reducing agent. For example, if leuco methylene blue is used, and the solution remains colorless throughout the experiment it is obvious that no reaction has taken place since the oxidation product of the leuco compound,—methylene blue itself,—is intensely colored. Dr. Clark (6) and his collaborators at the Hygienic Laboratory have prepared a whole series of dyestuffs and very carefully measured their potentials in solutions of varying acidity. Such compounds may be considered as forming a series of oxidation-reduction indicators. The use of such indicators promises to develop a whole new field in the quan-

titative study of the oxidation and reduction processes taking place in living organisms for which purpose they were particularly designed.

A third method which is less convenient than the use of indicators, but which has a somewhat greater range of applicability in the organic laboratory, involves the use of a series of reversible systems and an electrochemical cell very similar to that employed in the determination of the potential of reversible systems. In this method an equimolecular mixture of the reducing agent and its oxidized form is placed in the cell and the potential of an inert electrode measured in the usual way. The substance under examination which is to be irreversibly reduced is added. If it reacts with the reducing agent when introduced into the solution, changes in the composition of the reversible system will result and will affect the potential. The rate of change of potential will be a function of the speed of the irreversible reduction; if no change takes place one can conclude there has been no reaction. It is necessary to use as reducing agents only those substances which form strictly reversible systems which rapidly come to equilibrium. A number of organic compounds and certain inorganic compounds are suitable, but the electrode equilibria adjust too slowly in the case of a number of common reducing agents, such as stannous chloride. A series of suitable reversible systems (including many of Dr. Clark's dyes) are shown in figure 1; the potential of an equimolecular mixture of the two components in dilute solution (the "normal" potential) is plotted against the hydrogen ion concentration. It should be noted that the organic reagents for the most part are designated by the name of the oxidized form; as a *reducing* agent the reduced form would be employed.

The reader is referred to Dr. Clark's article for an exposition of the principles underlying the change of potential of reversible organic systems with change of hydrogen ion concentration. It is immaterial for our purpose whether the reduced and oxidized components of these reversible systems are actually hydrogenated and dehydrogenated organic compounds of the type BH_2 and B , or salts derived from one or the other of these, or

inorganic ions. It is necessary only that these systems are reversible in the sense that in solution they rapidly react with one another coming to a definite equilibrium whose composition is a function of their respective potentials. It is obvious that since we are working always with solutions containing water that the requisite hydrogen in many instances must come from the hydrogen ion as illustrated by the *reversible* interaction of quinone and titanous chloride.



Whether the fundamental reaction of reversible organic systems is a transference of hydrogen atoms or of electrons is an interesting problem but one with which we are not here concerned.

The electrochemical method just outlined has been used in studying the irreversible reduction of a number of azo dyes, nitro compounds and unsaturated 1,4 diketones and related substances in dilute acid and in buffered alkaline solutions. No exceptions have been found to the statement that the reduction in homogeneous solution of such compounds is a function of the potential of the reducing agent. This generalization can hardly be the result of accident, since the reagents include a number of different types of organic and inorganic substances. It may be concluded, therefore, that the action of soluble reducing agents on these compounds may be formulated in terms of the potential of the reagent. This is conveniently done by considering a hypothetical system which would just cause "appreciable reduction" (20 to 30 per cent, in thirty minutes); the potential of such a "critical reagent" may be considered as "the apparent reduction potential" of the substance being investigated under the conditions employed.

In most instances it has been found necessary to estimate "the apparent reduction potential" (A.R.P.) as lying somewhere between the potentials of two reagents about 100 millivolts apart, one of which causes less than 5 per cent reduction in thirty minutes, the other of which causes almost instantaneous reduction. In a few instances "border line cases," have been observed corresponding closely to the action of the hypothetical

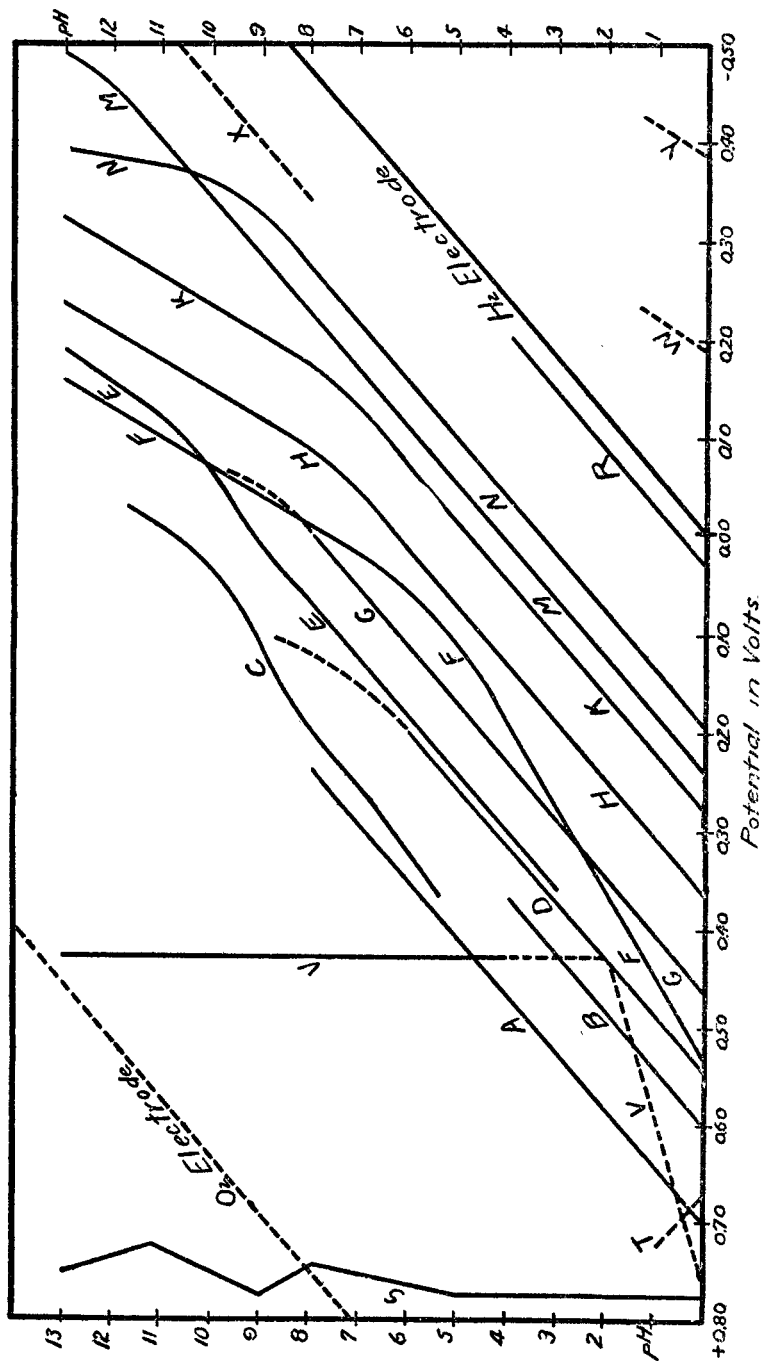
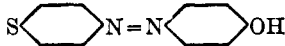
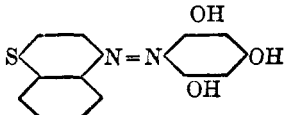
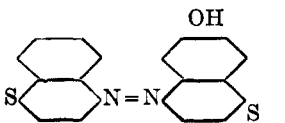
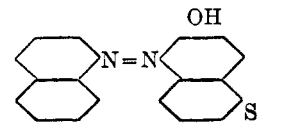


FIG. 1. NORMAL OXIDATION-REDUCTION POTENTIALS AT 25° OF SOME REAGENTS IN BUFFER SOLUTIONS OF VARYING PH
(Concentrations about 0.001 M; dotted lines indicate uncertain values)

- | | | |
|-------------------------------|--------------------------------------|---------------------------------------|
| A. Quinone | G. 1, 4-Naphthoquinone | S. $\text{K}_2\text{Mo}(\text{CN})_6$ |
| B. p-Xyloquinone | H. Indigo tetrasulfonate | T. $\text{Fe}^{3+} - \text{Fe}^{2+}$ |
| C. m-Bromoindophenol | K. Indigo disulfonate | V. $\text{K}_2\text{Fe}(\text{CN})_6$ |
| D. 1, 2-Naphthoquinone | M. 1, 8-Anthraquinone disulfonate | W. $\text{V}^{5+} - \text{V}^{4+}$ |
| E. 1-Naphthol-2-sulfonic acid | N. β-Anthraquinone sulfonate | X. $\text{Na}_2\text{S}_2\text{O}_8$ |
| F. Methylene blue | R. $\text{Ti}^{4+} - \text{Ti}^{3+}$ | Y. $\text{Cr}^{3+} - \text{Cr}^{2+}$ |

reducing system itself whose potential would be the "apparent reduction potential." It can be shown on the basis of the equations developed later in this paper, that a difference of 90 millivolts in the potential of two reagents would correspond to differences in rate of the irreversible step of about 1000 fold. A glance at Figure 1 will show that only in certain ranges and at certain pH values can we find, at present, reagents nearer together than 100 millivolts so that we are usually concerned

TABLE 1
Apparent reduction potential of certain azo-dyes in 0.2N HCl at 25°

FORMULA	A. R. P. (NORMAL HYDROGEN ELECTRODE = 0)
	volts +0.42
	+0.36
	+0.32
	+0.29

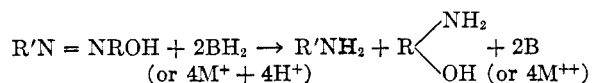
(S = SO₂H group.)

The results are significant within 10 millivolts.

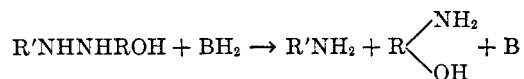
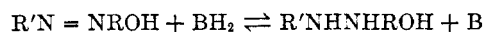
with distinguishing between reactions that are so slow as to be inappreciable and those that take place rapidly; an exact definition of "appreciable reduction" is at present not important.

The relationship between A.R.P. and structure is of considerable interest both practically and theoretically. When sufficient data along these lines have been collected such relationships should replace our present qualitative statements about the relative ease of reduction. The results given in table 1 are

representative of those obtained in a study of the reduction of some 30 azo dyes (13). The determinations were made for the most part in dilute acid solution (pH 0.76) but a number of experiments were also made with various buffer solutions to determine the change of A.R.P. with change in pH. The reaction takes place according to the equation:



It is extremely probable that the reversible step which controls the rate is the formation of the hydrazo compound which then undergoes further reduction with cleavage of the molecule, the last step being irreversible.



The difficulty of isolating ortho or para hydroxy and amino hydrazo compounds accords with this mechanism.

The influence of changes in structure on the A.R.P. is very well illustrated by a series of unsaturated 1,4 diketones and related substances (table 2) measured in acid solution containing a large percentage of acetone to render the compounds soluble (11). Here a slightly different series of reversible systems must be employed and the experimental difficulties are considerable. There is also a question what standard of potential should be chosen with such a solvent but the results are comparable with those in alcoholic and aqueous solution within 40 to 60 millivolts as measurements of the A.R.P. of benzoylacrylic acid in all three solvents have shown. A discussion of the significance of these results from the standpoint of the organic chemist is hardly justifiable in a general review of this sort, but a matter of some importance is involved in a comparison of the unsaturated 1,4 diketones and quinones, both of which contain the conjugated diketonic system $\begin{array}{c} -C-C=C-C- \\ || \quad || \\ O \quad O \end{array}$. If it be as-

sumed that the addition of hydrogen or removal of hydrogen with this particular system involves a 1,6 addition or elimination process, the reversible behavior of quinone on the one hand and the irreversibility of the diketones on the other is readily understood (11, 8). In the one case the di-enol which can be "dehydrogenated" being stable is always present in solution, in the other it has only a transient existence and is irreversibly

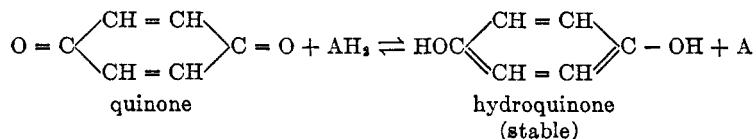
TABLE 2
Apparent reduction potentials

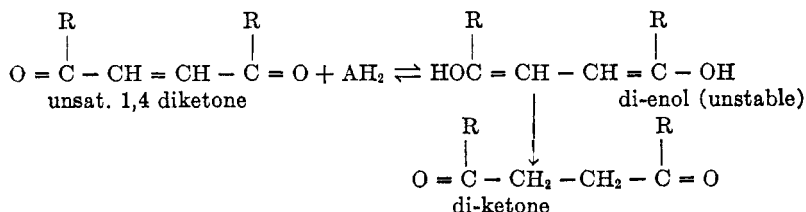
SUBSTANCE	SOLVENT	APP. RED. POT. (NORMAL HYDROGEN ELECTRODE = 0)
(A) Certain unsaturated compounds		
Dibenzoylethylene (cis and trans) ($C_6H_5COCH=CHCOC_6H_5$).....	a	+0.27 (± 0.02)
Benzoylacrylic acid ($C_6H_5COCH=CHCOOH$).....	a	+0.06 (± 0.04)
Benzoylacrylic acid.....	b	+0.06 (± 0.04)
Benzoylacrylic ester ($C_6H_5COCH=CHCOOC_2H_5$).....	a	+0.06 (± 0.04)
Maleic ester ($C_2H_5OOCCH=CHCOOC_2H_5$).....	a	-0.25 (± 0.06)
Maleic acid.....	a	-0.25 (± 0.06)
Maleic acid.....	b	-0.25 (± 0.06)
(B) Certain nitro compounds		
1, 3, 5 Trinitrobenzene.....	a	+0.26 (± 0.02)
2, 4 Dinitrobenzoic acid.....	b	+0.23 (± 0.02)
1, 3 Dinitrobenzene.....	a	+0.16 (± 0.01)
m-Nitrobenzoic acid.....	b	+0.06 (± 0.04)
Nitrobenzene.....	a	+0.06 (± 0.04)
Phenylnitromethane.....	a	-0.08 (± 0.06)

a = 75 per cent acetone, 25 per cent aqueous hydrochloric acid; total acidity 0.2N.

b = aqueous 0.2N HCl.

isomerized to the diketone; such an explanation is in harmony with our general knowledge of the keto-enol changes in such systems.





The few results obtained with nitro compounds(11, 13) are given in table 2 B; they are in agreement with the organic chemist's general experience that polynitro compounds are "easier" to reduce than mononitro compounds. It might be mentioned that the aromatic nitroso-hydroxylamine systems are reversible (11).

It is already possible to formulate certain more or less empirical rules relating apparent reduction potential and structure. With more data more general statements should be possible; it is not too much to hope that eventually it will be possible to develop a general theory embracing all those types of organic reductions which can be studied in this way. The organic chemist should then be able to decide with more certainty than at present, the best reagent and the most favorable conditions for bringing about the reduction of a new compound. Although this time has not yet arrived it may be well to consider from an electrochemical standpoint the use of the more usual soluble reducing agents.

The action of soluble reducing agents

There are some important differences between the "pure" reducing agents employed in the laboratory and the reversible systems composed of equal mols of reduced and oxidized compound which I have hitherto specified in this discussion. A solution of "pure" leuco indigosulfonate, for example, is electrochemically very different from an equimolecular mixture of leuco indigosulfonate and indigosulfonate. The former has theoretically an infinitely low potential, the latter a definite

potential called the "normal" potential (E'_o).³ At a definite hydrogen ion concentration the equation relating the potential of a reversible system (E_h) and the concentration of the components at 25° is as follows:

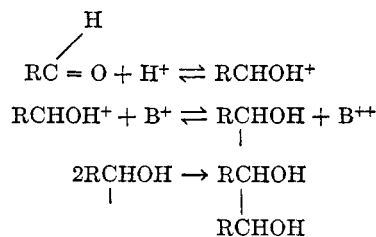
$$E_h = E'_o - \frac{0.059}{n} \log \frac{[\text{Red.}]}{[\text{Oxid.]}}$$

where n is the number of hydrogen equivalents involved in the process; as a rule with organic systems $n = 2$, with the inorganic ions $n = 1$. It is readily calculated that if a "pure" reducing agent contains 1 per cent of the oxidized form its potential will be $2 \left(\frac{0.059}{n} \right)$ volts greater than the normal (i.e., equimolecular) potential. What a reagent will accomplish by being oxidized from the "pure" state to 1 per cent oxidized is not very important so that we may consider the "working range" of a reagent to be its "normal" potential ± 0.059 volts if $n = 2$, or ± 0.118 if $n = 1$. It is to be noted that this "normal" potential must be taken as that of the equimolecular mixture under the conditions specified. The calculation of this from one set of experimental conditions to another is unfortunately complicated by the so-called "salt effect," the formation of complex ions, and the change in acidity of the solution. An exact treatment takes one far into the question of the difference between concentration and activity, evaluation of liquid junction potentials and other recondite electrochemical matters. The complications are not so great with most organic systems as they are where we are dealing with those inorganic salts, the activity of whose ions may vary tremendously with changes in concentration and solvent and whose "normal potential" may be more or less in the nature of an abstraction. If we confine our attention to those systems in which the activity coefficient of both components is not very different from unity and is affected to the same degree by slight changes in solvent, we are on relatively

³The European convention in regard to sign has been used throughout this paper; the potential of the chlorine electrode has been regarded as positive, that of the alkali metals as negative.

safe ground. In applying electrochemical ideas to a specific problem, one must be ever on one's guard that the simple equations involving concentrations and not activities are sufficiently close approximations for the purposes at hand. A test of this point may be obtained by studying the variations in potential with changes in concentration and solvent. It is difficulties of this sort, more than anything else, which limit the practical usefulness of the electrochemical ideas. However, the reagents listed in figure 1 are those which, *in the pH range plotted and in dilute solution* are generally free from large "salt effects" and are reliable enough for the purposes at hand.

If the irreversible step is a monomolecular reaction, then the "apparent reduction potential" will be essentially independent of the total concentration; this has been shown to be the case with the azo compounds and the 1,4 diketones, and is probably true in many other instances. Where this is not the case the situation is more complicated and the "apparent reduction potential" is a less satisfactory concept, necessarily varying with the concentration. The reduction of the "halochromic salts" of aryl carbinols, unsaturated ketones and certain aldehydes afford examples of this sort (9). Here the rate of the irreversible step which is bimolecular is a function of the total concentration; nevertheless even here one can state with some assurance that only reagents of less than a certain potential will bring about the reaction under definite conditions. The bimolecular reduction of an aldehyde by a homogeneous reducing agent (B^+) is illustrated below:



The temperature would be expected to affect the speed of the irreversible reduction but its effect on the "apparent reduc-

tion potential" can not be predicted in general as several different factors are involved. In the case of those compounds so far studied, a change of 50° has not very greatly affected the values which would be assigned to the A.R.P. (13).

In general, one can predict that the calculated quantity of the "pure" reducing agent should bring about rapid and complete irreversible reduction (under the conditions specified) if the normal potential is more than about 60 millivolts lower than the A.R.P. of the compound; if more than 100 millivolts higher the reagent will be "without action." A reagent falling between these ranges might be expected to cause slow reduction, the rate of which would decrease as the reaction proceeded for reasons given below.

With the exception of stannous chloride and sulfurous acid, most of the usual soluble reducing agents are shown in figure 1. A consideration of the potentials there plotted and the A.R.P.s given in table 1 and 2 lead to many obvious predictions to which as far as I am aware, there are no exceptions. The difficulties of the stannous-stannic system are of the type mentioned above and its potential is difficult to measure. In fairly concentrated hydrochloric acid an equimolecular mixture of stannous and stannic chlorides has a potential of $+0.4$ (20). Predictions based on this value *under precisely these conditions* will not be greatly in error.

The case of sulfurous acid is different from the other soluble reducing agents commonly employed; its oxidation to sulfuric acid is irreversible, and a study of it presents just the reverse of the problem we have been considering. No real oxidation-reduction potential can be given but an approximate "apparent oxidation potential" might be evaluated by methods to be discussed in a later section. One would expect that if the A.R.P. of a compound were about that of the "apparent oxidation potential" of sulfur dioxide under the same conditions, reaction would occur. The general information about the uses of sulfur dioxide as a reducing agent is in accord with this idea, but no special study has been made as yet.

The derivation of certain approximate equations

For the sake of completeness, the following simple algebraic analysis of the factors concerned in the preceding discussion is given; the discussion is based on concentrations throughout for simplicity's sake and because the requisite information in regard to activities is in most cases lacking. Where organic reversible systems are concerned the equations may be expected to hold fairly well, with inorganic salts for the reasons given above they are more uncertain. Since, according to our theory, it is the amount of material present as a result of the reversible reaction which governs the speed of the entire process, these equations are useful in making approximate predictions in regard to the effect of the relative amount of reducing agent and other variables. If we represent the reversible step by a general equation where AH_{nm} is the



material undergoing the irreversible transformation we obtain the following equations:

$$(E'_o)_A (\text{pot. } A/AH_{nm}) - (E'_o)_B (\text{pot. } B/BH_n) = \frac{0.059}{nm} \log \frac{[AH_{nm}] [B]^m}{[BH_n]^m [A]} \quad (5)$$

(reversible step) (reducing mixture)

If we start with z moles of reducing agent (BH_n), y moles of its oxidized form and c moles of A per liter and let x be the fraction of A converted to AH_{nm} as a result of the reversible step we have:

$$\text{Diff. in potential} = (E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log \frac{cx(y + mcx)^m}{(z - mcx)^{mc} (1 - x)} \quad (6)$$

If cx is small compared to c , z and y , we can write approximately:

$$(E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log x + \frac{0.059}{n} \log y - \frac{0.059}{n} \log z \quad (7)$$

If $z = y$ in equation 7 we have the simplified approximate equation:

$$(E'_o)_A - (E'_o)_B = \frac{0.059}{nm} \log x \quad (8)$$

The concentration of the substance undergoing the irreversible change is $x(c - s)$ where c is the initial concentration of the substance A and s is the amount of A which has been irreversibly reduced at any given time. We may, therefore, write the usual equation for a monomolecular reaction:

$$\frac{ds}{dt} = kx(c - s) \quad (9)$$

If a large excess of reducing agent containing an equal amount of the oxidized form is employed, x will be essentially constant throughout the entire reaction and we may integrate equation 9 obtaining equation 10 in which the usual constant k_1 is equal to kx . It is obvious that for a given transformation k is constant but x may be varied by using reducing agents of different potential: therefore the rate of the entire process (k_1) will be a function of the potential of the reagent employed.

$$k_1 = kx = \frac{2.303}{t} \log \frac{1}{1 - s} \quad (10)$$

If we use about an equivalent quantity of "pure" reducing agent (y small but not zero) a more complicated situation results; if $c = z$ and cx is small compared to y and c , equation 7 still holds approximately, but x is not constant during the course of the reaction. As the reaction proceeds z becomes smaller, y increases; these changes will continuously decrease the value of x and thus the rate of the irreversible step ($k_1 = kx$). If we start with one equivalent of reducing agent containing 1 per cent of oxidized compound, the value of x will fall throughout the course of the reaction from its initial value as follows ($n = 2, m = 1$); 10 per cent reduction $1/10$; 50 per cent, $1/100$; 90 per cent, $1/1000$. The decrease in speed of the reaction will correspond to these variations in the amount of labile reduction product formed by the continually readjusting equilibrium reaction. Between 10 and 90 per cent reduction, the rate will vary ten fold either side of that which is characteristic of the half-completed reaction. With larger amounts of oxidized form present in the reducing mixture this variation becomes less, until with an equimolecular mixture

Let us suppose that we were to treat the azo compound in dilute acid with a large excess of a reducing agent (BH_2) containing an equal amount of its oxidation product (B) (or an inorganic positive ion M^+ and its oxidized state M^{++}). The reversible reaction would proceed to a definite equilibrium (eq. A) the composition of which can be calculated from the known potential of the azo-hydrazo system (0.373 at 18° in 0.1 N HCl) and that of the reducing system ($BH_2 - B$, or $M^+ - M^{++}$). Since we have specified a large excess of a reducing agent containing its oxidation product, equation (8) above serves to calculate the fraction x of azo compound converted to hydrazo as a result of the reversible step and this fraction will be practically constant throughout the process. Biilmann has found the rate of the rearrangement process at 18° in the presence of acid to be such that $k = 0.076$ (half life of nine minutes). Substituting this value and that of x calculated from equation (8) into equation (10) we can predict the rate of semidine formation with any reducing agent we may choose to employ at 18° , always assuming that there are no disturbing influences on the rate of the irreversible step. The calculations are given below for a few hypothetical reducing agents of different potential: (E'_0 for the azo-hydrazo system being $+0.373$)

E'_0 OF REDUCING AGENT	x CALCULATED FROM Eq. 8 ($nm = 2$)	$k_t = kx = 0.076x$	TIME FOR 20 PER CENT OF REACTION CALCULATED FROM EQ. 10
			<i>minutes</i>
+0.402	0.1	0.0076	31
+0.431	0.01	0.00076	310
+0.460	0.001	0.000076	3100

It is evident that with the first reducing agent ($+0.402$) we would get an appreciable reduction in half an hour but with the other two it would take five and fifty hours respectively to reduce 20 per cent of the azo compound to the semidine; certainly in the last case evidence of reaction would be detected only by the most patient investigator! Reagents of lower potential than 0.402 (more powerful reducing agents) would cause still more rapid reduction, the maximum rate possible being that corre-

sponding to the rearrangement of the pure hydrazo compound (20 per cent in three minutes).

Here, then, is a very definite example of an irreversible transformation conditioned by the potential of the reducing agent employed. It should be noted, however, that this state of affairs would be true only if the reducing agent and the azo compound *react rapidly in comparison with the rate of the irreversible step*. If the speed of the irreversible step is faster than the speed of attaining equilibrium, then no equilibrium will be reached and the potential of the reagent employed will be of no significance. Since at 18° the formation of semidine is rather slow and the reaction of the azo compounds and most soluble reducing agents rapid, our predictions in this instance seem likely of experimental verification.

Catalytic hydrogenation and heterogeneous reduction

If a solution of one component of a reversible system is shaken with hydrogen and a catalyst (platinum or palladium, for example) an equilibrium will be established which can be predicted from the normal potential of the reversible system and the potential of the hydrogen electrode in the solvent in question (Denham (15)). This is merely a special case of two reversible systems interacting, the catalyst seeming to make the system H_2 gas/ H^+ truly reversible. If a compound whose irreversible reduction can be formulated in terms of reduction potentials is similarly treated, reduction will occur if the "apparent reduction potential" is somewhat above that of the hydrogen electrode. The hydrogen-platinum catalyst system is here functioning like any other reversible reducing agent, and reduction is presumably proceeding through the same two steps which are characteristic of the reactions hitherto discussed. However, if the catalyst-hydrogen combination is able to add hydrogen atoms to the compound by some mechanism *not involving a reversible step*, then the presumption is that the process may proceed although the "apparent reduction potential" is below that of the hydrogen electrode. Such a case has been experimentally examined in some detail (8). Of the known soluble reducing agents which

can be employed in aqueous solution *only* chromous chloride ($E'_{0} = -0.40$ at $\text{pH} = 1$) reduces maleic acid, yet hydrogen and a suitable catalyst ($E'_{0} = -0.059$ at $\text{pH} = 1$) hydrogenates the substance fairly rapidly. A large number of ethylene derivatives, which are not reduced even by chromous chloride, can be "catalytically hydrogenated" in solution at room temperature. Here plainly the process of catalytic hydrogenation is running a special course, which is in no sense a function of the potential of this reagent. Such hydrogenations or reductions, can not be formulated in electrochemical terms and probably involve the direct irreversible addition of hydrogen to the ethylene linkage. Catalytic hydrogenation is an excellent illustration of a process of reduction which as a rule can not be treated from the electrochemical point of view.

The use of two phase systems in bringing about reduction is very common, but very difficult to treat quantitatively. As the pioneer researches of Haber on reduction at a cathode showed, the rate of diffusion to the active surface is of great importance and may be the governing factor. The subject of cathodic reduction is amply treated, both from the practical and theoretical standpoint in texts on electrochemistry, it would be unwise to attempt to add to the discussion beyond calling attention to the fact that cathodic reduction may involve a reversible step or may like catalytic hydrogenation proceed directly and irreversibly. If the difficulties of diffusion rates could be eliminated or better controlled, these two possibilities might be investigated more fully in a number of instances; the results perhaps could then be correlated with the action of soluble reducing agents.

Much the same difficulties are encountered when one attempts to deal with the action of metals on solutions of organic compounds. To assign a definite potential to a metal against a solution of its ions is easy, but to say whether this potential actually controls the process of irreversible reduction is at present impossible. From the more or less qualitative evidence familiar to all organic chemists, it seems as if the reducing action of metals were a function of their potential. But more than

this can not now be stated. As in the case of catalytic hydrogenation and probably cathodic reduction, the possibility always must be considered that some special irreversible process is taking place that is in no direct way connected with the reducing potential of the metal.

OXIDATION AND DEHYDROGENATION

Of the great variety of transformations embraced by the term oxidation, only those which take place in solution at ordinary temperatures come within the scope of this review; how many of these can be formulated electrochemically is a matter for future experimentation to decide. At present it is possible only to indicate certain probable lines of attack and to suggest certain general principles which would seem to apply as surely here as in the much simpler field of reduction.

The reversible processes present little difficulty. The oxidation of hydroquinone, or of leuco indigosulfonate in solution by a component of another reversible system proceeds to a final equilibrium which can be as definitely predicted as any process in organic chemistry. The rate at which such equilibria are established when one is dealing with the usual soluble oxidizing agents happens to be very rapid, but concerning it our electrochemical equations give no information. Whether leuco indigosulfonate in a certain solution is "completely" oxidized or not is a question which can be answered satisfactorily in terms of electrochemical potentials if one uses such oxidizing agents as quinones, potassium dichromate or ferricyanide. If some compound not a component of a reversible system is taken as the "oxidizing agent" (e.g., an azo dye) then the problem is simply the one we have been considering under the head of irreversible reduction.

In line with the general theory developed in the introduction to this paper, we should expect to find a relation between the potential of the reagent employed and the rate of the irreversible oxidation only if some reversible step controlled. The oxidation of an ethylenic linkage by ozone (proceeding through the ozonide) or the addition of hypobromous or hypochlorous acid

to an unsaturated compound are specific reactions of these reagents which are obviously not controlled by their oxidizing potential; to attempt to connect these reactions with potentials would probably not occur to anyone. The same may probably be said of the addition reactions of the halogens which though formally an "oxidation" are often considered by organic chemists as closely akin to the addition of the halogen acids or "nascent" hydrogen. We seem here to be concerned with the addition of specific things to a non-polar molecule and are not dealing with

TABLE 3

Determination of A.O.P. of 1,2- and 1,4-Aminonaphthol in 0.2 N HCl at 25°

OXIDIZING REAGENT (CONTAINING EQUIMOLECULAR AMOUNT OF REDUCED FORM)	NORMAL POTENTIAL IN pH 0.76 (NORMAL HYDROGEN ELECTRODE = 0) OF REAGENT	CHANGE IN POTENTIAL (MILLIVOLTS) (ON ADDING HYDROCHLORIDE OF AMINOPHENOL)			
		1,4 aminophenol		1,2 aminophenol	
		5 minutes	30 minutes	5 minutes	30 minutes
Indigo tetrasulfonate.....	+0.326	0	0	0	0
1,4 naphthoquinone.....	+0.426	0	0	0	0
2,6 dimethoxyquinone.....	+0.471	3	12	0	0
1,2 naphthoquinone.....	+0.506	17	50	7	14
2,5 dimethylquinone.....	+0.449	70	95	38	43
Potassium ferricyanide.....	+0.631	170	185	126	132
Quinone.....	+0.656	185		136	
Ferric chloride.....	+0.726	250	280	220	228
K ₂ Mo(CN) ₈	+0.776	325	332	270	280

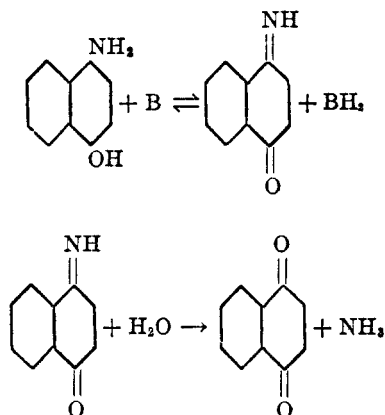
From the above, the A. O. P. in pH 0.76 at 25° of 1,4 aminonaphthol is + 0.460 ± 0.010 millivolts; of 1,2 Aminonaphthol is +0.500 ± 0.010 millivolts.

a series of more or less "powerful" reagents capable of bringing about the same change.

The irreversible changes which involve the elimination of hydrogen and which can be brought about in solution at room temperature are the most likely subjects for an electrochemical investigation of the type we are here discussing. To what extent are the rates of such reactions conditioned by the potential of the oxidizing agent employed?

The oxidation of the aminonaphthols to the corresponding

quinones (which is very rapid in acid solution at room temperature) has been investigated by means of a series of reversible oxidizing agents and an electrochemical device for following the reaction identical to that used in studying reductions (13). An "apparent oxidation potential" for these substances can be given with assurance as table 3 shows. The irreversible step is here undoubtedly controlled by the formation of an easily hydrolyzed quinone imine.

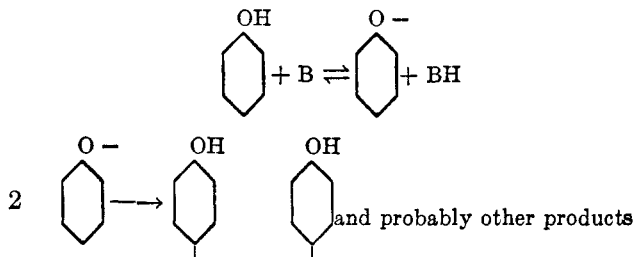


With this particular transformation, the potential of the reagent controls.

The oxidation of benzidine and certain other diamines to highly colored meroquinones has very recently been studied (Clark (5)) and found strictly reversible. These compounds, however, are very unstable in many solutions and pass irreversibly into other products. Such an irreversible transformation (under conditions where its rate is slower than that of the initial reversible oxidation) should be also capable of formulation in terms of "apparent oxidation potentials."

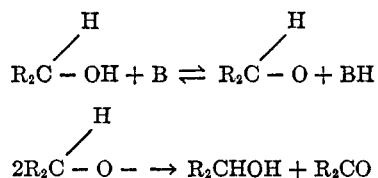
The researches of Goldschmidt (22) and Pummerer (30) have shown that in the oxidation of certain phenols the initial formation of a free radical can be demonstrated. It is probable that a similar process is involved in most irreversible oxidations of phenolic substances and if such a reaction is reversible it may

well control the rate of a subsequent irreversible rearrangement, as illustrated by the following equations:



The fact that it has been experimentally demonstrated that the formation of a free radical by reduction is a reversible reaction governed by the usual electrochemical laws (14) suggests that the formation of a monovalent oxygen radical by oxidation would also be reversible. Preliminary investigations have shown that the oxidation of many phenolic and enolic substances is dependent on the potential of the oxidizing agent employed (13). Similar considerations very probably apply to the oxidation of many amino compounds to hydrazines and mercaptans to disulfides. Considerable work has been done on the mechanism of amine oxidation from a strictly chemical viewpoint which would point to such a formulation as that given above (22a).

One is tempted to extend this mechanism to the oxidation of alcohols, hydroxy acids and aldehydes (in the form of their hydrates according to the Wieland concept (44)) and imagine that a second irreversible step consists of a "disproportionation" of the "odd molecule." Although such an extension is at present pure speculation, I venture to include it in this review as



being in the nature of a working hypothesis which may lead to interesting experiments.

It is important to establish whether or not the oxidation of such hydroxy compounds (which in contrast to the oxidation of the phenols may be called monomolecular oxidation) is governed by the potential of the reagent employed. For example, will the action of Fehling's solution or ammoniacal silver solutions on aldehydes be duplicated by any reagent of an equally high oxidizing potential at the same alkalinity? Is the oxidation of an aqueous solution of acetaldehyde by quinone in the presence of palladium (Wieland (43)) a specific action of quinone and closely related substances or does it merely depend on having a "powerful" enough oxidizing agent? What relation exists between the rate of oxidation processes brought about by palladium (43), platinum (2, 3), charcoal (41, 44), or ferments (see below) and the potential of the oxidizing agents employed? Similar questions can be raised in regard to the oxidation of unsaturated compounds with permanganate. Now that our knowledge of the potential of the reversible systems has become greater, we can look forward to obtaining an answer to these questions in the near future. In advance of such answers, we must be cautious in attempting to apply the electrochemical point of view to these reactions.

The work of Wieland during the last fifteen years (44) has strikingly demonstrated that many of our oxidation reactions are not concerned with the addition of oxygen but with the removal of hydrogen atoms,—“dehydrogenation” as he has called the process. The electrochemical study of reversible systems is entirely in accord with this viewpoint and, indeed, throughout this review I have used the word oxidation as almost equivalent to dehydrogenation. This separation of our ideas concerning oxidation from any idea of oxygen transfer is of the greatest importance and Wieland's work has done much to counteract the over-emphasis placed on oxygen as an oxidizing agent. Since the electrochemical point of view starts from the chemistry of ions capable of changing their valence by the gain or loss of one or more electrons, the term oxidation has never suffered in this branch of science from too close attachment to the element oxygen. I have preferred to use the term oxidation throughout

rather than dehydrogenation as the former term has become irrevocably attached to the change of ferrous to ferric salt and of ferrocyanide to ferricyanide. Although the latter process can be thought of as a loss of hydrogen when one considers the corresponding acids, the change of valence of positive ions is hard to fit into the "dehydrogenation" terminology. The same may be said of the oxidation of the triphenylmethyl ion to triphenylmethyl itself (hexaphenylethane) (14). The most satisfactory definition of oxidation in the electrochemical sense would seem to include the process of dehydrogenation, and the removal of electrons from positive and negative ions. At least we know examples of all three processes in organic chemistry which can be measured electrochemically. Personally, I am of the opinion that the *reversible* dehydrogenations are probably only special cases of reversible electron transfer, but until this point can be better established and we have more information concerning *irreversible* dehydrogenation it would seem to be unwise to use the electron picture too freely.

Oxygen as an oxidizing agent

Oxygen in the absence of a catalyst is not a component of a reversible oxidation-reduction system. Even in the presence of platinum and other catalysts it is very doubtful if a real equilibrium is reached involving oxygen and its reduction products (water or hydrogen peroxide or the hydroxyl ion). The potential of the hypothetical oxygen electrode can be estimated, however, indirectly; this potential would be characteristic of the behavior of oxygen only in the presence of a catalyst which functioned as surely as platinum functions with hydrogen. The reactions of oxygen under ordinary conditions are in no way connected with this more or less mythical potential.

A glance at figure 1 demonstrates that it is only because of this electrochemical inertness of oxygen that we are able to handle solutions of hydroquinone and similar substances in air without appreciable oxidation. Yet oxygen combines extraordinarily rapidly with free radicals containing trivalent carbon or with hemoglobin and more slowly with many aldehydes and other

action of quinone and palladium on aldehydes. Until such irreversible oxidations have been more thoroughly studied with a variety of oxidizing agents of definite potential a formulation of the action of oxygen is evidently impracticable.

The reduction of molecular oxygen by soluble reducing agents would seem to lend itself to an electrochemical inquiry. This process is usually attended by the formation of hydrogen peroxide or its salts. This is true both of the oxidation of solutions of many inorganic salts and also of alkaline solutions of phenols and hydroquinones (24). It appears that the rate of reduction of oxygen by a component of a reversible system (e.g., a hydroquinone or ferrous salt) is not a function of the potential of the system but of certain specific properties of the various reagents (17, 24). The fact that in alkaline solution anthrahydroquinone sulfonic acid reacts with oxygen more rapidly than does the more powerful reducing agent, sodium hyposulfite (Fieser (18)), is only one instance of this sort. Without attempting to review the enormous literature dealing with auto-oxidation in aqueous solution, it seems evident that a prediction as to the rate of oxygen absorption based on the potential would be very uncertain. Moreover, oxidations by atmospheric oxygen are very susceptible to homogeneous as well as heterogeneous catalysis, and an accurate statement as to the rate of oxidation by oxygen of a really pure substance would have to be made with caution.

Certain biochemical problems

Since the interaction of oxygen with reversible systems probably can not be treated electrochemically, there seems but faint hope of applying the methods to the irreversible oxidations (dehydrogenations) brought about by this gas in biochemical processes. Here again catalysis both homogeneous and heterogeneous plays an enormous rôle. Warburg (39, 40, 41) and Meyerhof (27, p. 16-22) have shown the startling effects of small amounts of metallic salts in promoting and small amounts of cyanide in retarding oxidations with atmospheric oxygen both in the living cell and in vitro (45). The biochemical oxidations

brought about by methylene blue, unlike those with oxygen, do not seem to be susceptible to this anti-catalytic influence of cyanide if we may judge from the experiments of Szent-Györgyi (34) and Fleisch (19) with muscle tissue and sodium succinate. The former investigator has developed the thesis that in biochemical processes there are two distinct types of catalysts at work,—one activating the oxygen, the other bringing about the oxidation (dehydrogenation) of the organic material. In Warburg's opinion (39, 40) complex iron compounds are the oxygen catalysts which function by virtue of an oxidation to the ferric state which is then reduced to the ferrous by the organic compound being burned in the living cell. Biochemically methylene blue is thus the equivalent of oxygen plus an iron catalyst. This separation of the problem of biochemical oxidation into two separate phases seems to be justified by all the facts now available and clears away much of the controversy between those who insisted on oxygen activation and those who laid emphasis on dehydrogenation (42, 36, 40).

The second aspect of the problem,—the so-called activation of the organic molecule—is closely allied to the action of metals, metallic colloids, and charcoal, in promoting the oxidation of organic compounds by quinone, methylene blue, etc. The living cell contains something that acts in a similar manner. (5, 25, 26, 27, 37). The possibility of attacking this problem of "catalytic irreversible oxidation" by the use of a series of reversible systems, I have already mentioned. Biochemical catalysis would seem to form merely one branch of this general subject.

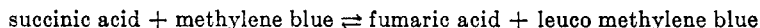
In connection with Warburg's theory, Spoehr's recent discussion (33) of the catalytic influence of iron phosphate on the oxidation of sugars is of interest as bringing out clearly two distinct mechanisms by which oxygen might be activated. He considers that in the case of his iron complex salt, oxygen may be either directly taken up (much as it is by hemoglobin) or may change the iron from the ferrous state to the ferric. According to the first hypothesis, the "oxygenated compound" would interact directly with the sugar; according to the second, the ferric

salt would be the oxidizing reagent and in turn would be reduced. If the latter mechanism is correct the oxidation-reduction potential of the complex may be of significance as Spoehr points out. If reversible oxidation-reduction systems of this type are present in nature, it is very probable that their behavior may be formulated ultimately in electrochemical terms. W. Palladin (29) has suggested a quinone-like system functioning in plants. Warburg's theory of iron complexes has been mentioned above and glutathione (isolated from tissue by Hopkins (23)) has been viewed as a reversible system aiding oxidation. Meyerhof (27, p. 35), however, believes that the simple change of the $-SH$ group to the $-S-S-$ involved in the oxidation and reduction of glutathione and cystine is not sufficient to account for its function in the living cell; his experiments indicate that some sort of a peroxide must be involved. The oxidation of such systems by oxygen seems to be subject to metallic salt catalysis (32).

It is uncertain whether or not the cystine-cystein and the glutathione system are reversible and can be formulated in electrochemical terms. Dixon (16) by using carefully prepared electrodes obtained reproducible potentials with solutions of cystine and cystein and glutathione and the corresponding disulfide, but the potentials were independent of the relative concentration of the two components of the system. Since dependence on relative concentrations is the most important criterion of reversibility one is inclined to believe that his potentials are not characteristic of the behavior of these systems. He suggests that we are here dealing with a new type of electrode system but the problem remains as to how one should interpret the potential of such a system. In this connection it should be mentioned that carefully prepared electrodes will often record fairly reproducible potentials in pure buffer solutions in the absence of any oxidation-reduction systems; the e.m.f. varies regularly with the hydrogen ion concentration. It is possible that some such "accidental" potentials were the source of the electromotive force measured in Dixon's work. A further study of disulfide systems using a series of reversible reagents of known potential seems advisable.

Evidence for the existence of reversible systems in living cells is afforded by recent studies from the Hygienic Laboratory (5). Dr. Clark and his coworkers have found that washed bacteria or yeast cells slowly develop the property of reducing a number of the colored oxidation-reduction indicators; the ability to reduce those of higher potential is first manifested. This development of reducing action is accelerated by the addition of a number of soluble substances such as sodium succinate; some water soluble substance seems to be formed in the cell which is oxidized by the dye. That this unknown substance is a component of a reversible system is strongly indicated by the fact that an inert electrode immersed in these same suspensions develops a fairly definite potential whose magnitude is commensurate with the behavior of the oxidation-reduction indicators. Further work in this direction will be awaited with interest.

Reference has already been made to the oxidation of succinic acid by methylene blue in the presence of muscle tissue. The work of the last few years indicates quite clearly that in the presence of finely divided muscle (Thunberg (37, 38)) or suspensions of certain bacteria (Quastel (31)), equilibrium is slowly attained in the following system at pH values near 7.



Quastel (31) estimating the extent of reduction colorimetrically finds the value for the equilibrium constant K (eq. 11) at pH 7.3 and 45° as 3.0; the value is independent of the amount of "rest-

$$\frac{(\text{fumaric acid})(\text{leuco methylene blue})}{(\text{succinic acid})(\text{methylene blue})} = K \quad (11)$$

ing" bacteria, and the extent of reduction varies with the relative concentrations of the reactants in accord with the mass action law.

Thunberg (37) has recently calculated the potential of the same system in the presence of muscle tissue at pH 6.7 from his observations on the extent of decolorization of methylene blue and Clark's value for the methylene blue potential; his value is +0.005. A value for the same system at pH 7.3 in the presence

of bacteria calculated from Quastel's K and Clark's data is -0.009 . The agreement is good when it is recalled that the change in 0.6 of a pH unit might be expected to lower the potential 36 millivolts (if the reversible process involves only the loss or gain of two hydrogen atoms); furthermore, the temperatures of the two experiments were not identical. The fact that two different investigators using different biochemical catalysts obtain essentially the same equilibrium constant is convincing evidence that the process is strictly reversible under these conditions. The catalytic action in the case of both the bacteria and muscle tissue is probably connected with the two phase system necessarily present but the discovery of any method of changing an irreversible system into a strictly reversible one is of much importance to the general theory of oxidation-reduction reactions quite apart from biochemistry. To what extent this reversible system is involved in other types of biological oxidation remains to be discovered. The fumaric-succinic acid system by itself can evidently not function as an "oxygen carrier" since, as has been stated above, the action of oxygen on succinic acid seems to be conditioned by some catalyst which is poisoned by hydrocyanic acid.

Criteria of reversibility

In future developments of the concept of oxidation-reduction potentials, both in connection with biochemical and strictly chemical problems, it would seem to be essential to determine at the outset whether a given system is reversible or not. If reproducible electrode potentials are obtained which accord with the logarithmic equation, one can hardly go wrong in accepting the reversibility of the system as established. However, as experience with a number of inorganic systems has demonstrated, there may be difficulties in measuring potentials even of reversible systems; other practical criteria are therefore of service. If one attempts to determine by the methods discussed in this review, the "apparent reduction potential" of the oxidized component and the "apparent oxidation potential" of the reduced form, certain peculiarities will manifest themselves in the case

of reversible systems. In the first place, if the electrochemical method of following the course of the reaction is employed, there will be a rapid initial change of potential when certain reversible systems are used and then no further change; this is in contrast to the behavior of a substance which is irreversibly reduced or oxidized in which case there is a progressive change in potential. In the second place, the particular oxidizing mixture which is appreciably affected by the reduced component, will have a potential *below* that of the mixture which first acts on the oxidized component. In other words, what appears to be the A.R.P. and A.O.P., overlap (actually these terms can only be applied if the processes are irreversible). This is made clearer by the case of a reversible azo-hydraso system. Thus azobenzenedisulfonate when introduced into a reagent of potential +0.375 (containing equal amounts of the oxidized and reduced form) causes an immediate change in potential corresponding to about a 40 per cent reaction; the potential of the same reagent is changed about the same amount, *but in the opposite direction* by the action of the hydraso compound. Reagents of lower potential reduce the azo compound practically completely; those of higher potential are without action. The normal potential estimated from these results is about +0.37; that actually measured by the method of mixtures +0.38 (13).

Thus the behavior of both components of a supposedly reversible system towards a series of known reagents gives information in regard to the reversibility; it further enables one to evaluate the potential of the system *if the system is reversible*. It would seem advisable to employ some such procedure in the case of those systems whose potentials have been reported as being independent of the concentrations of the components. The case of glutathione and cystine has already been mentioned; Rideal (21) by the use of specially prepared electrodes ("controlled electrodes") has obtained "irreversible potentials" of a number of sugars in aqueous solution. One is sceptical of the significance of all such potentials until they are checked by some such method as that just outlined which involves the use of reversible systems of known potential.

It may not be out of place to call attention again to the distinction made in the first part of this paper between reversible and irreversible systems. Since the distinction is in the last analysis based on rates of attaining equilibrium there may be some instances where one can draw the line only by means of some arbitrary rule,—these transitional cases (if they exist), however, are unimportant. We find that in aqueous solutions the transfer in both directions of hydrogen atoms or of electrons proceeds with great rapidity with certain compounds,—these are reversible systems and most common soluble reducing and oxidizing agents fall in this category. The equilibrium attained in the interaction of any two such systems might be investigated by sufficiently refined analytical methods (e.g., photochemical) and the results expressed without recourse to electrochemistry. As a general rule, however, these same systems come rapidly to equilibrium with an inert electrode (probably because of an interchange of electrons or possibly hydrogen atoms). Therefore, electrochemistry provides us with a powerful tool for investigating these systems; in order for this method to be effective, electrode equilibrium must be rapidly attained, but this is of importance *only in so far as it is of experimental assistance*. Speed of attaining electrode equilibrium and rapidity of chemical action seem usually to go hand in hand in oxidation-reduction reactions, *but need not necessarily*; the chemical behavior of the components of the system are not conditioned by their interaction with an electrode. Too great an emphasis on the reversibility of electrodes may therefore obscure important results; the testing of an unknown system by means of known reversible systems is therefore of great importance. Whether this experimentation involves electrical or colorimetric methods is of no theoretical importance.

CONCLUSION

Our increasing knowledge of the properties of a number of organic compounds which are rapidly oxidized or reduced in solution has greatly supplemented the list of soluble oxidizing or reducing agents of known potential. By using a graded

series of such reagents, we can decide whether the addition of hydrogen to a given type of compound under definite conditions is reversible or not. If it is an irreversible reaction, we can in many cases determine what reagent is necessary to bring it about; such information is of value in developing a quantitative account of organic reactions and enables us to correlate in such instances structure and chemical behavior. Similarly, a certain number of oxidation or dehydrogenation reactions can be investigated and precise data should be eventually available in this field. It is improbable that the methods will be universally applicable,—indeed, we can be quite confident that they will only avail when certain mechanisms are concerned; each new type of reaction, each new set of conditions must be experimentally examined. Although the road is thus hedged in by many complications and much arduous work remains ahead, the prospect is alluring to all those interested in the more precise formulation of the fundamental reactions of organic chemistry. It is evident that a consistent quantitative account of the oxidation and reduction of organic compounds will be of great practical value to both organic chemists and biologists. Furthermore, we may feel confident that on such foundation stones a general theory finally will be erected which will bring order out of the present chaos of qualitative information.

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