OXIDATION-REDUCTION SYSTEMS OF BIOLOGICAL SIGNIFICANCE.

I. THE REDUCTION POTENTIAL OF CYSTEINE: ITS MEASUREMENT AND SIGNIFICANCE.

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It is well known that tissue extracts or suspensions of cells establish a rather strong negative electric potential under anaerobic conditions at indifferent electrodes such as platinum, gold, or mercury. An observation of this sort was first made by Gillespie on bacteria; he inaugurated an interpretation based on the concepts of reduction potentials. The success met on application of the theoretical fundamentals of oxidation-reduction potentials (hereinafter to be referred to by us as "redox" potentials) as developed by Ostwald, Nernst, Haber, Luther, Fredenhagen, Leblanc, Peters, and others, to organic redox systems in the hands of BiiIlmann and more particularly W. M. Clark has given great impetus to more elaborate study of physiological materials. Among those investigators attempting to relate their studies of biological material to those of the reversible systems it may be sufficient to mention W. M. Clark, Conant, Dixon, Quastel, Tunnicliffe, Drew, Fleisch, E. N. Harvey, Kendall and Nord, Needham and Needham, Rapkine and Wurmser, Thunberg, Voegtlin, Johnson and Dyer.

The concept of a definite, finite redox potential and of a definite, finite redox system is limited to reversible systems and moreover to reversible systems at equilibrium. An attempt to apply the theory of redox potentials to physiological materials is met with the difficulty that oxidation in tissues is, on the whole, an irreversible process impossible of ending in a state of equilibrium, such equilibrium being contradictory to life. Is there then sufficient
reason to attempt to correlate biological reduction potentials with those inanimate redox systems capable of exact thermodynamic treatment?

There is one condition which permits this correlation. When a tissue, naturally aerobic, is deprived of its oxygen supply and when it has reduced the last small quantities of available oxygen as far as it can, then the reducing substance of the tissue acts on other of the tissue compounds and reduction goes on until a definite equilibrium is established. This equilibrium to be sure means death, but the final potential reached after its establishment must in some way be related to the chemical composition of the tissue in its living state. The potential established after withdrawal of oxygen supply may, therefore, be different for different kinds of cells and in addition be related to the individual properties of these cells when living.

Now let the problem be complicated by the presence of a normal oxygen environment and by the continuous, irreversible flow of oxidation processes consequent upon it. If there be some reversible redox system in the tissues, what conditions are essential to constancy of ratio of oxidized to reduced component of that particular redox system describable in terms of potential? A dynamic process at a constant level will be existent only when there is a constant ratio of reducible substances (say activated oxygen) furnished from latent sources (say air) to oxidizable substances (such as active glucose) from latent sources (such as glycogen). In so far as reaction velocities are involved in establishment of the constant level of which we speak, it is not necessarily true that any reversible redox system—natural or artificially added—would be kept at the same level of potential. There is, then, in the presence of oxygen, an important divergence from a tissue or a system at equilibrium.

For sake of greater clarity let us make a system of the kind we consider and let us examine its behavior. We add a definite amount of methylene blue to a measured quantity of tissue extract and maintain a constant oxygen pressure. We find in time that the dyestuff is partially reduced; a methylene blue-methylene white system has been established with its components in definite ratio. If we vary any of our quantities—dye, tissue extract, or oxygen—we can create a new methylene blue-methylene white
ratio. When the oxygen pressure is that of air, for instance, the methylene blue is present in a practically completely oxidized state; when the oxygen pressure is very low (as in an anaerobic Thunberg experiment) the methylene blue system is completely reduced; and there must be a range of intermediary oxygen pressures at which a definite ratio of methylene blue and methylene white will be established characteristic for the particular pressure. This is equivalent to the statement that by changing the oxygen pressure we can create a new potential of the methylene blue system.

In complete absence of oxygen the final condition may be considered a true equilibrium. In this case we may expect the potential as indicated by the added reversible dyestuff system to be independent of the particular chemical nature of the dye, provided the concentration of the dye is low enough to work only as an indicator and not to poise the system. In such a case, therefore, it is legitimate to speak of a definite reduction potential of the system as a whole.

When a definite oxygen pressure is maintained, however, no true equilibrium of the whole system will be reached but at best the methylene blue system will be kept in a constant condition. Reduction velocities are involved in establishment of the level of this condition, e.g. the velocity with which reduced dye is oxidized by molecular oxygen, and it is not necessarily true, as has been said, that different indicators be at the same potential. Nor is it necessarily true that the system as a whole have a definite potential; if there be more than one redox system present in tissue, each may have its individual potential.

Our problem of the moment, then, is to discover under natural conditions a substance or substances among the constituents of the tissue liquids which behave as the artificially added methylene blue, and which account for those biological reduction potentials, measurable at indifferent electrodes, of which we have spoken. A few systems corresponding to methylene blue have been described in tissues. Such, according to Cannan (1926, 1927), is the echinochrome of Arbacia eggs and the hermidin of Mercurialis perennis. The cytochrome described by Keilin (1925) and found widely distributed in mammalian tissues and in yeast, is as yet too little understood for certainty of its relationship to
tissue potentials. Biological systems demonstrated to behave like methylene blue are thus seen to be much restricted in their distribution and so of limited moment in the problem we have set ourselves. There is, however, a certain group of substances found ubiquitously in animals which are related to the methylene blue system in a peculiar way not yet fully understood and appreciated and which are moreover highly responsible for the potentials of noble metal electrodes dipping into tissue liquid. These substances are the sulfhydryl bodies. The most accessible representative of them is cysteine, and the one probably most responsible for the behavior of tissue is the glutathione of Hopkins. This substance was previously considered by the discoverer himself as reversibly oxidizable and reducible, and in this respect equivalent to the methylene blue system. This idea, however, was modified by further investigations from Hopkins’ laboratory as is told below.

The fact that cysteine (and reduced glutathione and sulfhydryl substances in general) can exhibit a strong reducing power led to an investigation of its potential at a noble electrode. Dixon and Quastel (1923) first attacked this problem; they observed a strong negative potential at an inert electrode dipping into a solution of cysteine or glutathione. Quantitative measurements led to the following formula in which RSH stands for cysteine (or reduced glutathione):

\[ E = E_0 - \frac{RT}{F} \ln [\text{RSH}] + \frac{RT}{F} \ln [\text{H}^+] \]

The potential \( E \) was measured at electrodes of solid gold; only such electrodes gave well defined values according to these authors. Platinum and gold-plated platinum gave inconsistent, drifting potentials. The massive gold electrode, too, failed of complete satisfaction. The constant \( E_0 \), and this should be emphasized even more than was done by Dixon and Quastel, was a constant only during a single titration experiment in the course of which either pH or cysteine concentration was varied. \( E_0 \) varied for different gold electrodes and moreover was variable without recognizable cause for the same electrode in different experiments to an extent of often as much as 60 millivolts.

Beyond the rather unusual difficulties encountered in measuring
the potential, it is both remarkable and interesting to note that
the formula is without reference to cysteine's oxidation product,
cystine, which may be symbolized by RSSR. The potential,
accordingly, of cysteine (and of reduced glutathione as well) is
independent of the presence or absence of its oxidation product.
Cysteine can be prepared readily from cystine by reduction and
cystine from cysteine by a simple oxidation; still they do not
constitute a simple reversible redox system. Dixon and Quastel,
therefore, justly considered the behavior of these substances as
very unusual and in consequence assigned them to a new type of
reduction-oxidation system.

The attempts of these authors to explain this behavior do not
appear to be wholly successful. They first resort to the plausible
hypothesis that there exists a primary oxidation product of cysteine
(from which cystine arises only through a secondary irreversible
process); they then proceed to the unlikely supposition that
this primary oxidation product is always present in invariable
concentration. This viewpoint, equivalent to considering the
solution at all times saturated with the primary oxidation product,
appears to have been abandoned by the authors themselves in a
later communication.

This second paper by Dixon (1927) attempts to interpret the
potentials from the standpoint of Wieland's theory of hydrogen
acceptors. Wieland has shown that palladium black, placed
into solutions of many different reductants, becomes charged with
hydrogen; the claim is made that cysteine is able to charge the
metal electrode with hydrogen but that cystine is not able to
withdraw hydrogen from the hydrogen-charged metal. The
process of charging the metal with hydrogen, in consequence, must
be considered irreversible; the counterbalancing effect of with-
drawal, evident in all reversible redox systems, is missing here.
The hydrogen charge of the metal, however, is limited by diffusion
of the gas out of the metal into the surrounding solution. Equilib-
rium of metal and hydrogen is reached when the rate of diffusion
of hydrogen into the solution from the metal exactly balances the
rate of supply of hydrogen for the metal by cysteine. The poten-
tial obtained is a hydrogen potential; the electrode works as a
hydrogen electrode of a definite gas pressure for each particular
environment.
This is the essence of Dixon's second attempt to explain the unique behavior of the cysteine potential. He went a step further and put his explanation to further test. The rate at which metals can be charged with hydrogen and the rate at which they will release the gas into the surrounding medium may well vary with the nature of the metal; the final potential, therefore, may be made to change by changing the metal electrode. Dixon found, indeed, that mercury gives a potential some 200 millivolts more negative than gold in the same cysteine solution; his ideas received still more support on consideration of the fact that mercury is capable of a much higher hydrogen overvoltage on cathodic polarization than gold. We shall show that these latter arguments of Dixon are faced with serious objection.

Another investigation of the cysteine potential has been made by Kendall and Nord (1926). We have not concerned ourselves with detailed study of the potential established in the presence of indigo carmine after treatment with oxygen or hydrogen peroxide; we shall not present, therefore, a critique of their theoretical treatment of this part of the subject. Suffice it to mention that Kendall and Nord confirmed, in an entirely qualitative way, the observations of Dixon and Quastel that cysteine does give a reduction potential when present alone in pure buffer solutions and that this potential is independent of the concentration of cystine. Kendall and Nord add no explanation of these observations to those already given by Dixon and Quastel.

It is quite unnecessary to emphasize the importance of complete understanding of the cysteine potential, to point out that it may well be correlated with the fundamental problems of tissue respiration. We could not be entirely satisfied with the results of Dixon and Quastel for their measurements suffered from failure of good reproducibility and their considerations, in consequence, were focused upon the findings of single titration experiments. Comparison and analysis of experiments independently performed were rendered difficult and uncertain. We sought, therefore, that method of procedure which brought with it exact reproduction of results, which gave us means of measurement capable of complete control. Such a method must be considered of first importance for an exact understanding of the problem at hand; it may be of service, we hope, in investigation of many closely
allied biochemical questions. We present in the following pages the development of such a method and the observations and considerations growing out of the experiments necessary to its establishment.

Technique.

It ensues from the considerations above that control of oxygen in systems to be investigated is of greatest importance. With many reversible systems a slight amount of this gas is often without effect on the electrode potential; this is particularly true of potentials in positive ranges such as those of quinone-hydroquinone, or ferric-ferrous ions, or ferrocyanide-ferricyanide ions. In other reversible systems, such as dyestuffs, lying in the negative range of potentials, oxygen is of much greater significance. Here, however, the reductant is usually enormously avid for molecular oxygen; the potential measured in presence of a trace of the gas suffers only from change of ratio of reduced to oxidized component consequent upon oxygen combination. This change may be insignificant when a fairly well purified nitrogen atmosphere is supplied, and the redox system is sufficiently poised to undergo no appreciable change of ratio of oxidant to reductant by consumption of the oxygen impurity. In solutions of substances like cysteine, however, even the slightest traces of oxygen have a great effect upon the potential. The reduction potentials of such solutions are always diminished, sometimes enormously, by presence of extremely small quantities of the gas. Spontaneous consumption of traces of oxygen cannot be relied upon in these cases. Cysteine even in presence of iron consumes oxygen with reasonable speed only over a limited range of pH; moreover, even at the optimum pH it appears to fail to remove the last traces of the gas.

The most important goal of the technique was, therefore, to remove oxygen as completely as possible from the cysteine system. This involved simply a supply of carefully purified nitrogen to a carefully sealed apparatus. Nitrogen, usually containing about 0.08 per cent oxygen, from a commercial tank was slowly led through NaOH and then through a Pyrex glass tube

1 Repeated analyses gave this value. It is lower for this nitrogen tank than that reported by other investigators.
Reduction Potential of Cysteine

60 cc. long tightly packed with short pieces of copper oxide wire. The copper oxide was previously reduced by hydrogen; this procedure was frequently repeated during the course of the experiments. The tube was maintained in an electric furnace at a temperature of 550–600°. Leads from the combustion tube to the electrode vessel were of copper tubing; connections between metal and metal were soldered; connections between metal and glass were sealed with De Khotinsky cement. The end of the copper tube was connected to the gas inlet of the electrode vessel by a mercury-sealed arrangement such as is shown in Fig. 1 or, in more detail, in Fig. 2. This is the only place where rubber was permitted on the electrode side of the furnace; here it was amply protected by mercury.

The purity of the nitrogen prepared and delivered in this way was occasionally tested with alkaline pyrogallol in the apparatus.
FIG. 2. Apparatus for testing the purity of nitrogen. Nitrogen is led through copper tubing (1) attached by means of short rubber tubing (2) to glass tubing (3); the rubber tubing is protected from air by a glass cup filled with mercury. Gas is then led via glass tube (8) through vessel (4), containing 10 per cent NaOH, after proper positions have been given stop-cock (11) and mercury valve (6). After NaOH is saturated with nitrogen, the gas is led via (8) into vessel (5) containing a pyrogallol solution acidified with a little acetic acid. Then with stop-cocks and mercury valves (6, 7) properly set, nitrogen is led over glass tube (9), and the liquid of (4) is pressed into (5) for mixing. In a similar way, the liquid is forced into vessel (4); after thorough mixing it is divided equally between (4) and (5). Vessel (5) is then permanently sealed by the 3-way stop-cock (11) and mercury valve (7) and gas is led via (8) to the outlet (6). Before the experiment is performed it is advisable to place a drop of dilute H₂SO₄ above stop-cock (11); this prevents premature appearance of color in case pyrogallol and alkali are mixed in spaces of the stop-cock.

Any trace of oxygen in nitrogen is shown by difference in color of (4) and (5) after some minutes of bubbling.
sketched in Fig. 2. This apparatus permitted washing out of oxygen by nitrogen from solutions of acidified pyrogallol and NaOH before mixture of the two; permitted mixture under a nitrogen atmosphere; and provided a standard of comparison for the color changes to be looked for. An hour of vigorous bubbling with purified nitrogen failed to produce detectable color change in the alkaline pyrogallol; 5 minutes slow bubbling with 1 part of oxygen to 40,000 of nitrogen brought an appreciable color difference.

Electrode vessels of various forms were used. That of Fig. 1 was both convenient and trustworthy. All leads were sealed with mercury.  

A calomel electrode in 4 M KCl was used as leading off electrode. It was frequently standardized against a hydrogen electrode in standard acetate buffer, the pH of which was taken equal to 4.62 in the temperature range between 18–38°C.

All measurements were made in a large air thermostat with electrically controlled thermoregulator and a strong air fan. Most measurements were at 38°C.

Potentials were read with nitrogen bubbling slowly through the electrode vessel. This precaution helps guard against accumulation of oxygen from the one unavoidable source of diffusion, the agar bridge connecting the electrode vessel to the calomel electrode. Moreover the nitrogen was bubbled at a definite rate. The amount of agitation of the cysteine solution is of great importance in measurement of potentials under known oxygen pressures; though insignificant in its effect in a pure nitrogen atmosphere, this safeguard was taken in the interest of complete control of experimental conditions.

In order to regulate the amount of agitation, it was first of all necessary to provide means of measuring and controlling the nitrogen flow. This was accomplished by use of a calibrated flowmeter of a type shown in Fig. 3 (one line of level difference in the arbitrary scale meant a flow of 1.56 cc. of N₂ per minute; the ratio of level difference and flow was constant over the employed range). Other obvious experimental conditions were then made such as to insure reasonable regularity in bubble size from experi-

\[2\] We are indebted to Mrs. Max Oates for the drawings in this communication.
ment to experiment. The absolute flow as indicated by the flow-meter could, therefore, be taken as a measure of the amount of agitation of the liquid.

A special series of experiments necessitated control of oxygen pressure. This was effected by a second flowmeter; oxygen either from an air tank or from a tank of analyzed, unpurified nitrogen, passed through it and so was measured, then, mixed with a measured quantity of purified nitrogen, was introduced into the copper tubing leading to the electrode vessel. In this way it was possible to vary the ratio of oxygen to nitrogen from 1:40,000 to that of air. The device was entirely satisfactory from point of view of facility of operation and constancy of the gas pressures it supplied.

A tank of hydrogen was permanently joined by way of a 3-way stop-cock to the copper tubing entering the combustion chamber. This gas, freed of CO₂ by NaOH, was, when occasionally needed, passed through the oven at a temperature of 550–600° to rid it of oxygen.
The cystine used was the commercial preparation of the Eastman Kodak Company. Cysteine hydrochloride was prepared from it by reduction with tin and HCl according to Baumann (1883–84); the final purification was made with acetone as suggested by Warburg.

The preparation was found to contain the theoretical amount of HCl by titration with NaOH, methyl orange being used as indicator. It also consumed the theoretical amount of iodine when, following Warburg (1927), titration was conducted in solutions of 95 per cent alcohol.

The potentiometry itself required no notable deviation from the standard methods. A Leeds and Northrup potentiometer type L and a galvanometer with telescope reading were used.

We are indebted to Dr. W. M. Clark for many personal suggestions used in development of the applied technique for purification of nitrogen.

**Cysteine Potential at Blank Platinum Electrodes.**

A long time is required for the establishment of the final, definite potential of a cysteine solution at a bright platinum electrode. A rather negative potential is established even in the presence of air; when the air is bubbled out with nitrogen, however, the potential becomes more and more negative. This shift is quite rapid in the beginning, then becomes progressively slower, and finally so slow that after the apparent end of an experiment 1 or 2 more hours are necessary for certainty that the final value has been reached. This is true both for temperatures of 22° and 38°. It is, therefore, necessary to make observations over a period of from 4 to 6 hours, depending on the electrode, before one is justified in calling a potential reading the ultimate one.

There are apparently two reasons for this slow establishment of the potential. As is to be shown in detail later on, the blank platinum electrode is extremely sensitive to even the smallest traces of oxygen; oxygen must, in consequence, be completely driven out of the solution. In agreement with this, if, after reaching the final potential, a trace of air was led into the electrode vessel, the potential was immediately shifted to the positive side. When bubbling with nitrogen was resumed, reestablishment of the end value took much less time than at the beginning of the experi-
ment; in fact, just about that time necessary for expulsion of the oxygen. This observation suggested that a change in the electrode itself is involved in the initial establishment of the potential. Recourse was had to the well known ideas concerning oxides or suboxides on the metal surface. From this point of view, the ultimate potential must await not only complete displacement of oxygen from solution but complete reduction of the surface layer of the metal as well. At the beginning of an experiment, the metal strips are all coated with oxides; it takes a long time for their complete reduction, and longer for the reduction of one strip than for another, for it is unlikely that the state of oxidation is identical for different samples. After complete reduction and establishment of the final potential, momentary introduction of a trace of air to shift the potential to the positive side produces little or no oxidation of the metal; the potential is quickly reestablished in consequence by bubbling with nitrogen.

Another experiment can be devised to demonstrate that the metal surface undergoes change on contact with the cysteine solution and reaches a stable state only after a long time. Two platinum electrodes were placed in the same vessel; one was immersed in, the other was left out of the solution. Oxygen was then bubbled out of solution with nitrogen and the bubbling continued until the immersed electrode reached its end value. The dry electrode was then pushed into the liquid; its potential was 100 to 200 millivolts positive to that of the first electrode, which remained unchanged during the manipulation. The positive potential changed after much time to the negative value established at the electrode which had been in solution from the beginning of the experiment. Several experiments verified these observations. It is certain, therefore, that contact of the electrode with the cysteine solution, freed of oxygen, induces a change necessary to the establishment of the final potential; this change, we repeat, very probably involves reduction of metastable oxides or suboxides of the platinum surface.

If the precautions growing out of the foregoing considerations were exercised, it was possible to reproduce at all platinum electrodes the potentials of definitive cysteine solutions to within 5 millivolts. Certain electrodes, of course, became unsatisfactory with time; this may have been due to minute cracks in the glass,
### TABLE I.

Potential observed at 38°, in volts. Potential of hydrogen electrode in standard acetate pH 4.62 and 1 atmosphere of hydrogen pressure taken equal to zero.

<table>
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<tr>
<th>pH</th>
<th>Concentration</th>
<th>Blank platinum.</th>
<th>Gold-plated platinum.</th>
<th>Mercury.</th>
<th>Solid gold.</th>
<th>Potential calculated on basis $E_0 = +0.284$.</th>
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TABLE I—Concluded.

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<tr>
<th>pH</th>
<th>Concentration</th>
<th>Blank platinum</th>
<th>Gold-plated platinum</th>
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<th>Potential calculated on basis of $E_0 = +0.284.$</th>
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</table>

Values marked (s) are from experiments in which electrodes were in the same vessel and so under exactly the same experimental conditions. Values marked (*s), from repetition of these experiments; those marked (**s) are from a third similar series. Values marked (+1) were obtained in one titration experiment; those marked (+2) came from another.

leading to establishment of secondary circuits or to other causes still unknown. Electrodes of irregular behavior, however, were not met with more often than in ordinary cases, e.g. in quinhydrone electrodes; moreover the faulty electrode was easily recognized from its behavior toward quinhydrone, and discarded. Reproducibility, in fact, was nearly as good as in any well established redox system. We worked for a long time with an electrode vessel containing six single platinum electrodes. One of these always showed a slight irregularity even in quinhydrone; the other five, which behaved regularly towards quinhydrone, all gave the same potential for cysteine though, as has been pointed out above,
The line drawn is that fixed by formula: \( E = +0.284 - \frac{RT}{F} \log [\text{RSH}] + \frac{RT}{F} \log [\text{H}^+] \). \( \Delta \) = values from individual experiments with blank platinum electrodes. \( \circ \) = values from individual experiments with mercury electrodes. \( +_1, +_2 \) = values from titration experiments with mercury electrodes. pH 4.62, temperature 38°. Potential of hydrogen electrode in standard acetate pH 4.62 and 1 atmosphere of hydrogen pressure taken equal to zero. To obtain values referred to normal hydrogen electrode subtract 285 millivolts. Each point is defined by an experiment.
Fig. 5. Variation of potential with variation of pH. The line drawn is that fixed by formula:

\[ E = +0.284 - \frac{RT}{F} \log [RSH] + \frac{RT}{F} \log [H^+] \]

\( \Delta \) = values from experiments with mercury electrodes. \( \circ \) = values from experiments with blank platinum electrodes. Concentration of cysteine = 0.01 M; temperature 38°. Potential of hydrogen electrode in standard acetate pH 4.02 and 1 atmosphere of hydrogen pressure taken equal to zero. To obtain values referred to normal hydrogen electrode subtract 285 millivolts. Each point is defined by an experiment.
different lengths of time were necessary for attainment of the final potential with different electrodes.

A. Variation of Potential with Variation in Cysteine Concentration.—The results in Table I and Fig. 4 show that at the pH 4.6 the potential is a logarithmic function of the concentration of cysteine. Within a rather large range of concentration, from 0.1 to 0.0002 M, an increase of the concentration by 10 times makes the potential at 38° more negative by 0.061 volt. All the results were obtained by individual experiments for each concentration, not by titration.

B. Influence of pH. Table I and Fig. 5 show that within the limits of reproducibility described before, the potential depends in a logarithmic way on the hydrogen ion concentration. Each change of a unit of pH at 38° produces a change of 0.061 volt.
This was observed over a range of pH from 1.2 to 9.4. The pH of the solution *in toto* was directly measured in each experiment. Hydrogen electrode values with freshly platinized platinum electrodes were checked by the Sörensen method with Clark indicators and by the method of Michaelis and Gyemant with the nitrophenol series.

**C. Influence of Oxygen Tension.**—The behavior of the cysteine potential in presence of oxygen shows three striking characteristics. 

1. The presence of oxygen makes the potential more positive; there is an increase in positiveness with increase in oxygen tension.
2. The establishment of a definite potential in presence of oxygen requires much less time than with purified nitrogen. 

(3) In the presence of a small amount of oxygen, the potential depends to a remarkable degree on the speed of gas bubbling through the solution or on the amount of stirring or shaking of the solution. This is especially the case for mixtures of nitrogen with very small amounts of oxygen (over the range of 1 part of oxygen to 40,000 of nitrogen, to 1 part of oxygen to 100 parts of nitrogen), whereas this shaking effect is little noticed in high oxygen tensions, such as that of air, and is insignificant in purified nitrogen.

The influence of variation in rate of bubbling is shown in Fig. 6. In these experiments the solution was saturated with gas from a tank of unpurified nitrogen; the ratio of oxygen to nitrogen was 1:1250. A difference of 70 millivolts was produced by variation in rate of bubbling. There was a maximum of negativity of the potential at a rather slow rate of bubbling; an extremely sharp decrease of negativity with further decrease in rate of bubbling; a much slower decrease, tending to an asymptotic value, with increase in the bubbling rate.

The effect of variation of oxygen pressure is shown in Fig. 7 and Table II. Such an experiment to be performed rigidly must take account of the agitation of the solution. This condition was not strictly controlled; it was regulated quite nicely, however; certainly closely enough to interfere in no important way with the results. The curve is selfexplanatory; even the slightest trace of oxygen pushed the potential to the positive side. An amount of oxygen amounting to 0.003 volumes per cent caused a decrease in negativity of 15 millivolts. The oxygen content of the solution is thus seen to be of enormous importance in determination of the potential.
Fig. 7. Variation of potential at blank platinum electrode with variation of oxygen tension. 0.0002 M cysteine at pH 4.62, temperature 38°C. Potential of hydrogen electrode in standard acetate pH 4.62 and 1 atmosphere of hydrogen pressure taken equal to zero.
D. Influence of Hydrogen on Potential.—There is an increase in negativity of the potential if the solution is bubbled with pure hydrogen after establishment of the final potential in presence of nitrogen. The value of this potential is always more positive than the hydrogen potential with platinized platinum; with practically all blank electrodes it is some 200 millivolts more positive. One receives the impression that bright platinum has the tendency to work as a true hydrogen electrode; i.e., that it attempts establishment of thermodynamic equilibrium between hydrogen gas and hydrogen ions but that the velocity of this process is very slow and the process itself incomplete. The relatively slight effect of hydrogen can be entirely dissipated by renewed bubbling with nitrogen. The presence of cysteine, then, in a buffer solution in no way alters the typical behavior of blank platinum as a hydrogen electrode in pure buffer solutions.

<table>
<thead>
<tr>
<th>Vol. per cent of O₂ added to purified N₂</th>
<th>Potential (volts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>+0.190</td>
</tr>
<tr>
<td>0.003</td>
<td>+0.205</td>
</tr>
<tr>
<td>0.1</td>
<td>+0.250</td>
</tr>
<tr>
<td>0.6</td>
<td>+0.308</td>
</tr>
<tr>
<td>1.0</td>
<td>+0.330</td>
</tr>
<tr>
<td>6.5</td>
<td>+0.328</td>
</tr>
<tr>
<td>20.0</td>
<td>+0.370</td>
</tr>
<tr>
<td>1.0</td>
<td>+0.425</td>
</tr>
<tr>
<td>0.5</td>
<td>+0.430</td>
</tr>
<tr>
<td>0.01</td>
<td>+0.325</td>
</tr>
<tr>
<td>0.02</td>
<td>+0.332</td>
</tr>
<tr>
<td>0.004</td>
<td>+0.240</td>
</tr>
<tr>
<td>0.000</td>
<td>+0.210</td>
</tr>
<tr>
<td></td>
<td>+0.188</td>
</tr>
</tbody>
</table>
E. Effect of Polarization.—Every care must be taken to avoid polarization in reading the potentials; even weak polarization brings about a change of some tenths or a whole of 1 millivolt. The displaced potential, however, rapidly returns to its normal value. This holds not only for the final value which is reached after hours of bubbling but in addition for those measurements taken during the course of the drift. The drift, it is to be recalled, is quite gradual; the potential returns so promptly and completely from momentary polarization that the disturbance alters its course in no appreciable way.

If the final, definite potential be forced up or down by say 200 millivolts on extended polarization, it returns spontaneously and exactly to its original value after polarization; this is an important fact for understanding the significance of the final potential. The velocity at which the potential returns varies greatly with the direction of polarization. After cathodic polarization, there is a rapid return to the true potential; after anodic polarization, the shift is slow and drifting.

These observations indicate that the cysteine potential is that of a system at equilibrium. Its full significance is lost, however, in our ignorance of the mechanism of establishment of the potential; their explanation must, in consequence, be postponed.

Cysteine Potential at Gold Electrodes.

The detailed description just given of the behavior of blank platinum electrodes fits quite well observations made with gold-plated platinum. There are only two differences: gold responds even less to hydrogen than does platinum and reaches its final potential even more slowly. The ultimate value of the cysteine potential in pure nitrogen is, within the limits of reproducibility, the same in the two sets of electrodes. Individual differences in the behavior of various gold-plated electrodes appear only in variation of the time necessary for establishment of the definite potential.

The behavior of electrodes of pure, solid gold is very different. A constant, final potential is more quickly established than in blank platinum or gold-plated platinum. There is even less variation in the potential with change in atmosphere from nitrogen to hydrogen than in the gold-plated electrode. The major differ-
ence, however, and the difficulty which presents itself in the case of solid gold strips, is that the end potential is altogether different for each gold electrode and never so negative as in the other electrodes.

The electrodes behave as though their surfaces contain oxygen compounds scarcely reducible by cysteine. The potential, following this view, corresponds to that of a platinum electrode incompletely reduced. Different gold strips are oxidized to different degrees; to each degree of oxidation there is correlated a definite potential. This peculiarity in the case of a particular electrode does not exclude a variation in potential with change in cysteine concentration and change in pH according to the formula of Dixon and Quastel. It does, however, make acceptance of the potential as an intrinsic property of the solution itself impossible and for this important reason we have discarded solid gold electrodes for measurement of the cysteine potential.

Cysteine Potential at Mercury Electrodes.

A resting surface of pure mercury was used as electrode; there was no call for a device permitting renewal of the surface. The essential result is that the final potential agrees nicely with that at blank platinum or gold-plated platinum. There are definite differences in behavior, however. The end potential at mercury is much more quickly established than at other electrodes; the electrode is far less sensitive to oxygen; the potential is unaffected to a detectable degree by a change from nitrogen to hydrogen.

The rapidity with which the ultimate potential is reached at mercury is quite surprising (see Fig. 8). Even in air the potential is much more negative than at platinum; the negativity very rapidly increases when the air is bubbled out with nitrogen and comes to within some 10 millivolts of its ultimate value in 15 minutes or so. The last few millivolts appear quite slowly, requiring a half to a whole hour, sometimes longer. All in all, however, the mercury electrode yields the final potential so much more quickly than platinum that the equivalent of a week's work with the latter can be done in a day with the former.

The effect of oxygen on a mercury electrode is similar to that on a platinum electrode in so far as the gas always renders the potential positive to that in purified nitrogen or hydrogen. The
Fig. 8. Potential establishment at electrodes of mercury and gold-plated platinum in cysteine of pH 4.6 and purified nitrogen atmosphere. ○ = values for gold-plated platinum. □ = values for mercury. Temperature 38°. Potentials referred to the normal H₂ electrode.
magnitude of the influence, however, is much smaller. A change from a purified nitrogen atmosphere to an atmosphere containing 0.45 volume per cent of oxygen made the potential more positive by 12 millivolts in a 0.0002 M solution of cysteine, and more positive by 15 millivolts in a 0.01 M solution at pH 4.6 in standard acetate and at 38°. Rate of bubbling at low oxygen pressures is of much less consequence than in the case of platinum. It becomes very conspicuous, however, in oxygen pressures of the order of that of air. When, for example, a 0.0002 M cysteine solution at pH 4.6 was saturated with air, its potential at mercury was 180 millivolts more positive at rest than on moderate bubbling. Interpretation of this observation is not difficult; in presence of oxygen, traces of mercury go into solution as Hg⁺ ions; a stationary condition is established when the rate at which Hg⁺ ions are furnished into the solution is compensated by the rate at which they are reduced by cysteine. On bubbling, the Hg⁺ ions are distributed throughout the entire liquid. At rest, they accumulate at the mercury surface. This explanation seems sufficient to account for the influence of bubbling on the potential at the mercury electrode in presence of oxygen. More detailed study of the phenomenon is thought beyond the scope of the immediate investigation.

The variation of the potential with concentration of cysteine and pH is to be seen in Table I and in Figs. 4 and 5. It is to be noted in accordance with an observation of Dixon and Quastel, that there is a deviation from the calculated potential at high pH. In our experiments this is first seen at a pH of 9.5; it is striking at pH 12.4. This deviation is apparently related to dissociation at the SH– group; its full discussion is reserved for a later communication. The quick establishment of the potential at mercury permitted titration experiments in which the concentration of cysteine was varied by addition to the electrode vessel from a pipette. The results of these titrations are to be seen in Table I and in Fig. 4 where the values are noted by +1 and +2. The two titration experiments noted agree not only with each other but also with experiments singly performed at mercury and blank platinum.
Reduction Potential of Cysteine

Cysteine Potential at Platinized Platinum Electrodes.

The drift to the end potential at the platinized platinum electrode is even slower than at the bright metal; after as long as 8 or 9 hours one is not certain that the measured potential is the ultimate one. An effort was made to check that value got on bubbling out air with nitrogen by establishing a hydrogen potential and then bubbling out the hydrogen with nitrogen. If the potential considered final be truly final, there should be no discrepancy between the value in a nitrogen atmosphere, be it derived from the air or from the hydrogen side. The minimal difference between the two values in six sets of experiments was 3 millivolts; the maximal, 25 millivolts; the mean, 12 millivolts.

Beyond these differences, evident after most painstaking precaution in experimental detail, many platinized electrodes in the same vessel and so under exactly equivalent conditions show potentials which vary widely. Little exact, in consequence, can be expected from the results; we discarded the platinized electrode after much unsuccessful labor to control it for the same reasons which led to abandonment of solid gold. Our thought that the black electrode might serve better to measure the reduction potential of cysteine than it does to measure the redox potential of systems such as quinhydrone is, therefore, without foundation.

Influence of Cystine on Potential.

Dixon and Quastel, working with solid gold electrodes, have stated that the oxidation product of cysteine, cystine, is without effect on the cysteine potential. Our results wholly confirm this finding. We made many attempts to test this statement; we present the following experiments as typical examples.

1. A solution of 0.0002 M cysteine hydrochloride in standard acetate buffer pH 4.62 at 38° was placed in the electrode vessel containing blank platinum electrodes. At this acid pH, even in presence of iron, oxidation of cysteine before complete removal of oxygen by nitrogen can be neglected. When the final potential was reached, the vessel was opened for a moment; an excess of cystine crystals poured in, the vessel was closed, and bubbling with nitrogen resumed. On the basis of Sano's (1926) figures for the solubility of cystine at 25° at pH 4.67, the solution is finally 0.00042 M with respect to cystine; actually cystine's molarity is
greater since the experiment is at 38°. Even on supposition that one-quarter of the original cysteine is impurity of cystine, certainly a high exaggeration, the concentration of cystine is changed 10 times by addition of the crystals. The ultimate potential, nevertheless, was not affected to a detectable degree by the addition of cystine.

2. Even more convincing is the following experiment in which arrangements permitted a much wider variation of concentration of cystine. The potential of a 0.0003 M solution of cysteine in 0.1 M HCl was measured at a mercury electrode; then a parallel experiment made with cysteine of the same concentration (0.0003 M) in 0.1 M HCl saturated at 38° with cystine by shaking with a great excess of finely ground cystine crystals. The solubility of cystine in 0.1 M HCl is, according to Sano, almost exactly 30 times greater than in standard acetate and therefore more than 30 times greater than the cysteine concentration of the experiment. No matter what the impurity of cystine in our cysteine, the concentration of the former was highly increased; still the potential remained within the limits of change brought by pH differences.

3. An electrode vessel with a mercury electrode was filled with borate buffer at pH 9.4. Through the rubber stopper of the vessel went a glass rod on the end of which was fused a glass spoon. The spoon was filled with 10 mg. of cystine and kept above the solution during the first part of the experiment. The buffer solution was thoroughly bubbled out with nitrogen; then the vessel opened and enough of a 0.02 M solution of cysteine hydrochloride added to give a 0.0005 M solution of cysteine. The vessel was promptly closed and the potential followed to its final value. Cystine was then added by pushing the spoon into the liquid. The amino acid readily dissolved but again failed to affect the potential. The quantity of cystine used was small enough not to alter the pH appreciably but large enough to change the cystine concentration to 0.002 M from that very low concentration due to impurity in the 0.0005 M solution of cysteine.

**Influence of Iron and of Cyanide on Cysteine Potential.**

It is known in large part from the investigations of Mathews and Walker (1909) and Warburg and Sakuma (1923, 1927), that heavy metals, especially iron, play a great part in the
physiological rôle of cysteine and that cysteine forms a complex both with ferric and ferrous iron. The deep violet ferric complex is unstable and undergoes a rapid change into ferrous iron and cystine. It is of no importance, therefore, in establishment of the potential. But the ferrous complex of cysteine may be of significance. The presence of a trace of iron could not be excluded under our experimental conditions and one may be inclined to attribute an important function to the metal in accord with Warburg's observations that even slight traces of it permit consumption of molecular oxygen by cysteine.

Ferrous sulfate added to give a concentration of 0.001 M to a 0.01 M solution of cysteine at pH 4.6 in standard acetate buffer was, however, without effect on the potential. The iron content of pure cysteine in pure buffer though not zero was negligible compared to the iron content of the above solution as shown by comparative thiocyanate reaction. Moreover, KCN added to a concentration of 0.01 M at pH 7.4 to a solution containing no more iron than its accidental traces effected no change in the potential. Variation of the iron content of the solution between extremely wide limits must, in consequence, be considered without effect on the potential established by the free cysteine. Obviously the amount of iron is concerned with the velocity of consumption of molecular oxygen by cysteine, and, according to S. Toda (in Warburg's laboratory (1928)), also in the rate of reduction of methylene blue by cysteine, but an extreme variation in its concentration has nothing to do with the magnitude of the potential established at an electrode in absence of oxygen.

DISCUSSION.

A. Physicochemical.

The potential of the cysteine system, to be in accord with the principles of reversible redox systems, should be a function of the logarithm of the cysteine concentration and of the logarithm of the reciprocal value of the square root of cystine's concentration. A function of these dimensions can be expected even though there be intermediary steps in the oxidation of cysteine to cystine. Experience shows, however, that the potential is completely independent of the concentration of cystine.
In our present knowledge we attempt explanation of this fact by assuming the process of potential establishment to be irreversible. This means that cysteine charges the electrode with hydrogen atoms but that cystine fails to withdraw hydrogen atoms from the metal. In so far as cysteine's tendency to charge the metal with hydrogen is not counterbalanced by a reverse reaction, the potential can be expected to reach an infinitely high negative value; or, since the potential in an aqueous solution cannot exceed a hydrogen potential (except for an overvoltage which is always finite), the potential should be expected to be at least that of a hydrogen electrode. This, obviously, is false.

Dixon, attempting an explanation, assumes that charge of the metal with hydrogen by cysteine is counterbalanced by diffusion of hydrogen out of the metal into the solution. It is unbelievable, however, that atomic hydrogen diffuses into the solution to be maintained in this state. If hydrogen does diffuse into the solution, it can be present there only in the form of dissolved hydrogen molecules. And if blank platinum and mercury, charged with hydrogen atoms, have the faculty of developing hydrogen molecules and so of establishing equilibrium between hydrogen molecules in solution and hydrogen atoms in the metal, then diffusion from the metal should be stopped by saturating the solution with hydrogen gas. A cysteine solution bubbled with hydrogen should, in consequence, establish a hydrogen potential. Cysteine solutions at electrode of blank platinum on being bubbled with hydrogen never reach the hydrogen potential; and at mercury there is not even a detectable difference in the potential, be the solution bubbled with hydrogen or nitrogen.

A more acceptable explanation in the light of this criticism would involve a diffusion of hydrogen atoms from the surface of the metal, not into the solution, but into the body of the metal itself. The capacity of metals, taking into consideration their entire bulk, is high for hydrogen atoms. This explanation, however, has to be discarded with the first for a continuous supply of hydrogen atoms to the electrode must result in progressive oxidation.

It is inconsequential whether we consider electrons from the molecule ionized at the SH-group or hydrogen atoms from the undissociated SH-group to leave cysteine and charge the electrode. For this reason it may suffice to say that cysteine charges the electrode with hydrogen.
of the cysteine, progressive diminution of its concentration, and a consequent drift of the potential to the positive side. According to our experience, however, the cysteine potential even in very dilute solutions is constant for as long as 24 hours. Moreover, it is highly improbable that the velocity of migration of atoms and protons is the same within platinum, gold, and mercury and so incompatible with the fact that the potentials at the three metals are equivalent. In consideration of these objections, diffusion of hydrogen atoms from the surface into the body of the metal must be negligible compared to the rate of supply of hydrogen atoms by cysteine.

In experimental support of his theory, Dixon pointed out that the magnitude of the cysteine potential depends on the nature of the "indifferent" electrode. He found the potential about 200 millivolts more negative at mercury than at solid gold; this observation was in accord with the high hydrogen overvoltage of mercury and in agreement with his theory. As our experiments show, however, the magnitude of overvoltage of various metals can in no way be correlated with the potentials established at them. The two metals, blank platinum and mercury, which are, when we disregard platinized platinum, the two limiting members of the overvoltage series of metals, give accurately the same potential in cysteine solutions and differ only in the speed with which the potential is established. It was probably accidental that Dixon compared mercury with solid gold; solid gold is, according to our experience, an exceptional and unacceptable case.

The shift of the potential in presence of oxygen and the variation in sensitiveness to the gas on the part of different metals can be accounted for in the following two ways. All of the noble metals have a tendency to behave as oxygen electrodes; that metal which is most efficient in dissociating molecular oxygen into atoms

4 We have adopted that theory of cathodic polarization and hydrogen overvoltage which states that hydrogen deposited by an electric current at the cathode is deposited in the atomic state and is loosely combined with the metal atoms. The theory makes the further assumption that transformation of atomic hydrogen into molecular hydrogen is dependent upon catalysis by the metal. Differences in catalytic capacity impart different overvoltages to different metals. (A summary of the theories of overvoltage is given by Baars, E., in Handbuch der Physik, Berlin, 1928, xiii.)
and of establishing equilibrium between the two will form the best oxygen electrode and will be most sensitive for oxygen. It is known from wide experience that platinized platinum, though far from ideal, is best fitted to these ends; next comes blank platinum, then gold.\(^5\) Our experiments with cysteine show a sensitivity of the potential to oxygen at these metals of exactly this order. The results can also be accounted for in another way, basically dependent on those properties of the metals just discussed. Oxygen oxidizes the hydrogen sticking in the electrode surface. The velocity of this process depends upon the catalytic property of the particular metal and therefore upon its capacity for oxygen and hydrogen and upon the facility with which it disrupts the molecules of gas into atoms. This catalysis is extremely poor at mercury. It is higher in blank platinum. In platinized platinum it is so great that permanent charge of the electrode with that amount of hydrogen corresponding to the reducing power of cysteine is almost impossible; the hydrogen, as it is supplied by cysteine, is eaten away from the electrode even by the smallest trace of oxygen.

In presence of a constant oxygen pressure there is a dynamic process at a rather constant level. Conditions, however, are somewhat unstable; change of degree of agitation of the liquid produces marked differences in potential. In a purified nitrogen atmosphere, on the other hand, the whole aspect of the potential as judged by constancy and reproducibility of its final definitive value, complete recovery from anodic and cathodic polarization, freedom from disturbance by changes in agitation, is that of the potential of a system in true equilibrium.

Objection may be made against considering the potential that of a true equilibrium rather than that of a dynamic process at a constant level in Dixon's sense. It is impossible to decide whether

\(^5\) Nothing can be said of mercury in this respect. Mercury acts as an indifferent electrode only in the presence of strong reducing agents which reduce mercurous ions to that level where the potential taken as a mercury electrode potential is equal to the potential of the redox system itself, mercury being considered as an indifferent electrode. The effect of oxygen at a mercury electrode may be accounted for by a production of mercurous ions above the level of equilibrium with the redox system or by a consumption of atomic hydrogen furnished to the electrode by the reductant.
the nitrogen used in our experiments was absolutely oxygen-free in the most rigid sense. We have shown that in presence of oxygen the potential is determined by the partial pressure of this gas. One may be inclined to the assumption, in consequence, that the anaerobic potential called by us final was really controlled by traces of oxygen too slight to be detectable. The following facts may refute such an assumption.

1. It has been shown that the change of potential at platinum with change in oxygen pressure is particularly steep in the range of the very lowest oxygen pressures. It will be remembered that the potentials established in presence of purified nitrogen were well reproducible at all times; in order to account for this in the light of the objection raised we would be forced to the assumption that the quantity of oxygen in our purified nitrogen was always the same. It is unreasonable to believe, however, that there was always accurately the same trace of oxygen present in purified nitrogen. This condition would have necessitated stricter constancy of temperature of the oven and constancy of the copper surface over which the nitrogen was led than was sought in our experiments. Moreover, it is unlikely that possible traces of oxygen would accompany purified nitrogen and purified hydrogen in accurately the same quantity; it will be remembered, however, that the potential at mercury was not to be varied by change from a nitrogen to a hydrogen atmosphere.

2. The potential at any definite oxygen pressure is very different at platinum than at mercury. We found, however, the same potential at platinum and at mercury in an atmosphere of purified nitrogen. We are forced to the conclusion again that our nitrogen contained either no oxygen at all or traces too slight to affect the potential in any way. So we feel justified, therefore, in considering the cysteine potential as that of a system in true equilibrium though we are unable to state that chemical reaction which is kept at equilibrium.

We have indicated that we think Dixon's theories impossible to accept. We are at a loss, however, to offer in substitution a comprehensive explanation of the peculiar behavior of cysteine. Elucidation of the observations and problems we have spoken of must await, therefore, further study and experimentation.
B. Physiological.

We return now to the problem set forth in the introduction: In absence of oxygen, are cysteine or other sulfhydryl derivatives indicators for what we can call the reduction power of the whole tissue system as is the methylene blue-methylene white system? In attempting to answer this question we are faced with an entirely new situation. Cysteine or its derivatives, natural constituents of the tissue liquids, certainly determine a definite potential in absence of oxygen. But, whereas in a system such as methylene blue the mechanism of potential establishment is clear, the potential of the sulfhydryl bodies is inexplicable. We can with good reason say, however, that the sulfhydryl bodies are present in high enough concentration to poise the tissue and to account for the reduction of all indicators by living cells recently demonstrated by Cohen, Chambers, and Reznikoff (1928). It is wholly likely that they impress their own level of reduction power upon those other tissue systems, the state of which can be expressed in terms of potentials.

It should be kept in mind that the sulfhydryl bodies play another important rôle in the organism dependent upon their activation of oxygen in presence of traces of heavy metal salts, as has been shown by Warburg. It is too early to build a hypothesis as to how oxygen activation and potential establishment are interwoven. Full explanation must await an understanding of the mechanism of the electrode potential.

SUMMARY.

A solution of cysteine in absence of oxygen establishes a definite potential at an indifferent electrode. This potential is independent of the concentration of cystine and depends logarithmically on the concentration of cysteine and of hydrogen ions. The potential is identical at electrodes of blank platinum, gold-plated platinum, and mercury, and reproducible to within 5 millivolts. Slight traces of oxygen displace the potential to the positive side. Mercury is much less sensitive to oxygen than platinum or gold-plated platinum and has the great advantage of yielding final values in a relatively short time. The anaerobic potential is negative enough to account for reduction of all indicator dyes by
cells under anaerobic conditions. The potential $E$, referred to the normal hydrogen electrode and measured in absence of oxygen, can be expressed in volts by the formula:

$$E = -0.001 - \frac{RT}{F} \ln \text{[cysteine]} + \frac{RT}{F} \ln \text{[H$^+$]}$$

No satisfactory theory can be offered for the mechanism by which this sharply fixed potential is established by cysteine which, in presence of its oxidation product, cystine, fails to form a truly reversible system.

BIBLIOGRAPHY.

Sakuma, S., Biochem. Z., 1923, clxii, 68.
Sano, K., Biochem. Z., 1926, clxxvii, 257; Ueber die katalytischen Wirkungen der lebendigen Substanz, Berlin, 1928.
OXIDATION-REDUCTION SYSTEMS OF BIOLOGICAL SIGNIFICANCE: I. THE REDUCTION POTENTIAL OF CYSTEINE: ITS MEASUREMENT AND SIGNIFICANCE
L. Michaelis and Louis B. Flexner


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