THE COUPLED REDOX POTENTIAL OF THE LACTATE-ENZYME-PYRUVATE SYSTEM*

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The reaction:

$$\text{succinate}^{=} + \text{methylene blue} \rightleftharpoons \text{fumarate}^{=} + \text{methylene white}$$

has been studied by a number of investigators. Quastel and Whetham (1) showed that a definite equilibrium was finally reached when resting *B. coli* were present. Thunberg (2) determined the oxidation-reduction potential of this equilibrium, when the muscle enzyme succinodehydrogenase was the catalyst. Lehmann (3) and later Borsook and Schott (4) worked out the thermodynamics of the equilibrium:

$$\text{succinate}^{=} \rightarrow \text{fumarate}^{=} + 2H^{+} + 2e,$$

and found that the free energy of the reaction as calculated from the equilibrium electrode potentials agreed very closely with the free energy calculated from the entropies and other physicochemical properties of the reactants. The fact that the calculated and observed values of free energy are so close is proof that the enzyme is a perfect catalyst (Borsook and Schott (4)).

The fact is well established, therefore, that the redox potential of a system such as succinate-enzyme-fumarate, which in itself will establish no definite potential on an electrode, may be correctly determined electrometrically by adding to the system a reversible redox compound having an $E'o$ in the same range. (The reversible redox compound should have an $E'o$ such that the logs of the ratios of its

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oxidant/reductant, at final equilibrium, will be less than ± 2, as within these limits it acts as a depolarizer and allows the actual potential to be determined.) Presumably, reversible redox compounds can exchange electrons directly with the electrode, whereas the succinate-fumarate system can only oxidize or reduce molecules subject to deformation on the surface of the enzyme (Quastel (5)). The coupled reactions between succinate-enzyme-fumarate, methylene blue-methylene white, and the electrode must, however, occur in a perfectly reversible manner, as from the *Eh* of the electrode the correct free energy of the first reaction may be calculated. It would be useful to have a special name for redox potentials determined thus indirectly and the name *coupled redox potential* seems to distinguish it properly from the apparent reduction potential of Conant (6). The *coupled redox potential* may be defined as the potential at which an irreversible system comes into equilibrium with a reversible system when a suitable catalyst is present.

The coupled redox potentials of any two compounds will determine how they will react provided suitable catalysts are present and this will largely determine the transfer of energy, as is implied by Knoop (7) in his article on "The mutual influence of organic compounds in the animal body."

It was with the view of adding a second coupled redox potential to our knowledge that Baumberger (8), in 1929, undertook the study of the lactate-enzyme-pyruvate system. In 1932 Wurmser and DeBoe (10) and Wurmser and Mayer (11) published a determination for this system, finding a coupled redox potential of -0.200 at pH 7.4 and 37°C., using as catalyst an autolysate of *B. coli*. Baumberger, Jürgensen, and Bardwell (9) presented a paper on this work at the International Physiological Congress in Rome.

Method

The electrode vessels used throughout the work were of a type quite similar to Lehmann's, consisting of a Pyrex U-tube (see Fig. 1) in which the two arms were connected by a capillary filled with agar-agar saturated with KCl. One arm contained the lactate-pyruvate system and the other served as reference cell. The two electrodes, one in each arm, consisted of platinum foil supported by platinum wire, thus eliminating the use of mercury. Oxygen was removed by attaching the stoppers of the two arms to a Y-tube connected to a water vacuum pump having a

nitrogen tank in the circuit. By intermittently evacuating and building up the pressure with nitrogen the oxygen was finally flushed out and the process was ended by evacuation until the vigorous bubbling of the fluid gave place to bumping, at which point the stop-cocks were turned off. The entire evacuation was carried out at 35°C. and required about 5 minutes.

Usually the reactants in the U-tube were added in the following sequence:

1. To the right arm:
   0.05 M KH phthalate in 0.15 M NaCl.
   Quinhydrone crystals.
2. To the left arm:
   4.0 ml. 0.2 M sodium phosphate buffer ± NaCl to ionic strength (μ) 0.8.
   1-X ml. 0.39 M lactic acid.
   X ml. 0.40 M pyruvic acid.
   1.0 ml. 0.002 M reversible redox indicator.
   2.0 ml. enzyme.

The osmolar concentration of (1) and (2) were equal. The reagents were all kept in the cold room at 3°C. ± 2°, protected from light, and fresh solutions were frequently prepared. The lactic acid was the usual *d-l* form. Pyruvic acid was freshly distilled at 70° and 16 mm. pressure. Lactic and pyruvic acids were made up to a concentration of 0.39 and 0.40 molar respectively, as determined by titration with standard alkali.

The enzyme preparation was made according to the method of Bernheim (12). Commercial dried yeast was ground to a powder in a coffee mill, treated with acetone, dried, and kept until used in a vacuum desiccator over $CaCl_2$. Each week a new enzyme preparation was made by extracting 10 gm. of the dried powder with 100 ml. 6.8 phosphate buffer and 0.1 ml. toluol with occasional grinding in a mortar for a period of 4 hours at room temperature. The extract was then centrifuged at 2500 R.P.M. for 15 minutes, and the supernatant fluid in 20 ml. lots was placed in cellophane (Visking Corporation, Chicago) sausage casings. These were placed in a jar containing 3000 ml. distilled water, which was kept stirred in the cold room. After 24 hours the water in the jar was replaced by fresh distilled water and the dialysis continued for another 24 hours. The contents of the dialyzers, which will be called the "enzyme solution," now showed a negative nitroprusside test for cysteine. The enzyme solution was centrifuged again as above and was placed in a stoppered flask to which a drop of toluol was added. This solution was protected from light and kept in the cold room. Its activity remained high for a period of 2 to 4 weeks but our experiments were carried out with enzyme during the first week after preparation and for each experiment the enzyme solution was recentrifuged immediately before use. Tests of the enzyme solution for cytochrome, lactic acid, pyruvic acid, reducing sugar, and aldehydes were all negative. When no lactate or pyruvate was added, the *in vacuo* experiments showed only slight reduction of methylene blue in many hours.

The vacuum U-shaped electrode vessels, a few minutes after evacuating, were

attached to the frame of a shaker enclosed in an electrically controlled air thermostat at 32°C. ± 0.1°. The shaker imparted a gentle circulatory motion to the fluid in the vessels and caused the electrodes to vibrate. Vessels were so connected that the E.M.F. could be measured while shaking continued.

The E.M.F. of the chain:

Platinum	quinhydrone NaCl KII phthalate	sat. KCl agar-agar,	enzyme lactate pyruvate sodium phosphate indicator	Platinum

was measured by a Leeds and Northrup potentiometer of the "hydrogen ion" type and an enclosed lamp galvanometer used as null point instrument. Care was taken to avoid polarization of the electrodes. Later a thermionic electrometer was employed to measure the E.M.F. The thermionic electrometer was developed in this laboratory by Mr. R. K. Skow.

The potential of the quinhydrone, NaCl, KH phthalate, was determined before and after the experiment by connecting it to a saturated calomel half cell by means of a saturated KCl agar-agar bridge. This potential was found to remain constant to within ±0.5 mv. for a period of 6 hours. If the upper portion of the vacuum electrode vessel is cooler than the lower portion the potential of the quinhydrone half cell continually becomes more negative. This change is due to the *in vacuo* distillation of quinone and its condensation on the cooler surface, where Dr. C. R. Noller collected it and identified it by melting point determination. The fact that quinone may be distilled off from quinhydrone under these circumstances is evidence against the idea that quinhydrone may be a half reduced quinone. It also suggests precautions that should be taken when quinhydrone electrodes are used.

The hydrogen ion concentration of the solutions was determined by means of the glass electrode. Glass electrodes were made of Corning 0.015 glass bulbs sealed to a double walled shank so that deviation film (Kahler and DeEds (13)) effects were obviated and the theoretical difference in potential between different pH's was obtained. The E.M.F. between the glass electrode in 0.05 M KH phthalate in 0.15 M NaCl and a saturated calomel half cell was determined by means of a thermionic electrometer. The glass electrode was then washed and placed in the lactate-pyruvate system from the left side of a vacuum electrode vessel and the E.M.F. against the saturated calomel half cell determined. The difference between the two E.M.F.'s divided by the temperature factor gave the pH difference, which, when added to the pH of KH phthalate, gave the pH of the lactate-pyruvate system. Determination showed no change in pH outside the limits of error, namely, ±0.02 pH.

The indicator used was indigo disulfonate (Clark *et al.* (14)) which was more suitable than any other reversible system because its molal electrode potential, $\tilde{E}$, in the physiological range of pH is approximately + 0.330 or only + 0.014 to

the $\tilde{E}$ of the lactate-pyruvate system according to our determination of it. Methylene blue (Clark *et al.*) had to be used in some of the more acid solutions as the indigo was out of range. Rosinduline (Michaelis (15)) was found to be too negative to be useful. β-anthraquinone sulfonate, (Conant (6)), though, with an $\tilde{E}$ of about + 0.240, falling into range at some ratios of lactate to pyruvate, was discarded because we found that this substance had a marked power of *photo-*

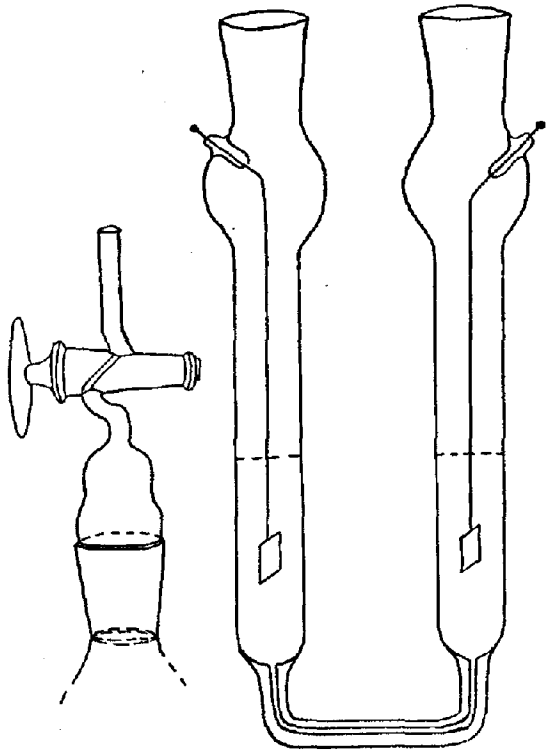


FIG. 1. Vacuum electrode vessel.

dynamic oxidation of both lactic and pyruvic acid with the accompanying reduction of methylene blue or indigo disulfonate. This effect is now under investigation by two of us. It is certain that results based on the use of β-anthraquinone sulfonate must be accepted with caution. No measurable difference was noticed when experiments were conducted in light or dark with the other indicators used, but the precaution of minimizing exposure to light was employed so that these experiments were conducted at a light intensity equivalent to twilight.

Rate of Change of Oxidation-Reduction Potential

Most of our experiments were made with four vacuum electrode vessels at once. Each vessel had a lactate-pyruvate system at a different pH or different ratio of lactate to pyruvate. The potentials were determined at intervals of about 20 minutes and the results

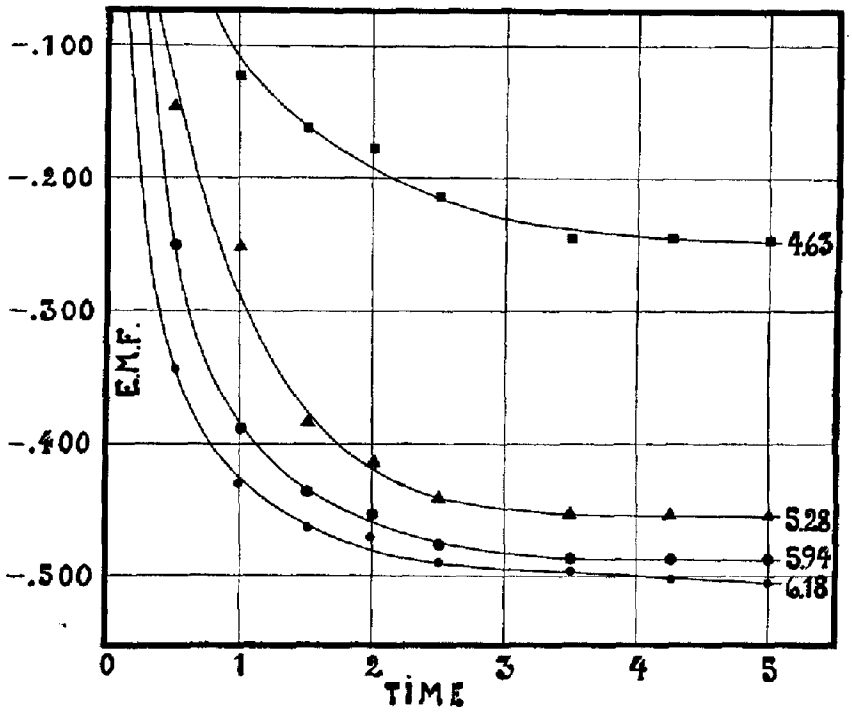


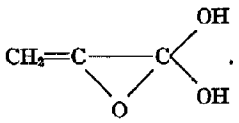
FIG. 2. Potential-time curves at different pH. Time in hours, E.M.F. in volts, pH indicated at right. E.M.F. is the P.D. between the quinhydrone half cell at pH 3.97 and the coupled redox system.

plotted against time. The potentials were found to become very rapidly more negative at first and then the rate of change became gradually slower until finally it became constant or negligible. Fig. 2 shows some typical potential-time curves. It can be seen from the curves that the rate of potential drop approaches zero in the case of relatively acid solutions and reaches a rather low value at pH 6.18.

The final constant rate of change of potential differs according to the pH as shown by the following table:

pH range	Rate of change of potential
	mv./hr.
4.6 −5.9	<1
6.2	1.5
6.6	2.5
6.7 −11.8	3.5

This continuous change is probably due to secondary reactions, most likely the formation of condensation products of pyruvic acid, which is accelerated at 37°C. according to Garino and associates (16) and a change in the keto-enol ratio which occurs with change in pH according to Henri and Fromageot (17) and the formation of



It will be necessary to evaluate these three effects before very exact determinations of the equilibrium potential may be made but we have assumed that the effect of the secondary reactions upon the potential may be minimized by considering the rate of change of potential throughout the experiment and selecting as the equilibrium potential the point where the rate of potential change becomes constant. This point is indicated by an arrow on each of the curves in Fig. 3. Values thus obtained are considered *equilibrium potentials* in the remainder of this paper.

Effect of pH on the Equilibrium Potential

When the ratio of lactate to pyruvate is kept at unity and the pH is varied by means of buffers, the equilibrium potential changes as shown by Fig. 4. This curve of $E'o$ (Clark *et al.* (14)) changes 0.060 volts per pH unit over the range of pH 5.2 to 7.2, in other words throughout most of the physiological range, and the formula fitting the curve is:

$$E'o - \frac{RT}{F} \ln [H^+] = \tilde{E} = 0.316 \text{ at } 32.5°C. \quad (1)$$

The molal electrode potential (Borsook and Schott (4)) $\tilde{E} = 0.316$ is based on twenty-two experiments at sixteen different pH values within the range 5.2 to 7.2. A maximum deviation of + 0.006 and −0.006 occurred but the deviations were random.

Outside the range of pH 5.2 to 7.2 we are unable to interpret the change of $E'o$ with pH. At pH < 5.2 the $E'o$ changes 0.160 volts per pH unit. To some extent this could be due to the acid dissocia-

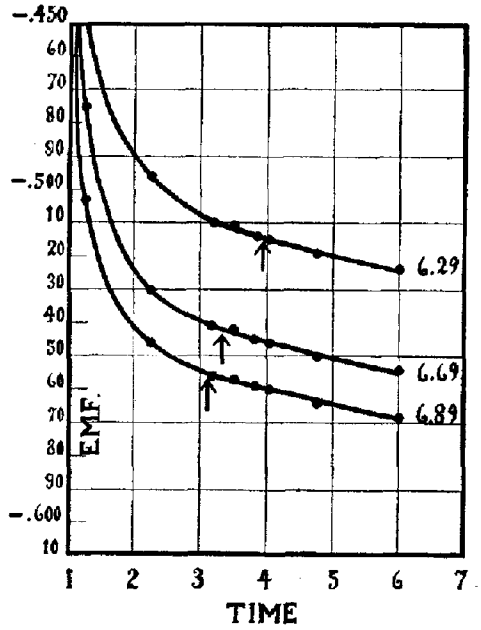


FIG. 3. Potential-time curves. Arrows indicate point where Δ E.M.F./Δ time becomes constant and is considered to be the *equilibrium potential*. (See description of Fig. 2 for further detail.)

tion of the lactic and pyruvic acid, although it is not clear to what degree dissociation of the carboxyl group will affect the oxidation-reduction potential. It is in this acid region, however, that the most marked shift of keto-enol ratio occurs, according to Henri and Fromageot (17), and this shift of ratio could very well affect the $E'o$. (Some consideration of the "influence of ionization at points unconcerned in oxidation-reduction" and the "influence of tautomerism" are given by

Clark *et al.* (14).) It does not seem likely that the reductant could have dissociation such as to account for the changes in slope of the $\Delta E'o/\Delta$ pH curves from 0.160 below pH 5.2 to 0.060 from pH 5.2 to 7.2 and to 0.030 from 7.2 to 12.0, and we have no other postulates to offer. The data in the pH ranges 2.9 to 5.2 and 7.2 to 12.1 are not as satis-

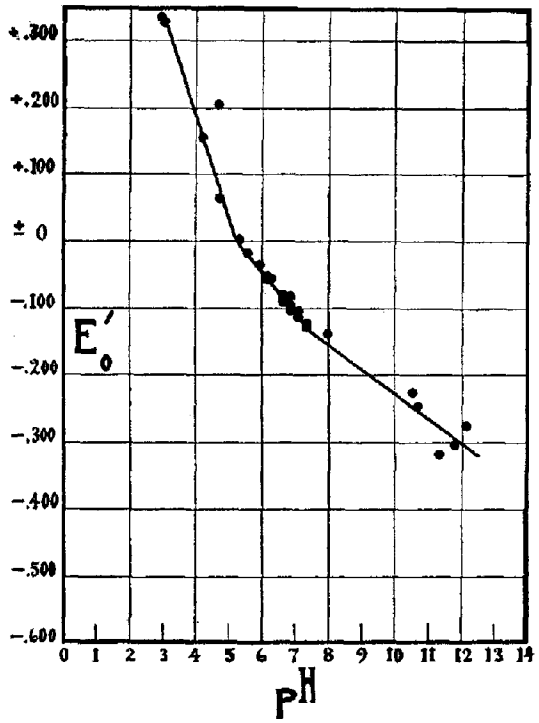


FIG. 4. $E'o$ − pH curve. Note that $E'o$ + 0.06 pH = 0.316 between pH 5.2 and 7.2.

factory as for the region pH 5.2 to 7.2 and we shall confine our attention to this more dependable information.

The Effect on the Equilibrium Potential of Changes in the Ratio of Pyruvate to Lactate

The proof of the reversibility of the reaction:

$$\text{lactate}^- \rightleftharpoons \text{pyruvate}^- + 2H^+ + 2e$$

lies in the determination of the effect on equilibrium potential of variations in the ratio of pyruvate to lactate. Experiments were carried out as before with ratios of pyruvate to lactate of 9:1, 5:5, 1:9. Since the stock solution of pyruvic acid had a concentration of 0.4 molar and the lactic acid 0.39 molar, at 32.5°C. the second term of equation (2) would give + 0.0291 for the 9:1 ratio and − 0.0285 volts for the 1:9 ratio. The expected difference between the two ratios would, therefore, be 0.0576 volts, and observations agreed closely with this, as shown in Table I.

TABLE I

pH	Pyruvate/lactate	0.316 − 0.605 pH + 0.030 log Pyruvate/lactate	Average observed Eh	No. observed
6.80	9:1	−0.066	−0.065	5
6.80	5:5	−0.095	−0.097	9
6.80	1:9	−0.123	−0.122	5

The observed and calculated *Eh* agreed within 1 or 2 mv. Since the log of the ratio of pyruvate/lactate if multiplied by 0.060, instead of by 0.030 as we have done, would change the values by about 29 mv., there can be no doubt that two electrical equivalents are required for the reaction; i.e., $n = 2$ in equation:

$$Eh = E'o + \frac{RT}{nF} \ln \frac{[\text{pyruvate}^-]}{[\text{lactate}^-]} \qquad (2)$$

Since the observed potentials fit the equation (2) when the ratio of pyruvate/lactate is varied, the process

$$\text{lactate}^- \rightleftharpoons \text{pyruvate}^- + 2H^+ + 2e$$

must be reversible. The process may proceed somewhat as follows: The lactate is oxidized by the indigo disulfonate until the dye is reduced to some degree. When the potential of the partially reduced indigo disulfonate is sufficiently low, the rate of reoxidation of dye by pyruvate will equal the rate of reduction by lactate and equilibrium will have been reached. That lactate is actually oxidized to pyruvate and pyruvate to lactate was not proven by direct analysis but it does not seem probable that equation (2) could hold unless the products

of oxidation of lactic acid and of reduction of pyruvic had the same free energy as pyruvic acid and lactic acid respectively.

Recovery of Pyruvate at End of Experiment

That pyruvate was not further oxidized in the course of our experiments was determined by analysis. Pyruvate was determined by the method of Wieland (18) and no change in this constituent was found in 6 hours. Tests with the Warburg apparatus for the presence of carboxylase (Warburg *et al.* (19)) in the enzyme preparations gave negative results. It may therefore be said that pyruvic acid is not removed during the experiment. An amount of pyruvate equivalent to the dye reduced or oxidized may appear or disappear, but the concentration of dye was kept below 0.01 per cent of the pyruvate, so these changes were within the experimental error of the analytical methods.

The Effect of Change in Temperature on the Equilibrium Potential

The $\frac{\Delta E}{\Delta T}$ of the process was studied by lowering the temperature from 32°C. to 16°C. after the equilibrium potential had been reached, but the reaction that must go on to reach an equilibrium at a new temperature is so slow that the results have so far been too variable to be of any significance. It is planned to carry out a new set of parallel experiments at two widely different temperatures in order to obtain this data. When such a value has been obtained, it may be used to calculate the heat absorbed during the reaction as by the Gibbs-Helmholtz equation:

$$E + \frac{\Delta H}{NF} = T\frac{dE}{dT}$$

and the increase in entropy (ΔS) of the system could then be calculated:

$$-\Delta H = -\Delta F - T\Delta S$$

Thermodynamic Calculations

The free energy (ΔF) of the process

$$\text{lactate}^- \rightarrow \text{pyruvate}^- + 2e + 2H^+$$

may be calculated as follows:

$$\begin{aligned} \Delta F &= -NFE \\ &= -2 \times 23057.5 \times -0.316 \\ &= 14{,}572 \\ -\Delta F &= -14{,}572 \end{aligned}$$

Data are not available for the calculation of the entropy of pyruvic acid, but the (ΔF°_{298}) standard free energy of formation at 25°C. for *d-l* lactic acid (l) is given by Parks and Huffman (20) as −124,400, and from this value and the ΔF of the reaction the ΔF°_{298} for pyruvic acid (l) may be calculated as follows:

Lactic ion (1*m*)	→ pyruvic ion (1*m*)	$= \Delta F_1 =$	+14,572
Lactic acid (1*m*)	→ lactic ion (1*m*)	$= \Delta F_2 =$	+5,410
Lactic acid (l)	→ lactic acid (1*m*)	$= \Delta F_3 =$	*x*
Pyruvic ion (1*m*)	→ pyruvic acid (1*m*)	$= \Delta F_4 =$	−3,709
Pyruvic acid (1*m*)	→ pyruvic acid (l)	$= \Delta F_5 =$	x^1
Lactic acid (l)	→ pyruvic acid (l)		$\Delta F = 16,273$

$\Delta F = \Delta F^{\circ}$ lactic acid (l) $- \Delta F^{\circ}$ pyruvic acid (l) = 16,273.
ΔF° pyruvic acid (l) = −124,400 − (+ 16,273) = −108,127.

This is to be considered as merely an approximation, as in the calculation the free energies of dilution X and X^1 are considered equal, since no adequate data are available for pyruvic acid (Massol (21), Simon (22), Meyerhof (23)).

The free energies of dissociation were calculated as follows:

$\Delta F_2 = RT \ln Ka_l$ where $pKa_l = 3.85$ (Clark (24), p. 678).
$\Delta F_4 = RT \ln Ka_p$ " $pKa_p = 2.65$ (Barmore (25), p. 41).

DISCUSSION

That the system lactate/pyruvate is irreversible in the absence of a suitable enzyme was shown by Barmore (25), who, working in the absence of enzymes, found that lactic acid is not oxidized by quinhydrone but is oxidized by $KMnO_4$ while pyruvic acid is not reduced by reduced 1-naphol, 2, sulfonate, nor by $TiCl_3$. We have found that lactate + pyruvate + enzyme does not establish a measurable potential on a bare platinum electrode except through the intermediary of a reversible system of suitable $E'o$, as for example indigo disulfonate, in the presence of which the coupled redox potential (as defined on page 962) is established.

The fact that this coupled redox potential obeys equation (2) proves that the system is reversible in the presence of the lactic acid dehydrogenase. Wurmser (27) and coworkers have also shown that this is the case by essentially the same method. These investigators added lactate to oxidized dye and pyruvate to reduced dye and followed the

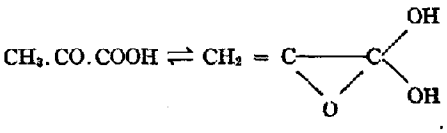
change in potential electrometrically. The enzyme present was Stephenson's (26) preparation of autolysed *B. coli*. The reduced dye (phenosafranine, $E'o$ −0.255 pH 7.4) was oxidized by pyruvate (but not by lactate) and oxidized dye (cresyl violet $E'o$ pH 7.4 −0.175) was reduced by lactate. The potentials of the two vessels approached each other but did not meet because the dyes did not sufficiently poise the electrode in the region of equilibrium.

When, after 24 hours, the reduction of cresyl violet by lactate had ceased, pyruvate was added until the ratio of pyruvate/lactate was 7 (Wurmser (27)). The Eh at 37°, pH 7.5, was then −0.165, rH_2 = 9.34, from which Wurmser calculates:

$$K = \frac{[CH_3.CO.COO^-]}{[CH_3.CHOH.COO^-]}[H_2] = 7 \times 10^{-9.34} = 10^{-8.49}$$

$$\Delta F = -RT \ln K = 12{,}040$$

This ΔF is 2,500 calories less than the value we obtain and the $\bar{E}$ 0.060 volts less than our determination. A correction for the 5°C. temperature difference might increase this discrepancy by a few millivolts; and it is quite possible that the pH was much greater than Wurmser indicates, because no statement is made regarding pH determinations except that in certain experiments 1 cc. of 7.4 phosphate buffer is added to 1 cc. *B. coli* autolysate, 1 cc. M/20 Na pyruvate, and 1 cc. M/1000 dye. Such a mixture could very well change pH, especially toward greater alkalinity. In all of our experiments the pH was determined at the end of the experiment with all of the reactants present and definite ratios of pyruvate/lactate were present from the start. Fromageot, Pelletier, and Ehrenstein (28) present evidence that the equilibrium:



shifts to the right at pH > 5.2. A difference in the proportion of this reactive compound, resulting from differences in methods of preparation, might account for our lack of agreement with Wurmser (27); *i.e.*, we may each be determining the ΔF of a different compound.

Although some discrepancy exists between Wurmser's data and our own, they both offer proof that the system lactate $\rightleftharpoons$ pyruvate is reversible.

Haldane (29) has pointed out that the regulatory mechanism that governs the process of cellular oxidation is still unknown. Understanding of this mechanism may be gained through knowledge of the coupled redox potentials of other metabolites, for the *Eh* at which the cell is poised may determine the ratio of reactants that can exist. Wurmser (30) has already made some progress in this direction.

Such a calculation can be made for the ratio of fumarate/succinate that can exist in muscle at 37°, assuming the pH 6.8, and assuming that muscle is poised at $Eh = 0.008$ ($\bar{E}\ 37° = 0.418$) at which methylene blue would be half reduced, the expected ratio of fumarate to succinate based on $\bar{E}\ 37° = 0.423$ (Borsook and Schott (4)) would be:

$$\frac{0.423 - 0.418}{0.0307} = \log \frac{[\text{succinate}^=]}{[\text{fumarate}^=]}$$

Therefore,

$$\text{succinate}^=/\text{fumarate}^= = 1.45.$$

Needham (31) found a ratio of 2 in pigeon breast muscle which is a very good agreement. In a similar manner the ratio of lactate to pyruvate in resting muscle would be:

$$\frac{\left(0.316_{38°} + \frac{\Delta E}{\Delta T}\,5\right) - 0.418}{0.0307} = \log \frac{[\text{lactate}^-]}{[\text{pyruvate}^-]}$$

which indicates that given sufficient time for the reaction to come to equilibrium, the system would go entirely to the oxidized form and no lactate would remain. The rate of activation of the lactate by the dehydrogenase would be a limiting factor which would permit of wide departures from the equilibrium state. During activity the *Eh* of muscle may drop to a low value, possibly within the range of finite proportion of lactate/pyruvate. The fact that some lactate is always present is due to the continuous glycolysis which keeps adding this reductant in competition with the penetration of oxygen into the cell. The oxygen combines with the hydrogen of cytochrome (or other inter-

mediary) reduced by the lactic acid in its oxidation to pyruvic acid. (Haarmann (32) has found that all tissues reduce pyruvate to lactate *in vacuo*.) The pyruvic acid may then be decarboxylated to acetaldehyde and CO_2 (Wieland (33)). Meyerhof (23) suggests that resynthesis of glucose in muscle goes mainly through the route lactic-pyruvic-acetaldehyde-glucose. It would not seem likely that pyruvate could be reduced to lactic acid in the cell in view of the coupled redox potential of this system and, therefore, the direction of Meyerhof's synthesis is given support by these thermodynamic considerations.

SUMMARY

1. The term "coupled redox potential" is defined.

2. The system lactic ion $\overset{\text{enzyme}}{\rightleftharpoons}$ pyruvic ion $+ 2H^+ + 2e$ is shown to be reversible (when the enzyme is lactic acid dehydrogenase) and its coupled redox potential between pH 5.2 and 7.2 at 32°C. is:

$$E'_o = 0.316 + \frac{RT}{F} \ln [H^+] + \frac{RT}{2F} \ln \frac{[\text{pyruvate}^-]}{[\text{lactate}^-]}$$

3. The free energy of the reaction:

$$\text{lactic ion } (1m) \rightarrow \text{pyruvic ion } (1m) = -\Delta F = -14{,}572.$$

4. The standard free energy of formation (ΔF°_{298}) of pyruvic acid (l) is estimated at $-108{,}127$. This is merely an approximation as some necessary data are lacking.

5. The importance of coupled redox potentials as a factor in the regulation of the equilibrium of metabolites is indicated.

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