

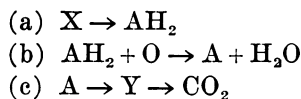
# KINETICS OF AN INTRACELLULAR SYSTEM FOR RESPIRATION AND BIOELECTRIC POTENTIAL AT FLUX EQUILIBRIUM

GORDON MARSH

(WITH FOUR FIGURES)

## Introduction

The oxidation-reduction theory of bioelectric currents formulated by LUND (14) has received much experimental confirmation (16, 17). The theory assumes the normal aerobic respiratory process in the living cell to give rise to the electromotively active materials, as follows:



X is a precursor substance;  $AH_2$  and A are the reductant and oxidant, respectively, of the electromotive material.

If we assume the dissociation  $AH_2 \rightarrow A + 2H^+ + 2e$  we may write

$$[e] = \sqrt{\frac{K [AH_2]}{[H^+]^2 [A]}}$$

where  $[e]$  is the electron concentration of the solution. The equation for the electromotive force developed at a locus (phase boundary) within the living cell may then be written:<sup>1</sup>

$$E_h = E_o - \frac{RT}{2F} \ln \frac{[A]}{[AH_2]}$$

where  $E_h$  is the potential referred to the hydrogen electrode,  $E_o$  a constant (containing  $\frac{RT}{2F} \ln \frac{[H^+]^2}{K}$ ). The difference of potential across the living cell is assumed to consist of the difference between the oppositely oriented potentials of at least two loci, or

$$E_p = E_{h_1} - E_{h_2}$$

The E.M.F. measured across a tissue will be the algebraic sum of the polarity potentials of all the cells included in the measuring circuit, or

<sup>1</sup> The convention of sign is that of LEWIS and RANDALL (12, pp. 389, 402), which is the simple negative of the more commonly used European convention (20, p. 465). An increase in the ratio  $\frac{[A]}{[AH_2]}$  increases the negative value of  $E_h$ . The European convention, however, has been followed in expressing the measured E.M.F. of cells and tissues. In consequence, the locus of high negative potential according to the equation is *measured* as the point of high positive potential. This has led to LUND'S formal error of statement that the value of the ratio is smaller at that locus which is positive in the measuring circuit.

$$E = \sum E_p$$

For a given rate of oxygen uptake the ratio  $\frac{[A]}{[AH_2]}$  will possess a constant value, a condition termed by LUND a flux equilibrium. The E.M.F. will vary with oxygen pressure and other factors influencing the rate of metabolism, although the variation is not defined precisely in LUND's formulation (in part due to the inclusion of an oxygen concentration term in the equation).

The present paper supplies a quantitative connection between the velocity of the oxidative reactions and the measured E.M.F. of a cell or tissue. Certain properties of the intracellular system thus emerge which are not otherwise obvious. The system also yields a satisfactory equation linking the rate of oxygen consumption to oxygen pressure.

At the risk of promoting a sterile controversy, it seems necessary to introduce the question of intracellular electrodes. The one objection urged against the oxidation-reduction theory of bioelectric phenomena is that metallic electrodes are absent from the cell (2). This argument is entirely *a priori*. It should be unnecessary to point out that the platinum (or other metal) electrode is *not* essential to the formulation of equations expressing the potential of an electrochemical system, nor to its manifestation (6, p. 378). "Metallic" conduction is not restricted to "metals," and a normal cell structure possessing the *essential properties* of an electrode is physically possible.

LUND's theory assumes the existence of such structures (or interfaces). Aside from its implications there is no evidence upon this point.<sup>2</sup> In view of the unanimity of the experimental support which the theory has received and its power to unite an extensive body of data into a coherent whole, the writer prefers to accept the existence of intracellular electrodes rather than to dismiss as irrelevant the evidence which LUND and his coworkers have amassed.

### Relation of $E_h$ to respiratory velocity at flux equilibrium

The steps in the oxidative reaction may be amended<sup>3</sup> to read:

<sup>2</sup> An attempt to provide direct evidence (21) has proved abortive. The color change reported at the borders of *Tradescantia* cells proved to be due primarily to retraction of the protoplast from the cell wall, not to a reversible pH change. The conclusion that conductors of the first class exist within the cell is unsupported (see also BLINKS, 3).

<sup>3</sup> LUND's equation (b) was not intended to specify the intervention of atomic oxygen. Consequently no great violence is done in altering the reaction to equation (2) above, while the mathematical treatment may be considerably simplified. The extra oxygen atom is here combined in A; for purposes of formulation the reaction might be written in any of a number of other ways. Until our knowledge of the specific chemical details of the respiratory process is greatly enhanced, the dynamics of the system cannot with profit be elaborated beyond some simple formulation like the present one.



Because of the existence of (a) "basal metabolism" and (b) an asymptote to the oxygen consumption: oxygen pressure curves (equation (15) below), we may write  $[X] = C$ . ( $C$  varies with the nutritional state, but appears constant for a given state.) The number of equivalents (per liter) of  $X$  transformed irreversibly in time  $t$  will then be  $\frac{-dx}{dt} = k_1C = K_1$ .

At flux equilibrium the velocities of production and removal of  $AH_2$  and  $A$  and the velocity of oxygen consumption will all equal  $K_1$ , unless respiration be primarily determined by rate of diffusion of oxygen, a condition which theoretical and experimental investigations indicate to be non-existent, or extremely rare (11, 9). Under these conditions the E.M.F. at a locus will vary with oxygen pressure but the rate of oxygen consumption will be constant. Since, experimentally, consumption does vary with pressure,  $X \rightleftharpoons AH_2$  must be reversible.

Let  $[X] = C$ . Then  $k_1C$  = the tendency of  $X$  to form  $AH_2$ , and  $k_2[AH_2]$  = the tendency of  $AH_2$  to form  $X$ . The number of equivalents (per liter) of  $X$  changing to  $AH_2$  in time  $t$  will be:

$$\frac{-dx}{dt} = k_1C - k_2[AH_2]$$

This is also the rate of production of  $AH_2$  due to reaction (1), or

$$\frac{d[AH_2]}{dt} = k_1C - k_2[AH_2] \quad (4)$$

The rate of disappearance of  $AH_2$  according to reaction (2) may be written

$$\frac{-d[AH_2]}{dt} = k_3P_c[AH_2] \quad (5)$$

where  $k_3$  is the velocity constant of reaction (2) and  $P_c$  is the flux equilibrium oxygen tension at a locus within the cell.

At flux equilibrium (4) = (5), whence

$$[AH_2] = \frac{k_1C}{k_2 + k_3P_c} \quad (6)$$

The rate of disappearance of  $AH_2$  equals the rate of production of  $A$ . Combining (6) and (5)

$$\frac{d[A]}{dt} = \frac{k_3k_1CP_c}{k_2 + k_3P_c} \quad (7)$$

The rate of disappearance of  $A$  due to reaction (3) is then

$$\frac{-d[A]}{dt} = k_4[A] \quad (8)$$

where  $k_4$  is the velocity constant of reaction (3). As before, at flux equilibrium (7) = (8), and

$$[A] = \frac{k_3 k_1 C P_c}{k_4 (k_2 + k_3 P_c)} \quad (9)$$

The ratio of concentrations of oxidant and reductant at a locus (at flux equilibrium) will then be

$$\frac{[A]}{[AH_2]} = \frac{k_3 P_c}{k_4} \quad (10)$$

and the electromotive force developed at a locus becomes

$$E_h = E'_o - \frac{RT}{2F} \ln P_c \quad (11)$$

$-\frac{RT}{2F} \ln \frac{k_3}{k_4}$  is included in the constant  $E'_o$ .

$P_c$  may be expressed as a function of the velocity constants of the reactions and the oxygen pressure of the medium surrounding the living cell. We may write the rate of diffusion of oxygen into a locus, in accordance with Fick's law, as

$$\frac{do}{dt} = k_o (P - P_c) \quad (12)$$

where  $P$  is the oxygen tension of the medium in contact with the cell and  $k_o$  is a diffusion constant for oxygen. The rate of oxygen consumption at a locus  $\left(\frac{-do}{dt}\right)$  may be expressed by (7). At flux equilibrium the rate of diffusion equals the rate of consumption; equating (7) and (12) we obtain the expression

$$k_o k_3 P_c^2 + (k_3 k_1 C + k_o k_2 - k_o k_3 P) P_c - k_o k_2 P = 0$$

whose roots are

$$P_c =$$

$$\frac{k_o k_3 P - k_3 k_1 C - k_o k_2 \pm \sqrt{(k_1 k_3 C + k_o k_2)^2 + P^2 k_o^2 k_3^2 - 2P(k_o k_1 k_3^2 C - k_o^2 k_2 k_3)}}{2k_o k_3}$$

This may be simplified by writing

$$\frac{k_1 k_3 C + k_o k_2}{k_o k_3} = a, \text{ and } \frac{k_1 k_3 C - k_o k_2}{k_o k_3} = b$$

The constants are all positive in sign as written, and not zero. When  $P = 0$ ,  $P_c = 0$ , hence

$$\frac{-a \pm \sqrt{a^2}}{2} = 0$$

Only the positive root satisfies this condition. Therefore

$$P_c = \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \quad (13)$$

and

$$E_h = E'_o - \frac{RT}{2F} \ln \frac{P - a + \sqrt{a^2 + P^2 - 2Pb}}{2} \quad (14)$$

Figure 1 shows the variation of  $P_c$  with  $P$  for certain values of the con-

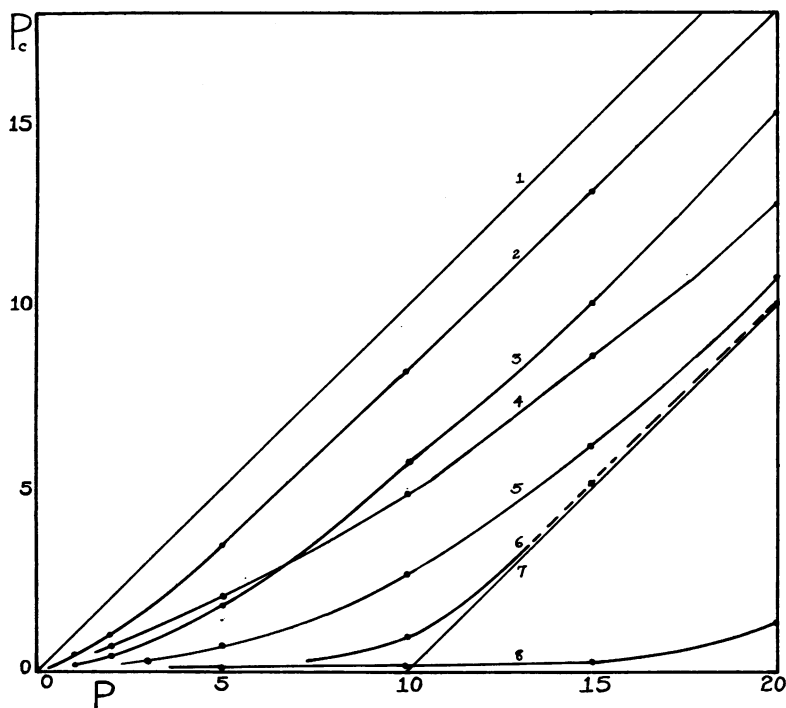


FIG. 1.  $P_c$  vs.  $P$  for the following values of the constants: (2)  $a=3$ ,  $b=1$ ; (3)  $a=6$ ,  $b=4$ ; (4)  $a=15$ ,  $b=5$ ; (5)  $a=11$ ,  $b=9$ ; (6)  $a=10.1$ ,  $b=9.9$ ; (8)  $a=20.1$ ,  $b=19.9$ . (1) is the line  $P_c = P$ ; (7) is the line  $P_c = P - \frac{a+b}{2} = P - 10$ , to which (4), (5), and (6) are asymptotic.

stants.  $P_c = P$  at  $P = 0$ ; from zero  $P_c$  increases with  $P$  to become asymptotic to  $P_c = P - \frac{a+b}{2}$ . The smaller the value of  $a-b$  the more rapidly the curve approaches the asymptote, as may be seen in curves (4), (5), and (6). The reference line  $P_c = P$ , curve 1, is included in the figure to show how the flux equilibrium oxygen tension,  $P_c$ , differs from the external oxygen pressure,  $P$ , for different sets of values of the constants. The larger

the sum  $a + b$  and the smaller the difference  $a - b$  the more widely does  $P_c$  depart from  $P$ .

$E_h$ , correspondingly, increases with increasing  $P$  (in harmony, it might be noted, with biological redox potential measurements). The locus in the living cell becomes, under this treatment, a modified oxygen electrode, and the ratio  $\frac{[A]}{[AH_2]}$  cannot be combined with  $P$  (or  $[O]$ ) in the equation for  $E_h$ , as done by LUND (16, p. 244).

#### Electrical polarity of the cell, $E_p$

The polarity potential of a cell will evidently be

$$E_p = \frac{-RT}{2F} \ln \frac{P_{c_1}}{P_{c_2}} = \frac{-RT}{2F} \ln r'$$

$$= \frac{-RT}{2F} \ln \frac{P - a_1 + \sqrt{a^2 + P^2 - 2Pb_1}}{P - a_2 + \sqrt{a^2 + P^2 - 2Pb_2}}$$

where the subscript 1 designates the locus of high positive potential in the measuring circuit. (The negative sign of  $E_p$  comes from the convention used, as explained above.)

Variation of  $r'$  with  $P$  for four sets of arbitrary values<sup>4</sup> of the velocity constants is shown in figure 2.  $r'$  increases from 1 at  $P = 0$ , passes through a maximum, and again decreases to 1 as  $P$  becomes indefinitely large.  $E_p$  correspondingly would increase from 0 at  $P = 0$ , pass through a maximum, and again return to 0. Figure 1 illustrates why this must be so. With the difference between the two loci determined by  $k_1$  (thus avoiding intersection of the  $P_c$  curves), equal increments of  $P$  cause a relatively greater increase in  $P_c$  for the positive locus (as measured externally) in the lower portions of the curves, and the ratio  $r'$  will increase. As the curves approach their parallel asymptotes, equal increments of  $P$  produce approximately equal increments in both  $P_c$ 's, and  $r'$  decreases.

The position of the maximum on the  $P$  axis and the absolute value of the maximum are functions of the values of the constants. No general solution for either position or value has been found. Differentiating  $r'$  with respect to  $P$  yields an equation which is too involved to admit of solution by ordinary methods. It can only be demonstrated that substitution of appropriate values for the velocity constants furnishes numerical results which are in harmony with the experimental facts.

For the ascending limb of the curve the polarity potential increases with increasing  $P$ . The position of the maximum for systems whose variation

<sup>4</sup> For purposes of illustration it is assumed that the difference between two loci is solely determined by the velocity constant  $k_1$ , which is smaller at the locus of greater  $E_h$ . No significance is to be attached to such a choice of constants.

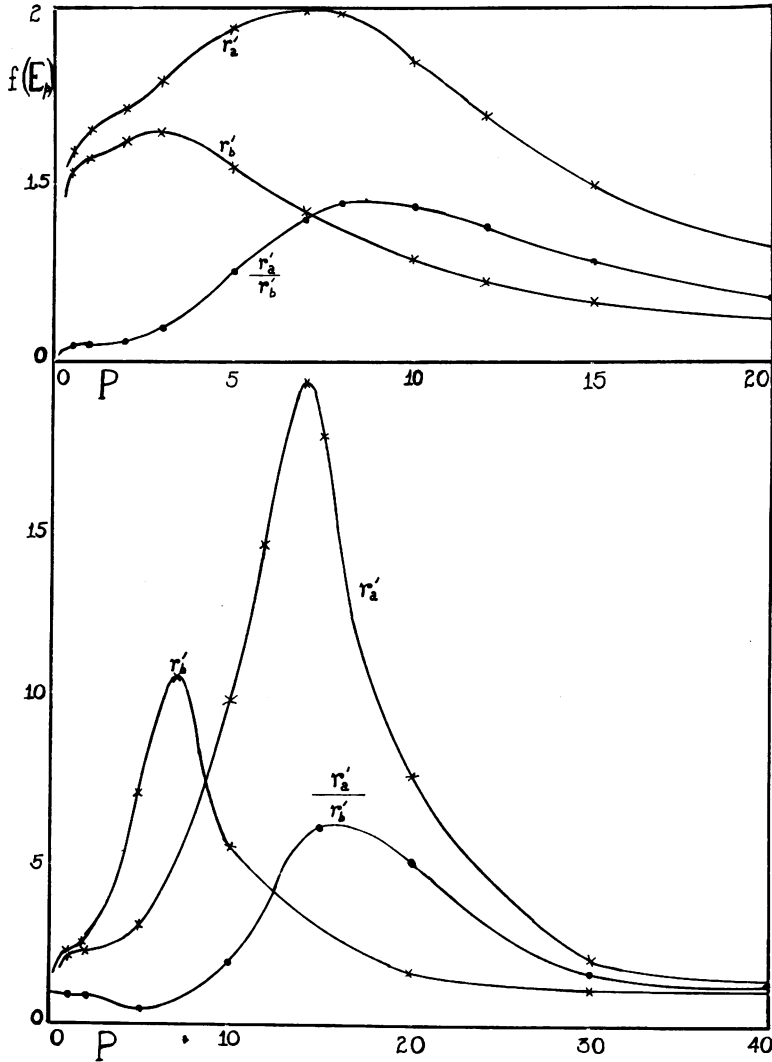


FIG. 2. Variables of the equations for  $E_p$  and  $E$  plotted against oxygen pressure.  $r'_a = f(E_p)$  for apical cell;  $r'_b = f(E_p)$  for basal cell.  $r'_a/r'_b = f(E)$ , the E.M.F. being oppositely oriented in the two cells. B shows reversal of apico-basal polarity at low values of  $P$ .

with  $P$  has thus far been investigated (frog skin, 15, 8; onion root, 22) evidently lies outside the range of oxygen pressures used.

If the difference between the two loci giving rise to  $E_p$  involves one or more constants in addition to  $k_1$  and  $C$ , the  $P_c$  curves may intersect (curves (3) and (4), fig. 1). Let  $P_{c_1}$  follow curve (3) and  $P_{c_2}$  follow curve (4).

$E_p$  for such a cell would show, in addition to the behavior just described, complete reversal of polarity below  $P = 6.5$  (approximate). The critical pressure for reversal, or intersection of the curves, would be determined by the magnitudes of the constants.

Such a reversal of polarity has recently been found for the apical 1.5 mm. of the onion root tip exposed to  $H_2$  gas (22). The potential gradient of this region of the root is unidirectional as a rule. If basally oriented regions existed the reversal may have been of the type discussed in the following section.

#### Measured E.M.F. of a tissue or cell group

The E.M.F. measured across a tissue may evidently be (1) the sum of the elements of a unidirectional potential gradient. In this case the variation of  $E$  with  $P$  will not differ fundamentally from the variation of  $E_p$  with  $P$ . Or (2)  $E$  may be the algebraic sum of the elements forming two (or more) oppositely directed potential gradients, *e.g.*, the axially growing organs or organisms such as *Obelia* stem, roots and shoots of plants, etc. Analysis of the behavior of  $E$  for this type of tissue leads to a relation between respiratory rate and the polarity potential which gives a theoretical foundation to a number of observed facts.

In tissues and organs possessing apico-basal polarity the apex is electro-positive to the base in the external circuit. The rate of oxygen consumption per unit mass is higher for apical than for basal tissues, and LUND (16) has demonstrated unequivocally for *Obelia* (and later work on the onion root) that this difference is due to the presence of a higher concentration of oxidizable material ( $AH_2$ ) in the apical cells. These facts suggest that the magnitude of the electrical polarity of a cell or tissue is a direct function of its oxygen consumption rate (at constant oxygen pressure), where the differences in oxygen uptake between cells are caused by differences in value of their respective constants, leading to differences in flux equilibrium level of concentration of  $AH_2$ .<sup>5</sup> The properties of the system as outlined above are such as to yield this result.

The relation may be demonstrated as follows: Assume, as before, the difference between two loci in the cell to be determined by  $k_1$ . Let the differences in level of  $[AH_2]$  (between cells) be caused by variation in  $C$ . For convenience of handling we may write  $o'$  for  $\frac{-do}{dt}$  at a given locus. Then from (12) and (5)

$$o' = k_o P - k_o P_c = k_3 P_c [AH_2]$$

<sup>5</sup> The conclusion of LUND and MOORMAN (19) that the potentials of twelve frog skins bore no obvious relation to their rate of oxygen consumption offers no conflict to this view, if only upon statistical grounds. In fact their data suggest that for a large number of skins such a relation might appear.



or

$$P_c = \frac{k_o P}{k_o + k_3 [AH_2]}$$

Substituting into (5) we obtain

$$o' = \frac{k_o P k_3 [AH_2]}{k_o + k_3 [AH_2]}$$

At any fixed and finite value of  $P$ ,  $[AH_2]$  will be determined by  $C$ , and

$$\lim_{C \rightarrow 0} o' = 0 \quad \text{and} \quad \lim_{C \rightarrow \infty} o' = k_o P$$

From (12)

$$P_c = \frac{k_o P - o'}{k_o}$$

and

$$r' = \frac{k_o P - o'_1}{k_o P - o'_2}$$

where the subscripts 1 and 2 designate respectively the positive and negative loci as measured externally. Then

$$\lim_{C \rightarrow 0} r' = 1 \quad \text{and} \quad \lim_{C \rightarrow \infty} r' = 1$$

For  $0 < P < \infty$ ,  $o'_1 < o'_2$  (equation 15), hence  $r'$  varies for fixed  $P$  and changing  $C$  in the same way as for fixed  $C$  and changing  $P$  (fig. 2), and  $E_p$  rises from 0, passes through a maximum, and again descends to 0 as  $C$  increases.

The rate of oxygen consumption of the cell will increase with  $C$ . Under these conditions, and for the ascending limb of the  $E_p$  vs.  $C$  curve, the cell having the higher oxygen consumption will possess the larger  $E_p$ , and this will be true for all values of  $P$  between 0 and  $\infty$ . Thus the net electropositivity of the apex of polar tissues over the oppositely oriented basal regions is susceptible of explanation.

This relation carries the implication that the state of nutrition of a tissue determines the magnitude of its measured E.M.F., for it is well known that the nutritional state affects the rate of oxygen uptake. Direct experimental proof has recently been presented by LUND (18), who showed that skins from frogs fed on liver gave potentials 83 per cent. higher (and oxygen consumption 28 per cent. greater) than those from starved animals. This is supported by observations that captivity results in lowered potential (5, p. 362; 8, p. 381). The clearest indication of a connection between magnitude of potential and concentration of oxidizable material exists in the phenomenon of "rebound" or "overshooting," a temporary increase in P.D. when  $P$  is changed from a low to a high value (15, 8, 22). The corresponding over-

shooting of oxygen consumption rate is of general occurrence and is evidently due to accumulation of  $AH_2$ .

The "apical" cell will also show a relatively greater increase in  $E_p$  with  $P$ . For simplicity let the E.M.F. represent the algebraic sum of the oppositely oriented polarity potentials of an apical and a basal cell. Then

$$E = \sum E_p = \frac{-RT}{2F} \ln \frac{r'_a}{r'_b}$$

The subscripts a and b respectively designate the apical and basal cells. The difference between two loci is determined by the constant  $k_1$  and this difference is the same for the basal as for the apical cell. The apical cell is assumed to have the higher value of  $C$ . The variation of  $\frac{r'_a}{r'_b}$  with  $P$  is plotted in figure 2, using the values of the constants given in table I.  $\frac{r'_a}{r'_b}$

TABLE I  
CONSTANTS OF THE CURVES IN FIGURE 2

	2A: $(k_1)_1 = 3, (k_1)_2 = 5$		2B: $(k_1)_1 = 5, (k_1)_2 = 10$	
	APICAL CELL ( $r'_a$ )	BASAL CELL ( $r'_b$ )	APICAL CELL ( $r'_a$ )	BASAL CELL ( $r'_b$ )
C .....	2	1	2	1
$a_1$ .....	7	4	10.1	5.1
$b_1$ .....	5	2	9.9	4.9
$a_2$ .....	11	6	20.1	10.1
$b_2$ .....	9	4	19.9	9.9

increases from 1 at  $P = 0$ , passes through a maximum, and returns to 1 at  $P =$  indefinitely large. The E.M.F. would increase from 0, pass through the maximum, and again return to 0. The curve is similar in form to  $r'$  vs.  $P$  and the position of the maximum lies slightly beyond that for the  $r'_a$  curve. The rate of oxygen consumption of the apical cell in both cases is greater than that for the basal cell (calculated by equation 15 below). For the ascending limbs of the curves, the system satisfies the observed dependence of apico-basal polarity upon  $P$ .

Two cases are theoretically possible for the ascending limb. In figure 2A the apical cell is everywhere positive to the basal save at  $P = 0$  and  $P = \infty$ . When the value of the ratio  $\frac{k_2}{k_3}$  is small compared with the value of  $\frac{k_1 C}{k_0}$ , however, the tissue will show an inverted polarity at low values of the oxygen

pressure, as appears in figure 2B. Reversal of tissue polarity under these conditions is due to the fact that  $E_p$  increases more rapidly in the vicinity of  $P = 0$  for the basal cell than for the apical.

### Velocity of cell oxidation and oxygen pressure

The treatment given above yields a theoretical equation linking the velocity of cell oxidation to the oxygen pressure of the medium.<sup>6</sup> The rate of oxygen uptake at a locus is expressed by equation (7)

$$\frac{-do}{dt} = \frac{k_1 C k_3 P_c}{k_2 + k_3 P_c}$$

Upon substituting the value of  $P_c$  from (13) we obtain

$$\frac{-do}{dt} = \frac{k_1 C k_3 (P - a + \sqrt{a^2 + P^2 - 2Pb})}{2k_2 + k_3 (P - a + \sqrt{a^2 + P^2 - 2Pb})}$$

Dividing the numerator and denominator by  $k_3$  and performing the substitution  $\frac{2k_2}{k_3} = a - b$ , gives

$$\frac{-do}{dt} = \frac{k_1 C (P - a + \sqrt{a^2 + P^2 - 2Pb})}{(P - b + \sqrt{a^2 + P^2 - 2Pb})} \quad (15)$$

Equation (15) is plotted against  $P$  in figure 3 for six sets of values of the constants.  $\frac{-do}{dt} = 0$  at  $P = 0$ ; it increases rapidly at first, then more slowly, and approaches  $k_1 C$  as a limit as  $P$  becomes indefinitely large.

The measured respiratory rate of a tissue or cell will be the sum of the  $\frac{-do}{dt}$ 's for all the loci. For simplicity we may write<sup>7</sup>

$$O' = \sum \frac{-do}{dt}$$

The characteristics of the curve  $O'$  vs.  $P$  will be similar to those of the individual  $\frac{-do}{dt}$  curves, and the curve will approach  $\sum k_1 C$  as a limit as  $P$  increases. This may be seen in figure 3, where curve (1) is obtained by adding together the points on curves (2), (3), and (4). The summation curve will follow the course of an equation identical in form with equation (15), or

$$O' = \frac{\sum k_1 C (P - a' + \sqrt{a'^2 + P^2 - 2Pb'})}{P - b' + \sqrt{a'^2 + P^2 - 2Pb'}} \quad (16)$$

<sup>6</sup> For a recent review see TANG (24).

<sup>7</sup>  $O'$  is introduced to avoid possible confusion of the more generally used  $A$  with the same symbol for the oxidant of the electromotively active material.  $O'_m$  corresponds to the function  $A/A_0$ .

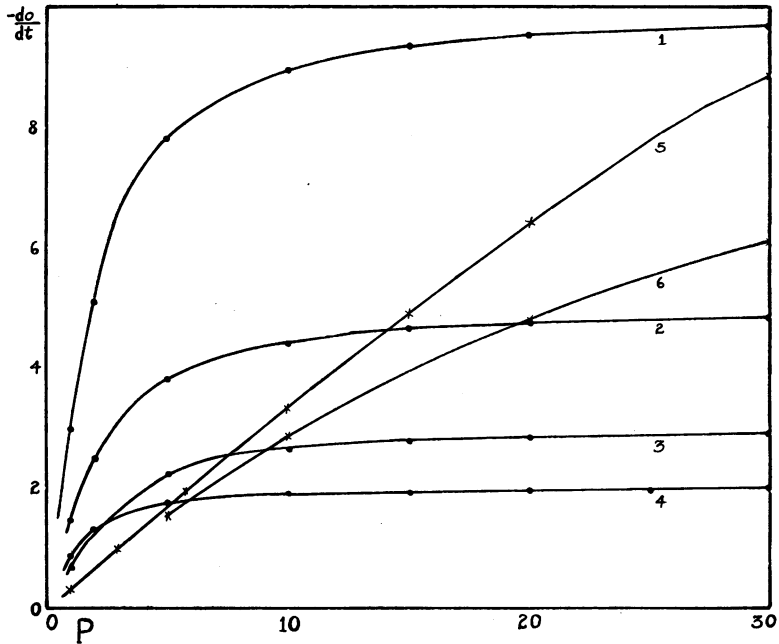


FIG. 3. Rate of oxygen consumption *vs.* oxygen pressure for the following values of the constants: (2)  $k_1C=5$ ,  $a=3$ ,  $b=1$ ; (3)  $k_1C=3$ ,  $a=4$ ,  $b=2$ ; (4)  $k_1C=2$ ,  $a=2$ ,  $b=1$ ; (1), the summation curve of (2), (3), and (4) is fitted by the same general equation; values of the constants are  $\Sigma k_1C=10$ ,  $a'=2.98$ ,  $b'=1.167$ . (5) shows a nearly linear relation between consumption and pressure,  $k_1C=10$ ,  $a=30$ ,  $b=29$ . (6)  $k_1C=10$ ,  $a=30$ ,  $b=5$ .

$a'$  and  $b'$  will lie within the extremes of the values of  $a$  and  $b$  respectively for the different loci. No general expression has been obtainable for determining their value.

$\Sigma k_1C$  may be eliminated in the following manner:

$$\text{Let } O'_m = \frac{O' \text{ at } P}{O' \text{ at its maximum value}} = \frac{O' \text{ at } P}{\Sigma k_1C}$$

Then,

$$O'_m = \frac{P - a' + \sqrt{a'^2 + P^2 - 2Pb'}}{P - b' + \sqrt{a'^2 + P^2 - 2Pb'}} \quad (17)$$

The behavior of  $O'_m$  *vs.*  $P$  for different values of  $a'$  and  $b'$  may be inferred from figures 3 and 4. The larger the value of  $a'$  the more slowly does the curve approach its asymptote. For a given value of  $a'$  the curve approaches its asymptote rapidly when  $a' - b'$  is small, slowly when  $a' - b'$  is large. With  $a'$  and  $b'$  very large and  $a' - b'$  very small, the rate of oxygen consumption will appear linear over even a considerable range of  $P$ . In describing

experimental results of the linear type equation (16) would be employed, since the maximum rate of oxygen consumption is not determined.

Equation (17) has been applied to the majority of the data to be found in the literature, and in most cases yields a satisfactory fit. Space limitations prevent complete analysis of all available data; enough only is presented to illustrate the advantages and weaknesses of the equation as a description of experimental results.

Six sets of data are plotted in figure 4. The curves represent the course

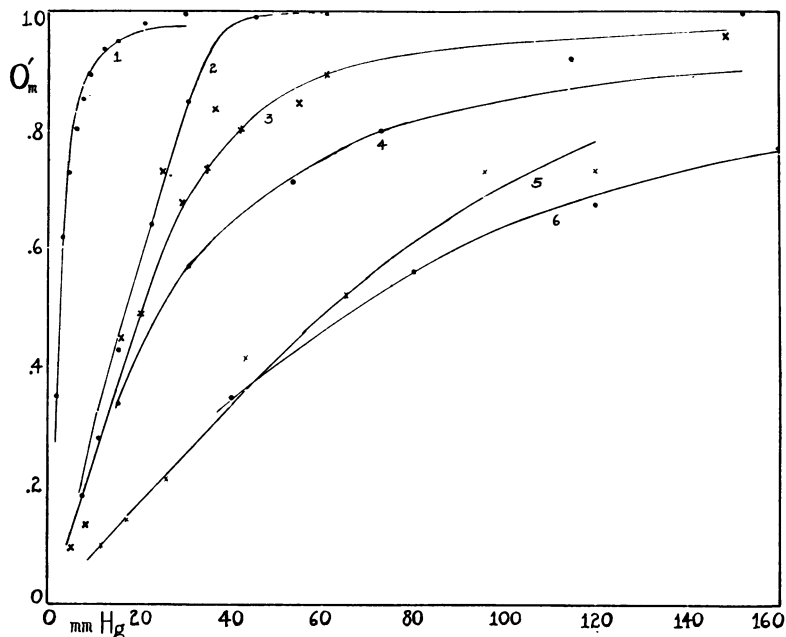


FIG. 4. Rate of oxygen consumption vs. oxygen pressure for six sets of published data: (1) SHOUP (23) luminous bacteria,  $a' = 4$ ,  $b' = 2.7$ ; (2) TANG and GERARD (25), fertilized *Arbacia* eggs,  $a' = 35.4$ ,  $b' = 35.26$ ; (3) LUND (13), *Planaria agilis*,  $a' = 35.5$ ,  $b' = 27.5$ ; (4) MEYERHOF (GERARD, 9, p. 267),  $\text{NO}_3$  bacteria,  $a' = 37.2$ ,  $b' = 8.41$ ; (5) AMBERSON, MAYERSON, and SCOTT (1), *Homarus*,  $a' = 113$ ,  $b' = 90$ ; (6) THUNBERG (26), *Limax*,  $a' = 93.6$ ,  $b' = 17$ . The curves follow the calculated values; observed values indicated by crosses or dots.

of equation (17); experimental points appear as crosses or dots. An arbitrary maximum was estimated for curve (5). For curves (2) and (6) the correspondence of observed and calculated values is nearly complete. SHOUP'S (23) results for luminous bacteria, curve (1), reach the maximum somewhat earlier than the calculated curve. Curves (3) and (5) show good correspondence except for the scattering of the experimental points. An incomplete fit is afforded the data of MEYERHOF on  $\text{NO}_3$  bacteria (table in 9,

p. 267), shown in curve (4). Calculated and observed values correspond almost exactly until  $P$  reaches about 80 mm. Hg, beyond which the observed points deviate, reaching  $O_m=1$  in advance of the theoretical curve. Similarly data by TANG on unfertilized *Arbacia* eggs (9, p. 266) can be accurately fitted save for the upper three values. Determination of the point of 100 per cent. respiration is not always easy, and a moderate error may considerably alter the shape of the resulting  $O_m$  vs.  $P$  curve.

The equation will not follow data which show an upward concavity when plotted as above, e.g., those of AMBERSON, MAYERSON, and SCOTT (1) for *Nereis*. In the modified form, however, it will describe the linear relation between  $O'$  and  $P$  found by HALL (10) for the toadfish. Reasonable correspondence is found for other data (scup and puffer, 10; fertilized *Arbacia* eggs, 25; frog's skin, 15; termite, 7; and others). An almost point to point fit is given the curve for  $NO_2$  bacteria, MEYERHOF (table in 9) and to data by BODINE (4) on embryos of *Melanoplus differentialis*.

The theoretical equations relating oxygen consumption to oxygen pressure through the effect of oxygen upon the reaction velocity of the oxidative process are summarized by TANG (24). All take the hyperbolic form

$A = \frac{P}{K_1 + K_2 P}$  where  $A$  is the oxygen consumption rate,  $P$  the oxygen pressure, and  $K_1$  and  $K_2$  are constants. GERARD (9), using the equation in the form

$$\frac{A}{A_0} = \frac{[O_2]}{K + [O_2]}$$

found  $K$  only roughly constant, if at all, for data by SHOUP, TANG, and MEYERHOF. Except for TANG's data the variation in  $K$  was greatest in the lower range of  $P$ . The graphical application of the equation to twenty-four sets of data by TANG (24) likewise is least successful at small values of  $P$ . (A scale for the ordinate in TANG's figures would make it possible to judge how well the equation really fits.)

In the "hyperbolic" equation  $P$  is interpreted to be the oxygen pressure of the medium, although upon the underlying assumptions it must be the intracellular oxygen pressure. The equation is identical with (7) above, and is valid only when  $P_c/P$  approaches 1. This will occur as  $P \rightarrow 0$  and as  $P \rightarrow \infty$ ; it will be true for all values of  $P$  only for cells in which  $\frac{a+b}{2}$  is very small. It would be expected, therefore, that for small values of  $P$  the hyperbolic equation would show a poor fit for numerous experimental data, as GERARD and TANG apparently have found.

The estimation of the relative applicability of similar mathematical formulas to experimental results is uncertain. Nevertheless, because of its competency to follow experimental values in the lower range of  $P$  where

the hyperbolic equation tends to deviate, and because of the somewhat sounder theoretical foundation, the writer is of the opinion that the equation developed in this paper will prove more generally valid.

Finally it may be remarked that the derivation of a valid oxygen consumption: oxygen pressure relation upon the basis of the formulation of the reacting system for E.M.F. fulfills a necessary condition for the oxidation-reduction theory of bioelectric currents.

### Summary

A quantitative connection is derived between the velocity of respiration and inherent cellular E.M.F. upon the basis of the amended steps in the oxidative reaction proposed by LUND. The system shows the following properties:

1. Direct dependence of the potential at a locus upon oxygen pressure.
2. Dependence of the electrical polarity of a cell (difference between two or more loci) upon oxygen pressure.
3. Direct dependence of the magnitude of cell polarity upon the concentration of the oxidizable material, *i.e.*, upon rate of oxygen uptake at constant oxygen pressure.
4. Relatively greater increase in cell polarity with increased oxygen pressure for the cell possessing the higher concentration of the oxidizable material, *i.e.*, dependence of apico-basal polarity upon oxygen pressure.
5. Reversal of cell or tissue polarity at low oxygen pressures under appropriate conditions.

An oxygen consumption-oxygen pressure equation is obtained which satisfactorily describes existing data.

ZOOLOGICAL LABORATORY  
STATE UNIVERSITY OF IOWA

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