ABSOLUTE REACTION RATE THEORY AND THE RESPIRATORY REBOUND*

by

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Experiments in physical chemistry are generally designed to take advantage of a unique property of a system; thus, those experiments designed to show the presence of unpaired electrons on free radicals must exploit the magnetic properties of these unpaired electrons in order that their existence may be established; as pointed out by SIMON (quoted by PAULING[1]), those techniques designed for a closer and closer approach to the absolute zero of temperature depend upon the utilization of phenomena which appear at these low temperatures in order that they might be achieved. Similarly, a kinetic study of a biological system becomes in the final analysis an exhibition of the kinetic properties unique to a living system. Perhaps the greatest difference between kinetic studies in this type of system and a purely chemical system (of the usual type) is that in the former all reactions proceed in such a fashion as to give rise to the "steady state". This was first noted by HILL[2] and has been defined[3] as existing "... if the rate of change with time of any given parameter, say the concentration of a given reactant of the system, is small compared to the rate at which that reactant is being transformed in one direction..." Its properties may be more clearly demonstrated by a comparison with the properties of a system in equilibrium, which it resembles in superficial aspects only. BURTON[3] has indicated that the differences are:

1. Energy is never dissipated in an equilibrium, but it is dissipated in the steady state.
2. In an equilibrium system forward and back reactions proceed at the same rates; in the steady state there is always a difference in the absolute values of the forward and backward rates.
3. When a system is in complete thermodynamic equilibrium, the decisive factor for the position of the equilibrium is the combined chemical potential energy of the reactants and the products of the system; in a steady state, the "position of the steady state" is determined not only by the potential energy of the particular steady state reaction, but also by a quantity termed the "flux energy" of the steady state.

The existence of a chemical potential factor in the position of the steady state does

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explain why the behaviour of a living system may be influenced by gravitation forces[4], by electrical forces[5] or by any force which has as its resultant the distortion of the chemically reacting masses bounded by phase produced surfaces. It should also be true that capillary forces might change the position of the steady state, although there seems to be no experimental work extant in this connection, *per se*. This does not include, of course, work with surface active agents, ionic materials, or any demonstration of the effects of osmotic pressure variants on a living system, which certainly partake in their nature of effects on capillarity.

As BURTON[3] has shown, the existence of the "flux rate", R, serves to characterize further the steady state. In practice, evaluation of this R quantity provides a convenient means for estimating individual reaction rate constants or concentrations of reactants and products. In the following an attempt will be made to show that in the flux rate we have a quantity of greater theoretical significance than the equations as they stand at present would indicate. The catenary reactions of metabolism have a property peculiar to all reactions of the chain type in that the energies and entropies of the products of one reaction are the energies and entropies for the reactants in the subsequent reaction. In a reaction series of the equilibrated type since there is equality of rates in both directions, and since the universal frequency factor, k'T/h (k' is the Boltzmann constant, h, Planck's constant, and T, the absolute temperature) which appears in all expression for the reaction rate constant[6] is in identity, there must be a finely balanced relationship between the exponential energy and entropy terms of activation of the forward and reverse reactions; in the steady state, however, there can be no such balance and the conclusion is forced that the flux rate, R, must actually be determined in part by the difference in the energies and in the entropies of the activated complexes.

The equation given by BURTON[3] for the evaluation of the flux rate is of the form:

$$k_0 S^{S_1} - k_0' x_1 = k_1 x_1^{n_1} - k_1' x_2^{n_2'} = \ldots = k_z x_n - k_z Z = R \tag{1}$$

in which the k-terms represent the specific reaction constants for the reaction sequence:

$$S \underset{k'_0}{\overset{k_0}{\rightleftharpoons}} A \underset{k'_1}{\overset{k_1}{\rightleftharpoons}} B \underset{k'_2}{\overset{k_2}{\rightleftharpoons}} C \underset{k'_3}{\overset{k_3}{\rightleftharpoons}} D \rightleftharpoons \ldots \underset{k'_{n-1}}{\overset{k_{n-1}}{\rightleftharpoons}} N \xrightarrow{k_z} Z \tag{2}$$

in which x_1 is the concentration of A, x_2 is the concentration of B, etc. From the theory of absolute reaction rates[6], this may be written as:

$$\begin{aligned}&\left\{e^{-\Delta \overset{\neq}{E_0}/RT}\, e^{\Delta \overset{\neq}{S_0}/R}\, e^{\Delta \overset{\neq}{n_0}-1}\right\} S^{S_1} - \left\{e^{-\Delta \overset{\neq'}{E}/RT}\, e^{\Delta \overset{\neq'}{S_0}/R}\, e^{\Delta \overset{\neq'}{n_0}-1}\right\} x_1^{n'_1} = \\ &\left\{e^{-\Delta \overset{\neq}{E_1}/RT}\, e^{\Delta \overset{\neq}{S_1}/R}\, e^{\Delta \overset{\neq}{n_1}-1}\right\} x_1^{n_1} - \left\{e^{-\Delta \overset{\neq'}{E_1}/RT}\, e^{\Delta \overset{\neq'}{S_1}/R}\, e^{\Delta \overset{\neq'}{n_1}-1}\right\} x_2^{n'_2} = \ldots \text{etc.} = R\end{aligned} \tag{3}$$

in which $\Delta \overset{\neq'}{E}$s represent the internal energy of the active configuration, $\Delta \overset{\neq'}{S}$s, the entropies of the activated configurations, and the final exponentials represent the increase in the number of molecules involved in the formation of the activated complex. Equation (3) may be simplified. Every concentration appearing in the reaction sequence may be expressed as a function of the concentration of the reactant just immediately preceding it. This statement applies, of course, to only the ***simplest*** possible steady state reaction sequence, and does not take into consideration the existence of feedbacks, or any other complicating factor[7]. Extension of this substitution leads to:

$$[\{e^{-\Delta E_0^{\neq}/RT}\, e^{\Delta S_0^{\neq}/R}\, e^{\Delta n_0^{\neq}-1}\} - \{e^{-\Delta E_0^{\neq\prime}/RT}\, e^{\Delta S_0^{\neq\prime}/R}\, e^{\Delta n_0^{\neq\prime}-1}\}\, l\, (x_1^{n_1})^{s_1-1}]\, S^{s_1} =$$

$$[\{e^{-\Delta E_1^{\neq}/RT}\, e^{\Delta S_1^{\neq}/R}\, e^{\Delta n_1^{\neq}-1}\}\, m\, (x_1^{n_1})^{s_1-1} - \{e^{-\Delta E_1^{\neq\prime}/RT}\, e^{\Delta S_1^{\neq\prime}/R}\, e^{\Delta n_1^{\neq\prime}-1}\}\, n \qquad (4)$$

$$(x_2^{n'_2})^{s_1-2}]\, S^{s_1} = \ldots .\, R$$

in which every concentration is expressed in terms of the concentration of the primal material, S, of the reaction sequence, multiplied by a series of factors, l, m, n, p, etc., to account for the differentiation of numbers of molecules with each successive reaction; introduction of these factors takes into account the fact that it may, for example, require one, two, or three molecules of A to produce a molecule of B, or that two or three molecules of B are produced from the decomposition of one molecule of A. At first glance it may seem that it is also necessary to introduce another transformation factor from equation to equation, but inspection will show that any factor such as this is already contained in the exponential term, which may be regarded in this instance as a factor of proportionality.

Equation (4) shows that the value of the flux rate, R, actually depends upon the difference in the exponential terms of the forward and back reactions, upon the concentration of the initial material, S, and upon a succession of small factors which will have finite values of the order of $^1/_3$, $^1/_2$, 1, 2, or 3. This is equivalent to the statement that the value of the flux rate, R, depends chiefly upon the value of the difference of the exponential terms to the extent that this difference is equal or nearly in each successive reaction. (In the ideal scheme postulated both for aerobic and anaerobic metabolism it can be seen that the majority of the reactions involve a one-to-one transformation of molecules)[8, 9]. The fact that the last step in the metabolic chain is irreversible leads to a further simplification in the observed flux rate, for the exponential term representing the proportionality factor must be equal to the difference in exponential terms for all previous reactions.

Because of the bifurcation of metabolic pathways permitting the "ideal" biological system to derive energy both aerobically and anaerobically, it is necessary to set up two values of the flux rate for two series of reactions. The bifurcation may be represented as

$$\begin{tikzcd}[column sep=small] & & W \arrow[r, leftrightharpoons] & \ldots \arrow[r, leftrightharpoons] & X \arrow[r] & Y & & & \\ A \arrow[r, leftrightharpoons] & B \arrow[ur, leftrightharpoons] \arrow[r, leftrightharpoons] & \ldots \arrow[r, leftrightharpoons] & C \arrow[r, leftrightharpoons] & D \arrow[r, leftrightharpoons] & \ldots \arrow[r, leftrightharpoons] & N \arrow[r] & Z \end{tikzcd} \qquad (5)$$

in which the X series is the anaerobic series of reactions, the A series, the aerobic. From (5) it can be seen that a portion of the series is common to both pathways, which fact implies a flexibility in the value of the exponential terms in expression (4). Since there is a series of reactions common to both aerobic and anaerobic metabolism, since it is likely that these two pathways do not of necessity have the same value of the flux rate, and, finally, since in the reactions common to both pathways there is no reason to assume that the nature of the reactants or products are in any way chemically altered, the value of the flux rate must depend primarily upon the differences in the entropies of activation, for it is possible to produce variations in the internal energies of activation only by changing the nature of the materials involved. This point will be discussed later.

The existence of the steady state gives rise to "rebound" or "overshoot" phenomena in respiration, equivalent in effect to the more familiar oxygen debt of muscle physiology. If a respiring system is suddenly deprived of one of the constituents (in this case, oxygen) necessary for normal metabolism for a short period of time, and then, after this time has elapsed, is returned to an environment where this constituent is present in adequate amounts, it has been observed that for an interval dependent upon the duration of the anaerobic period, respiration will proceed at a far greater rate than it would have under normal conditions. It is this increased rate that is termed "rebound". In the rebound reaction the order of the respiratory reaction has changed, the composite exponential term, the so-called critical increment, has changed, and finally, the rate determining factor or factors in the total reaction sequence appear to have changed temporarily. The change in concentration in the reaction series may be brought about in several ways; first, by actually withholding one of the necessary reactants, second, by suddenly changing the pressure on the reacting series, and third, by suddenly changing the temperature of the system. All three of these methods of inducing rebound have been employed.

BURTON[3] had indicated that overshoot phenomena arise if it is possible to vary the individual values for the specific reaction rate constants of diffusion. He indicated further that the possibility for rebound depends upon an exponential, which, he states, is related to a variation in the potential energy of the system. Because of the properties of the steady state described above, it would not seem that the overshoot depends upon fluctuations in the energies and entropies of activation, and, of these two factors, the variation in entropy of activation is the one which is the most decisive.

It may be postulated that within the first few seconds (or smaller time interval) of the anaerobic period, the aerobic reaction chain will proceed at its normal rate. As the concentration of the next to the last metabolite increase, the back reaction involving its destruction becomes increasingly important. This shift in "importance" of reactions may be transferred back through the chain, because of the accumulation of metabolites, until the entire system has assumed the equilibrium position, or exceeded it slightly in a direction reverse to that of normal aerobic metabolism. When this occurs, and it may occur very rapidly, it may be that it is possible to call into play the normal chain of reactions of anaerobiosis, which must themselves exist in a steady state. The exponential terms of the two steady states must now be equal; in particular, they must be equal to the exponential terms of equilibrium of the aerobic chain, and the value of this new flux rate must be determined by this exponential term. When the switch from aerobiosis to anaerobiosis has been made *within the tissue*, the products of anaerobic metabolism accumulate within the cell or cells until the tissue is again placed in an environment where aerobic metabolism is possible. The anaerobic metabolites may, of course, merely accumulate, or be removed from their locus of production by chemical destruction and transformation, or be removed by means of a physical process, such as diffusion.

A concept such as this may explain why aerobic metabolism is the preferred mechanism when oxygen is present. The pathway of aerobic metabolism beyond that common to both pathways may involve a higher entropy or series of entropies of activation inasmuch as the terminus of the aerobic chain involves several enzymes. The nature of enzymatic reactions seems to be such that a fine degree of spatial organization is necessary for reaction to occur[7]. Such an organization might impose lowered probability in reactions involving these enzymes, manifested by a lowered entropy factor (which enters the equations as a positive exponential). The pathway of anaerobic metabolism, on the other hand, might place high energy requirements upon the system.

There is considerable experimental evidence available to support these theoretical conclusions. COLLIP[10] has reported some interesting rebound observations for the tissue *Mya arenaria*. He reported that when this clam was exposed to anaerobic conditions for periods of some days in length, and then returned to fresh, oxygen-containing sea water, the rate of oxygen absorption far exceeded the normal rate; in some cases the normal rate did not appear until after three days. In addition, the rate of oxygen consumption after the anaerobic period varied directly with the temperature. Thus, for example, after four days without oxygen, the rate of absorption was 7.06 ml/100 g drained clam tissue/hour, at 5° C; at 14° C, it was 15.10 ml/100 g drained clam tissue/hour and at 22° C the rate was found to be 21.21 ml/100 g drained clam tissue/hour. The normal rate at 14° C was 1.40 ml/100 g drained tissue/hour. If the tissue was removed from the 14° C environment and placed in an environment at 26° C, the consumption jumped to 11.92 ml/100 g/hour; upon return to the 14° C temperature, the respiration was found to be 7.93 ml/100 g/hour. In these cases the rate of oxygen absorption was determined by the WINKLER method. The data demonstrate the possibility of producing a temperature caused overshoot reaction.

Demonstration of the fact that the overshoot reaction may involve both the end products of aerobic and anaerobic metabolism is found in the work of JATZENKO[11] with *Spaerium corneum*, although he reports temperature fluctuations for his measurements from 14° C to 18° C. He sealed his specimens in a solution of known oxygen concentration (determined by the WINKLER method) for periods of as long as 46 days and then returned them to a solution of high oxygen concentration. He stated that the rate of oxygen consumption was greatly increased and did not return to the normal rate until after 2 days. In these two days and the shell was opened and the siphon was extended. Unfortunately, when a specimen is sealed in a constant environment for periods as long as this, it may be that excreted products appear in the surrounding medium. The effect of these on metabolism is unknown. In spite of this difficulty, and that posed by the fluctuation in temperature, one startling fact is reported by JATZENKO. He found that for the first 14–17 days of anaerobiosis the shells were tightly closed and the animals quiescent in the container. Between the 14th and 17th days, the shells opened up, the siphon was extended and the animals were seen to travel about the vertical walls of the vessel. Simultaneously with this occurrence, the shells and the tissue began to darken in colour. Subsequent analysis showed that this darkening was probably due to the deposition of iron sulphide, both in the shell and in the tissue. On the basis of the theoretical treatment it is suggested that these facts might indicate a switch to an anaerobic type of metabolism *within the tissue*. Such a metabolism must be unique for this tissue inasmuch as it involves the irreversible deposition of iron sulphide.

In this same connection the work of LESSER[12] might be mentioned. He found that the earthworm, *Lumbricus terrestris*, produced fatty acids during an anaerobic period which are not produced in normal oxidative metabolism. When the animals are returned to high oxygen concentration, he found that the oxygen was consumed at an increased rate and that the fatty acids disappeared rapidly. It may be that this, too, can be explained on the basis of the theoretical considerations.

LUND's work[13] on the respiratory overshoot of *Planaria agilis* likewise may fall in line with the theoretical predictions, for he found that the percentage acceleration of oxygen consumption "is more marked in starved than in fed animals". His measurements were made with the WINKLER titration; there is no statement as to temperature control; the anaerobic period lasted from 8–10 days.

The work of CHOUDHURY[14] on rebound reactions in potatoes (whole) was followed by determining the rate of carbon dioxide production by means of the PETTENKOFER titration. He feels that the existence of an overshoot may be due to one or more causes; "it may be that some oxidizable substance is produced during the anaerobic period and is used up when sufficient oxygen is available, or it may be that some substance is produced in nitrogen which afterwards acts merely as a stimulant and increases the aerobic respiratory rate . . . A third explanation can be that a temporary protoplasmic change . . . is brought about when nitrogen is replaced by air, which leads to an increased availability of sugar for oxidation." The second suggestion, which is based (as CHOUDHURY indicated) on a previous statement of MEYERHOF's[15] might be without validity. The only known method for a material to act as a "stimulant" in a chemical change is for it to function as a catalyst. To be sure, catalysis involves actual participation of the catalysing molecule in the reaction, but, barring poisoning, or other accidents, this catalysis should continue. This is not borne out by the fact that the rebound reaction persists for only a fairly short period of time, and that the rate returns once again to its normal value. The concept of a protoplasmic change (attributed to BLACKMAN[16]) certainly is concerned with a changing entropy value. To attribute this to sugar molecules alone may not be completely justified, on the basis of the previous theoretical treatment.

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SUMMARY

The treatment of BURTON[3] of the respiratory overshoot is reexamined in the light of the absolute reaction rate theory. The value of the flux rate is shown to depend upon the differences in the energies and entropies of activation of the forward and reverse reactions of the individual steps. A mechanism is suggested for the switch within a tissue from aerobic to anaerobic metabolism.

RÉSUMÉ

Le traitement de BURTON[3] de l'excès respiratoire est réexaminé à la lumière de la théorie de la vitesse absolue des réactions. On montre que la valeur de la vitesse du flux dépend des différences des énergies et des entropies d'activation des réactions inverses des pas individuels. Un mécanisme est proposé pour le passage à l'intérieur d'un tissu du métabolisme aérobique au mécanisme anaérobique.

ZUSAMMENFASSUNG

Der Ausdruck des Atmungsüberschusses von BURTON[3] wird im Lichte der Theorie der absoluten Reaktionsgeschwindigkeit einer erneuten Betrachtung unterzogen. Es wird gezeigt, dass die Grösse der "Flussgeschwindigkeit" (flux-rate) von den Unterschieden in den Aktivierungsenergien und -entropien der entgegengesetzt verlaufenden einzelnen Teilreaktionen abhängt. Es wird ein Mechanismus für den innerhalb der Zelle stattfindenen Umschlag vom aeroben zum anaeroben Stoffwechsel vorgeschlagen.

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