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Hypothesis: The electrochemical regulation of metabolism

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Abstract — An Electrochemical model of metabolism is described that takes account of a variety of metabolic phenomena observed in our laboratory. These include a utilisation by hepatocytes of oxygen, substantially in excess of ATP requirements; energy-dependence of the [lactate]/[pyruvate] ratio; non-equilibrium behaviour of the components of the lactate dehydrogenase reaction during ethanol oxidation, and linear relationships between cellular potentials and metabolic fluxes. In the light of these findings, we propose as an extension of the Mitchell chemiosmotic hypothesis, that metabolic pathways are under the control of opposing far-from-equilibrium chemical and electrical forces that poise the pathways in a balanced state of apparent equilibrium, allowing flux to be regulated by changes in the magnitude of cellular potentials.

INTRODUCTION

As a result of our studies of cellular metabolism spanning more than 30 years, it has become apparent to us that many of the metabolic processes observed in cells cannot readily be explained solely on the basis of solution chemistry. We have therefore made an attempt to devise an alternative to the chemical model that will provide a more satisfactory explanation for observed metabolic behaviour. The new model takes account of the fact that although thermodynamic equilibrium is incompatible with life, many individual pathways of living cells show the properties of systems operating close to equilibrium, such as efficiency, sensitivity and stability (1,2).

The concepts and experimental work to be presented form the basis for the model which we term an Electrochemical model of metabolism, and which essentially represents an extension of the Mitchell chemiosmotic hypothesis (3). The concepts and experimental work to be presented form the basis for an Electrochemical model of metabolism which essentially represents an extension of the Mitchell chemiosmotic hypothesis (3). Mitchell's hypothesis postulates that the flow of electrons through the electron transport chain from substrate to oxygen is coupled to the vectorial translocation of protons from the mitochondrial matrix to the cytoplasmic surface of the inner membrane. In this manner an electrochemical proton gradient is created that stores a substantial portion of the free energy liberated in substrate oxidation. According to the hypothesis, dissipation of the gradient, associated with the flow of protons back to the matrix, is tightly coupled to the synthesis of ATP. The chemiosmotic hypothesis thus firmly establishes the concept that utilisation of the energy stored in an electric field enables the ATP synthase to catalyse the formation of ATP from ADP and inorganic phosphate, a reaction that in the absence of electrical energy input would be thermodynamically highly unfavourable.

The Electrochemical model presented here envisages that not only the ATP synthase, but other key mitochondrial inner membrane enzymes such as the nicotinamide nucleotide transhydrogenase (4) and the NADH dehydrogenase (5) also come under the influence of electric fields. Furthermore, it is proposed that electric fields are not confined to the mitochondrial inner membrane, but are present in all membranes in which redox reactions take place. The association of key enzymes, particularly dehydrogenases and kinases, with such membranes is considered to result in these enzymes, too, coming under the influence of electrical forces. Electrical interactions of this kind are regarded as playing a fundamental role in the regulation of metabolism.

The vectorial nature of an electric field implies that it can exert an influence on a susceptible enzyme only if that enzyme maintains a fixed geometry in relation to the field. Hence our model requires the existence of a high degree of cellular organisation. There is abundant evidence that this exists, in the form of a complex "cytoskeleton", consisting of an intricate network of microtubules, intermediate filaments and microfilaments (6), and a highly cross-linked "microtrabecular lattice" which envelopes all subcellular organelles (7). The cytoskeletal elements are not confined to the cytoplasm, but extend within the nucleus and the plasma membrane, thereby forming a network that pervades and interconnects every region of the cell (8). A further degree of organisation appears to exist at the molecular level, in the form of enzyme complexes involving protein-protein interactions. Some of these protein associations, such as those formed by the enzymes of fatty acid or pyruvate oxidation in animal mitochondria, represent stable enzyme complexes whereas others are of an evanescent nature (9), being created by the temporary association of enzymes with each other, with membrane structures or with the cytoskeleton. Their formation and dissociation may depend on metabolic circumstances (10-12). Our own studies with isolated hepatocytes, employing Control Analysis (13), provide additional support for intracellular enzyme organisation (14).

The postulated action of intracellular electric fields on enzymes is a fundamental element of our argument that the equilibrium behaviour exhibited by living systems does not represent true thermodynamic equilibrium, but rather an apparent dynamic equilibrium achieved by a balanced interplay of far-from-equilibrium forces (2). Thus, each far-from-equilibrium chemical reaction that occurs in living cells is balanced by an opposing electrical reaction. In effect, electrical energy is utilised to do work on cellular enzymes, thereby shifting the mass action ratio of the components of the reactions that they catalyse away from equilibrium. Favourable chemical reactions, i.e. those with a large negative ΔG, will be retarded whereas energetically unfavourable reactions will be promoted. As discussed in this paper, the resultant apparent equilibrium behaviour has dramatic effects on overall cellular metabolism.

RESULTS AND DISCUSSION

Measurement of cellular oxygen uptake

An important premise of the Electrochemical model is that a substantial portion of the O_2-consumption of the living cell will be related to electrical energy generation, storage and flow rather than to ATP synthesis. In keeping with this a noteworthy feature of the respiratory behaviour of hepatocytes is their high rate of basal O_2-uptake. The basal respiration, obtained with hepatocytes derived from fasted rats and incubated in a bicarbonate-saline medium, is more than 40% that of cells synthesising glucose and urea at maximal rates (15). This high rate of O_2-uptake in the absence of added substrates is difficult to explain on the basis of the resting cell's apparent low requirement for ATP turnover, and can be compared to the substantial basal respiration of the whole animal at rest (16). It is also a well-recognised phenomenon that the oxidation of fatty acids by liver induces a stimulation of O_2-uptake that is considerably in excess of the increase that appears necessary to meet any additional requirements for cellular ATP resulting from the presence of fatty acid (17). Thus, there is good evidence that a substantial proportion of cellular respiration is not linked to ATP synthesis. In hepatocytes from normal rats the highest respiratory rate is more than twice the respiratory requirement for maximal ATP synthesis, and ATP-independent O_2-uptake is even greater in cells from hyperthyroid animals (15). It seems reasonable to conclude that the extra respiratory capacity observed under various experimental conditions is associated with the ability to generate and dissipate cellular electrical energy. It seems probable that not only the mitochondria but other organelles of the cell are involved in respiration not linked to ATP-turnover.

Investigation of the postulated near-equilibrium network

A number of workers in the field of metabolism have suggested that a network of near-equilibrium reactions exists within the living cell (18), but this concept is not compatible with our Electrochemical model, which envisages living cells as open, highly organised systems, rich in vectorial processes, and therefore not amenable to exact description in terms of classical equilibrium thermodynamics. Determination of whether or not near-equilibrium exists for any major cellular reaction would therefore appear helpful in establishing the correctness or otherwise of our model. One group of studies that throws doubt on the validity of the near-equilibrium model of metabolism indicates that maintenance of cellular redox state is energy-dependent. Thus, it is well established that agents that impair energy transduction inhibit the reduction by hepatocytes of pyruvate to lactate (19,20). An energy requirement for establishment and maintenance of the resting [lactate]/[pyruvate] ratio was confirmed by experiments in which hepatocytes incubated with lactate were exposed to successive increases in the concentration of the uncoupling agent 2,4-dinitrophenol, which dissociates mitochondrial electron transport from ATP synthesis. This treatment brought about incremental rises in the normal resting steady-state ratio (21). The [lactate]/[pyruvate] ratio could also be increased by impairment of cellular energy state with the respiratory inhibitor, rotenone, but was restored to near-normal by addition of a rotenone-insensitive energy source (21,22). These observations indicate that the cytoplasmic [NAD^+]/[NADH] ratio is under energy-dependent control and therefore is not at thermodynamic equilibrium; hence the [lactate]/[pyruvate] ratio, which equilibrates with the cytoplasmic NAD pool and reflects its redox state, likewise cannot be considered to represent thermodynamic equilibrium.

In recent further studies to clarify whether or not the major redox reactions of living cells are maintained near thermodynamic equilibrium, we have carried out an examination of the effects of hepatic ethanol oxidation on cytoplasmic redox state. The rate of ethanol oxidation ($J_{ethanol}$) was varied by exposing the cells to 4-methylpyrazole, an inhibitor of alcohol dehydrogenase. Consistent features of these experiments were the rapid steep rise and subsequent more shallow decline in the [lactate]/[pyruvate] ratio that took place immediately following ethanol addition; this decline continued even after $J_{ethanol}$ had become constant and maximal. In view of this unexpected response, the effects of graded concentrations of 4-methylpyrazole on the relationship between $J_{ethanol}$ and the [lactate]/[pyruvate] ratio were examined. The results of a representative experiment are shown in Fig. 1. In the absence of 4-methylpyrazole, the pyruvate concentration fell sharply following ethanol oxidation and remained depressed for the remainder of the incubation period. In consequence, the [lactate]/[pyruvate] ratio rose steeply to reach a peak of just over 200/1 within 5 min and then, as lactate was taken up by the hepatocytes, gradually declined towards its former value, but was still close to 80/1 after 60 min. For incubations where graded concentrations of 4-methylpyrazole were present, the fall in pyruvate levels in the incubation media was less severe and the rise in the [lactate]/[pyruvate] ratio correspondingly less steep. At these slower rates of ethanol oxidation, the return of the [lactate]/[pyruvate] ratio towards steady-state was facilitated by release of pyruvate by the cells and therefore occurred earlier, the minimum level reached being an inverse function of $J_{ethanol}$, so that at the highest degree of inhibition of ethanol oxidation observed, the steady-state [lactate]/[pyruvate] ratio had declined to near control values after 30 min (Fig. 1).

These findings indicate that the redox state of the cytoplasm, as determined from the [lactate]/[pyruvate] ratio, was dependent on the rate of ethanol oxidation and not on ethanol concentration which was the same under all experimental conditions. This response of the [lactate]/[pyruvate] ratio to $J_{ethanol}$ is not in accord with the concept that lactate dehydrogenase catalyses a near-equilibrium reaction. Rather, the data can be explained on the basis that the magnitude of the ratio is determined not only by the interactions of lactate and alcohol dehydrogenases but also by the activity of the shuttle(s) responsible for transferring reducing equivalents from the cytoplasm to the mitochondria. The higher the shuttle activity, the faster will reducing-equivalents arising in ethanol oxidation be removed from the cytoplasm and this will tend to counteract the rise in the [lactate]/[pyruvate] ratio induced by ethanol oxidation. Shuttle activity is depressed at the commencement of the incubation due to a lack of shuttle intermediates, but accelerates as these accumulate (23). Hence the [lactate]/[pyruvate] ratio will peak early in the experiment and subsequently decline (Fig. 1).

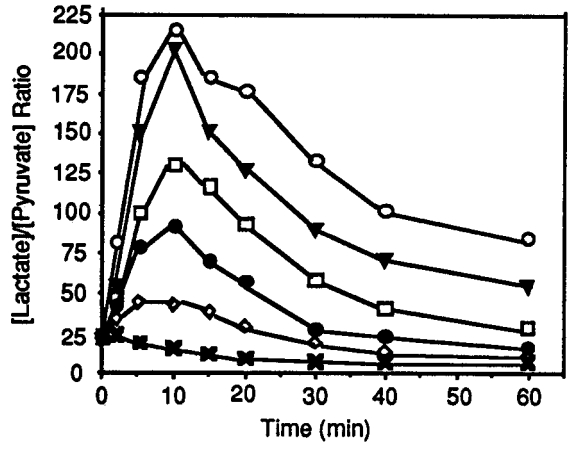

Figure 1 Change in [lactate]/[pyruvate] ratio in hepatocytes metabolising ethanol at different rates. Hepatocytes from fasted rats were incubated with Lactate 10 mM (✱) or Lactate, 10 mM plus Ethanol, 12 mM in the absence (○) or presence of 2 (▼), 4 (□), 6 (●) and 10 μM (◇) 4-methylpyrazole.

Confirmation of the influence of shuttle activity on cytoplasmic redox state was achieved by exposing hepatocytes to graded concentrations of inhibitors of the malate-aspartate shuttle or of energy transduction. Depression of shuttle activity with either group of inhibitors caused a rise in the [lactate]/[pyruvate] ratio and a substantial fall in the $J_{ethanol}$. On the other hand, it was possible to stimulate shuttle activity in isolated hepatocytes by adding shuttle intermediates or their precursors to the incubation medium. We found that asparagine (24) was by far the most potent agent in this respect. Asparagine almost entirely relieved the effects of ethanol, so that pyruvate accumulated and the peak [lactate]/[pyruvate] ratio remained close to control values (data not shown).

Our conclusion is that these results are not compatible with the existence of near thermodynamic equilibrium between cytoplasmic free NAD and the components of the reactions catalysed by the predominant cytoplasmic dehydrogenases. If the components of the reactions of lactate and alcohol dehydrogenases approached near equilibrium in a cytoplasmic compartment of the hepatocyte acting virtually as a closed system, it can be predicted that for any given concentration of lactate and ethanol, the same peak [lactate]/[pyruvate] ratio would be achieved and maintained; although when alcohol dehydrogenase activity was impaired with 4-methylpyrazole it would be anticipated that this ratio might be attained more slowly.

Linear relationships between cellular flows and forces

During our examination of ethanol metabolism we observed a number of interesting relationships between $J_{ethanol}$ and the cytoplasmic NAD-linked redox potential (E_{hc}) as derived from the [lactate]/[pyruvate] ratio. For example, during incubation of the cells with ethanol, with or without 4-methylpyrazole, we noted that at any given time-point $J_{ethanol}$ was a linear function of E_{hc} (Fig. 2). Furthermore, linear relationships were observed in the presence of the inhibitors aminooxyacetate (Fig. 2) or rotenone. We have observed the existence of similar linear relationships between cellular potentials and metabolic flows on numerous other occasions. For example, we found that rates of lactate formation from glycogen, fructose or pyruvate; glucose synthesis from lactate, pyruvate or fructose; and urea synthesis from ammonia, all show a linear response to changes in the magnitude of cellular potentials such as phosphorylation potential (ΔG_p), redox potential (E_h) and inner mitochondrial membrane electrical potential ($\Delta\Psi$) (25-27). ΔG_p (which normally mirrors $\Delta\Psi$) provides an indirect measure of the free energy change (ΔG) of cellular ATP-coupled reactions. Likewise E_h provides an indicator of ΔG for redox reactions. Hence changes in one or both of these potentials can be taken to signify corresponding changes in the ΔG of ATP- or NAD(P)-coupled reactions within the cell's metabolic pathways.

Two important questions arise from this. Firstly, can a change in the activity of one or two reactions within a pathway bring about a linear change in the rate of flux through the overall pathway? The answer to this has already been given in the affirmative as a consequence of studies (14,27) which showed that a linear change in the activity of a single enzyme can lead to a linear response for the whole pathway. When this type of behaviour is observed for several enzymes of an individual pathway, a phenomenon we have noted for both glycolysis (14)

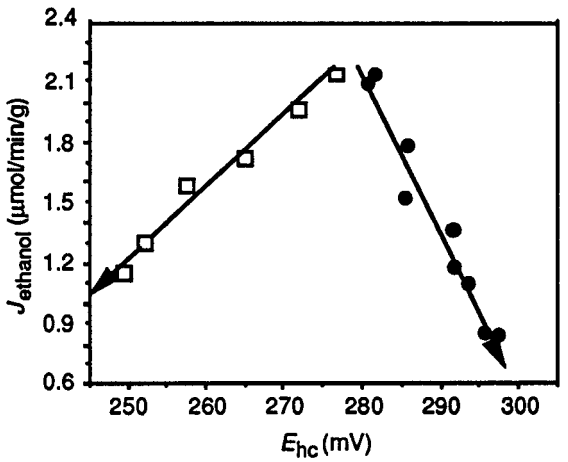

Figure 2 Relationship between $J_{ethanol}$ and cytoplasmic redox potential.
Hepatocytes from fasted rats were incubated for 40 min with Lactate, 10mM plus Ethanol, 12 mM in the presence of 0-10 μM 4-methylpyrazole (□) or 0-100 μM aminooxyacetate (●).
The arrowhead indicates increasing concentration.

and gluconeogenesis (14,27), it can be taken to imply that the enzymes of that pathway form a single complex (28), and this is further compelling evidence for the existence of enzyme organisation. The second question is why should changes in ΔG invoke a linear response in relation to enzyme activity? According to kinetic theory, ΔG is related to the flux through a reaction (or reaction sequence) only when the reaction is poised close to equilibrium (2). It is obvious that the major energy-transducing reactions of the hepatocyte cannot be near or at equilibrium, else little or no flux could occur. Moreover, as already argued, our experimental findings are not compatible with the concept of the existence of a network of near-equilibrium reactions with the cell.

The results can be satisfactorily explained by assuming that the observed linear response behaviour reflects a balanced interplay of energy-coupled far-from-equilibrium chemical and electrical forces giving rise to apparent equilibrium (2). This balance of forces determines the resultant magnitude of the cellular redox potentials, which not infrequently are regarded as equilibrium potentials (29), but need to be perceived as poised in a far-from-equilibrium steady-state by the continuous dissipation of electrical energy. This concept is embodied in the chemiosmotic hypothesis (3,30), and is manifest experimentally in the phenomenon of "reversed electron transfer" (31-33). For an understanding of the mechanism involved, it may be helpful to take as an example the nicotinamide nucleotide transhydrogenase (30) and envisage this membrane-bound redox enzyme, which spans the inner mitochondrial membrane, as a polarisable electrode. The transhydrogenase catalyses the conversion of mitochondrial NADH to NADPH, a reaction which thermodynamically can be expected to have an equilibrium constant of 1. However, under physiological conditions, the influence of the electric field across the inner membrane shifts the reaction strongly in favour of NADPH formation, thus bringing about a very substantial energy-dependent increase in the [NADPH]/[NADH] ratio. Analogous actions of the inner membrane electric field on the NADH dehydrogenase can be anticipated to greatly influence the redox potential of the mitochondrial matrix NAD pool, as measured by the ratio [β–hydroxybutyrate]/[acetoacetate] (29).

A large amount of experimental work has shown that under many conditions reducing-equivalents are readily transferred from cytoplasm to mitochondria and, furthermore, in other circumstances reducing-equivalents pass from mitochondria to cytoplasm. It follows that the magnitudes of the redox potentials of the two compartments must be maintained functionally similar, despite the apparent large differences obtained by measurement of the steady-state [lactate]/[pyruvate] and [β–hydroxybutyrate]/[acetoacetate] ratios. We conjecture that this near-to-isopotential state of the two compartments is achieved by the expenditure of electrical energy and assume that the site of this energy dissipation is the inner mitochondrial membrane. Although as yet the exact mechanism cannot be delineated, our working hypothesis on which present experimental work is based postulates that the dissipation of a portion of the energy stored in the electric field(s) across the inner membrane is coupled to a process of intercompartmental hydrogen transfer that favours reduction of mitochondrial, and oxidation of cytoplasmic, NAD. This process can also be regarded as analogous to that mediated intramitochondrially by the nicotinamide nucleotide transhydrogenase (30).

Maintenance of the components of the ATP synthase reaction far-from-equilibrium

This discussion of the consequences of the postulated balance between electrical and chemical forces has focussed on the influence of electric fields on cellular redox reactions. However, linear relationships are also observed between phosphorylation potential and the rates of many mitochondrial and cytoplasmic metabolic processes, some of which do not involve redox reactions. We conclude from this that the cellular phosphorylation potentials are also maintained in a state of apparent equilibrium. As argued in the chemiosmotic hypothesis, the establishment of an electric field across the inner mitochondrial membrane (3) provides the mechanism by which the mitochondrial ΔG_p can be maintained in this state. It must be assumed that the cytoplasmic ΔG_p is also held at apparent equilibrium, by linkages between the mitochondria and cytoplasm, yet to be defined, but undoubtedly involving the adenine nucleotide translocase (34). Because in the context of the Electrochemical model all individual reactions of metabolic pathways must normally be poised at apparent equilibrium, it follows that other reactions with a high negative ΔG, such as those involving phosphatases, must also in some manner come under the influence of electric fields.

The role of electric fields in the control of metabolic flux

The maintenance of the NAD-linked redox potential of the cytoplasmic and mitochondrial compartments in a functionally isopotential condition, will have important consequences for metabolism. Much of the metabolic flux in both these compartments requires intercompartmental transfer of reducing-equivalents, which is mediated by various "shuttles" (35,36). Following electrodic principles (37) it can be concluded that the rate of such transfer, and hence of overall metabolic flux will be determined in part by the difference in potential between the two compartments. This conclusion provides a straightforward explanation for the many linear force-flow relationships described in our studies. If, as we argue, energy-dependent electric fields are harnessed to overcome the redox potential barrier between the two compartments and thus promote otherwise energetically unfavourable intercompartmental transfer of reducing-equivalents, it follows that any agent that interferes with energy transduction will impede this transfer, and thus reduce metabolic flux. Under normal conditions, interference with energy transduction does not take place, so that the most usual physiological mechanism for altering cellular redox potentials and thereby changing the rate of flux will be exposure of a cell to a substrate at a concentration that causes a shift in the redox potential of either the cytoplasmic or mitochondrial compartment, and hence a functional difference in potential between the two compartments. This will initiate intercompartmental reducing-equivalent transfer and flow until the compartments are once again functionally isopotential. Phosphorylation potential will also be susceptible to chemical agents which impair energy transduction, and particularly to hypoxia. The degree to which changes in physiological conditions can affect phosphorylation potential remains to be clarified.

This argument does not discount the regulatory role of factors affecting enzyme activity. The total amount of enzyme protein, the availability of substrate at levels below saturation, the presence of allosteric effectors or inhibitors can all separately or together have a major impact on metabolic flux] (38). The capacity of transport systems may also be rate-limiting (39). These factors are extensively discussed in textbooks of biochemistry, but the likely function of electric fields in the regulation of metabolism is not generally recognised. It is hoped that this paper will stimulate interest in this possibility.

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REFERENCES

1. J.W. Stucki, Eur. J. Biochem. 109, 269-283 (1980).
2. H.V. Westerhoff and K. van Dam, Thermodynamics and control of biological free-energy transduction, Elsevier, Amsterdam (1987).
3. P. Mitchell, Biochem. Soc. Trans. 4, 399-430 (1976).
4. H. Sies, S.T. Ta, B. Brauser and Th. Bücher, Adv. Enzyme. Regul. 10, 309-322 (1972).
5. H. Weiss, T. Friedrich, G. Hofhaus and D. Preis, Eur. J. Biochem. 197, 563-576 (1991).
6. J.P. Ruppersberg, J.K. Hörber, C. Gerber and G. Binnig, FEBS Lett. 257, 460-464 (1989).
7. K.R. Porter, Prog. Clin. Biol. Res. 295, 15-20 (1989).
8. J.C. Pinder, Biochem. Soc. Trans. 19, 1039-1041 (1991).
9. J.E. Wilson, Trends. Biochem. Sci. 3, 124 (1978).
10. P.A. Srere, Annu. Rev. Biochem. 56, 89-124 (1987).
11. P.A. Srere, Trends. Biochem. Sci. 15, 411-412 (1990).
12. C.J. Masters, S. Reid and M. Don, Mol. Cell Biochem. 76, 3-14 (1987).
13. H. Kacser and J.A. Burns, Biochem. Soc. Trans. 7, 1149-1160 (1979).
14. Berry, M.N., Gregory, R.B., Grivell, A.R., Henly, D.C., Phillips, J.W., Wallace, P.G. and Welch, G.R. In: Control of Metabolic Processes (Cornish-Bowden, A. and Cardenas, M.L., Eds.) pp. 343-350, Plenum Press, New York (1990).
15. R.B. Gregory and M.N. Berry, J. Biol. Chem. 267, 8903-8908 (1992).
16. M. Kleiber, The Fire of Life: An Introduction to Animal Energetics, Wiley, New York (1961).
17. M.N. Berry, D.G. Clark, A.R. Grivell and P.G. Wallace, Eur. J. Biochem. 131, 205-214 (1983).
18. D.F. Wilson, M. Stubbs, R.L. Veech, M. Erecinska and H.A. Krebs, Biochem. J. 140, 57-64 (1974).
19. M.N. Berry, E. Kun and H.V. Werner, Eur. J. Biochem. 33, 407-417 (1973).
20. A.J. Meijer and J.R. Williamson, Biochim. Biophys. Acta 333, 1-11 (1974).
21. M.N. Berry, A.R. Grivell and P.G. Wallace, FEBS Lett. 119, 317-322 (1980).
22. M.N. Berry, FEBS Lett. 117 Suppl, K106-K120 (1980).
23. N.W. Cornell, P. Lund and H.A. Krebs, Biochem. J. 142, 327-337 (1974).
24. T. Sugano, K. Nishimura, N. Sogabe, M. Shiota, N. Oyama, S. Noda and M. Ohta, Arch. Biochem. Biophys. 264, 144-154 (1988).
25. M.N. Berry, R.B. Gregory, A.R. Grivell, D.C. Henly, J.W. Phillips, P.G. Wallace and G.R. Welch, FEBS Lett. 224, 201-207 (1987).
26. M.N. Berry, R.B. Gregory, A.R. Grivell, D.C. Henly, C.D. Nobes, J.W. Phillips and P.G. Wallace, Biochim. Biophys. Acta 936, 294-306 (1988).

27. M.N. Berry, R.B. Gregory, A.R. Grivell, D.C. Henly, J.W. Phillips, P.G. Wallace and G.R. Welch, FEBS Lett. 231, 19-24 (1988).
28. H.M. Sauro and H. Kacser, Eur. J. Biochem. 187, 493-500 (1990).
29. D.H. Williamson, P. Lund and H.A. Krebs, Biochem. J. 103, 514-527 (1967).
30. J. Rydström, Biochim. Biophys. Acta 463, 155-184 (1977).
31. B. Chance and G. Hollunger, J. Biol. Chem. 236, 1577 (1961).
32. M. Klingenberg and P. Schollmeyer, Biochem. Z. 335, 243 (1961).
33. J.M. Tager, Biochim. Biophys. Acta 77, 258 (1963).
34. W. Bogner, H. Aquila and M. Klingenberg, Eur. J. Biochem. 161, 611-620 (1986).
35. P. Borst, Proc. 5th Int. Cong. Biochem. 2, 233-247 (1963).
36. E. Zebe, A. Delbruck and Th. Bücher, Biochem. Z. 331, 254-272 (1959).
37. J.O'M. Bockris and A.K.N. Reddy, Modern Electrochemistry, Plenum/Rosetta, New York (1973).
38. E.A. Newsholme and C. Start, Regulation in metabolism, Wiley, London (1973).
39. A.C. Schoolwerth and K.F. LaNoue, Annu. Rev. Physiol. 47, 143-171 (1985).