Dependence of Gluconeogenesis, Urea Synthesis, and Energy Metabolism of Hepatocytes on Intracellular pH*

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The relationship of intracellular pH to extracellular pH has been measured in suspensions of isolated hepatocytes at 25 °C. The internal pH was found to be a linear function of external pH and it changed by 0.45 pH unit per 1.0 unit change in external pH. The internal [H⁺] was equal to the external [H⁺] at approximately pH 7.1. Gluconeogenesis, urea synthesis, and oxidative phosphorylation showed different dependencies on the intracellular pH. Gluconeogenesis was the most sensitive to changes in [H⁺] and it declined by 80% when the intracellular pH decreased from 7.1 to 6.9. Urea synthesis was less pH-dependent, decreasing by about 30% for the same change in the intracellular [H⁺] whereas respiratory rate showed very little dependence on pH at this temperature. Intracellular [ATP]/[ADP] decreased linearly from 8.5 to 1.5 as the intracellular pH increased from 6.8 to 7.6, while intracellular [P_] was essentially constant at 3.2 nmol/mg of cells, wet weight. Cytochrome c became more reduced with increasing intracellular pH, from less than 10% at pH 6.8 to 35% at pH 7.7. The calculated free energy of hydrolysis of ATP was nearly independent of pH as was the free energy of electron transfer from the intramitochondrial NAD couple (calculated from the [acetoacetate]/[3-OH-butyrate] ratio) to cytochrome c.

Maintenance of an intracellular milieu very different from the external one, is a characteristic feature of living matter. The cell interior is separated from the external environment by the plasma membrane which provides a permeability barrier to the movement of ions, metabolites, and other small molecular weight substances. Operation of several active transport systems and exchange reactions maintains the concentrations of ions and metabolites in the two compartments at different values. Hydrogen ions present a special situation because they are formed continuously inside the cell during metabolism and must be exported as rapidly as they are produced to prevent cellular acidosis (for review see Refs. 1 and 2). Under physiological conditions, the intracellular concentration of hydrogen ion (the value of pH) appears to be maintained in a relatively narrow range of values characteristic for each organ and not vastly different from that of the extracellular environment. On the other hand, systemic acidosis and alkalosis are not uncommon clinical findings in pathological conditions and in some situations marked acidosis of the tissues has been observed. These observations mean that, in some tissues at least, changes in extracellular pH are reflected in alterations in the intracellular [H⁺]. Moreover, since many enzymatic reactions are known to be dependent on ambient pH, the functioning of several organs of the body may be affected by changes in [H⁺]. One of the most important of the tissues to consider is the liver which participates in several metabolic pathways, some of which are located exclusively or predominantly in this organ.

Our understanding of the biochemical effects of systemic alkalosis and acidosis on hepatic function requires elucidation of the relationship of liver metabolism on pH. Cohen and co-workers (3-5) have addressed this question by examining the relationship between the intracellular pH of perfused liver and that of the perfusion medium. They established that when the organ was exposed to different pressures of CO₂ at constant bicarbonate concentrations, the intracellular pH varied as a linear function of the negative logarithm of the CO₂ pressure. However, the intracellular [H⁺] changed substantially less than the perfuse pH, the ratio of ΔpH/ΔP being approximately 1.8 (see, for example, Refs. 4 and 5).

Many types of metabolic studies can be carried out more effectively with suspensions of isolated hepatocytes than with perfused liver, because, in the latter, changes in the vascular system may alter delivery and removal of metabolites. In the present work we have investigated the relationship of intracellular pH to that of the suspending medium in suspensions of isolated hepatocytes and the impact of changes in pH on cellular energy production and on the behavior of three crucial metabolic pathways: oxidative phosphorylation, gluconeogenesis, and urea synthesis. We find that both gluconeogenesis and urea synthesis are strongly dependent on the concentration of H⁺, while the metabolic energy state and the respiratory rate are much less pH-dependent. The metabolic consequences of systemic acidosis and alkalosis on hepatic function are discussed.

MATERIALS AND METHODS

Isolation of Liver Cells—Forty-eight-hour starved male Sprague-Dawley rats (250-280 g) were used for isolation of the liver cells. The liver cells were prepared by the method of Berry and Friend (6) incorporating the modifications described by Cornell et al. (7) and Krebs et al. (8). The rats were anesthetized with pentobarbital (5 mg/100 g). The flow rate of oxygenated perfusion solution during collagenase infusion was 50 ml/min. The collagenase (0.4 mg/ml) was dissolved in Krebs-Henseleit solution (8) and perfused at 37 °C. After isolation, the cells were suspended in Krebs-Henseleit saline containing 2% dialyzed bovine serum albumin. Stock suspensions of cells

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were maintained at room temperature by gently shaking with a 95% O$_2$:5% CO$_2$ atmosphere.

**Incubation and Quenching Method**—Incubations were carried out in 25-ml Erlenmeyer flasks containing 2 ml of reaction mixture (1 ml of cell suspension (about 40 mg, wet weight/ml of cells) and 1 ml of additions (substrates and medium containing albumin)) with constant shaking (100-1000 cycles/min) at 25 or 37°C. The incubation medium contained 5 mM NH$_4$Cl, 2 mM lactate, 2 mM ornithine, 1 mM oleate, and 2% bovine serum albumin, unless otherwise noted. The pH of the cell suspension was adjusted using CO$_2$-bicarbonate or a Hepes-N'-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid.

The pH was measured from the chemical shifts of the $^19$F resonance lines of a-difluoromethylalanine and a-trifluoromethylalanine inside the hepatocyte, as described previously (10, 11). A-difluoromethylalanine methyl ester or, in one case, both di- and trifluoromethylalanine methyl esters, were added to the cell suspension immediately prior to NMR measurements. The esters were taken up rapidly by the hepatocytes and cleaved within minutes to the free acid by endogenous esterases. The hepatocyte suspension was circulated continuously between the NMR probe and an external oxygenation chamber.

The reactions were quenched by injecting 0.2 ml of 40% HClO$_4$ at the end of the incubation period (usually 30 min) and immediately cooling the samples in ice water. The HClO$_4$-treated samples were centrifuged to remove precipitated protein and the clear supernatant neutralized with 2 N K$_2$CO$_3$, 0.38 M triethanolamine. Aliquots of the extracts were used for assay of metabolites. The dry weight of the cell suspension and the medium was determined for each preparation. A factor of 3.8 was used to convert dry weight into wet weight of cells.

**Measurement of the Intracellular pH Using $^19$F NMR**—Intracellular pH was measured from the chemical shifts of the $^19$F resonance lines of a-difluoromethylalanine and a-trifluoromethylalanine inside the hepatocyte, as described previously (10, 11). A-difluoromethylalanine methyl ester or, in one case, both di- and trifluoromethylalanine methyl esters, were added to the cell suspension immediately prior to NMR measurements. The esters were taken up rapidly by the hepatocytes and cleaved within minutes to the free acid by endogenous esterases. The hepatocyte suspension was circulated continuously between the NMR probe and an external oxygenation chamber.

The di- and the trifluoromethylalanine and their methyl esters were obtained from Lee's Bioorganic Laboratories, Marcus Hook, PA. All other reagents were of the highest purity commercially available.

**RESULTS**

The Intracellular pH of Suspensions of Isolated Hepatocytes—For measurements of the intracellular [H$^+$] at different external pH values in the range 6–8, the hepatocytes were suspended in Krebs-Henseleit buffer containing various HCO$_3^-$ concentrations and the appropriate methyl esters of the fluorine-labeled amino acids (for details, see "Materials and Methods"). Since the liver contains highly active intracellular esterases, the methyl esters of the amino acids which entered the cell were rapidly hydrolyzed, usually within 2 min, and the amino acids which were formed inside the cell then leaked into the external medium. Hence, even the initial spectra, which were accumulated for 5 min after the preliminary 2–3-min manipulations, did not usually show the presence of the methyl esters.

A representative $^19$F spectrum of hepatocytes suspended with the methyl ester of difluoromethylalanine is shown in Fig. 1. The proton-decoupled spectrum of the difluoromethylalanine, in a homogenous environment at a given pH, is a quartet of resonances which arises from the two nonequivalent fluorine atoms. The presence of two quartets in Fig. 1

\[ E_n = E_{n0} + 0.0297 \log \frac{\text{[acetate]} \times \text{[3-hydroxybutyrate]}}{\text{[3-hydroxybutyrate]}} \]

Using the $E_n$ of 0.235 V (17), this value is pH-independent over the range of intracellular pH values observed in this work.

**Measurement of the Redox State of the Cytochrome c**—A sample of the incubated cell suspension was added to a 10-mm spectrophotometric cuvette in a dual wavelength spectrophotometer (Johnson Research Foundation) using the wavelength pair 550–540 nm. The half-bandwidth of the measuring light was 1.6 nm. In order to prevent sedimentation, the suspension of cells in the cuvette was continuously mixed with a vibrating stirrer. After the initial spectrophotometric reading had become stable, 10 ml of a 2 mg/ml solution of antimycin A was added to cause complete oxidation of cytochrome c. This value was taken to correspond to 100% oxidation of this cytochrome (18, 19). The fully reduced state of cytochrome c was determined by the addition of 2 mM cyanide. The calculation of the redox state of cytochrome c in liver cells is described by Wilson et al. (18).

**Reagents**—Enzymes (collagenase, pyruvate kinase, lactate dehydrogenase, and glutamate dehydrogenase) were obtained from Boehringer Mannheim, and other enzymes (hexokinase Type IV, glucose-6-phosphate dehydrogenase Type IX, phosphoglucomutase, phosphopyruvate e, and urease) were obtained from Sigma.

The di- and the trifluoromethylalanine and their methyl esters were obtained from Lee’s Bioorganic Laboratories, Marcus Hook, PA. All other reagents were of the highest purity commercially available.
indicates that the amino acid was located in two compartments with differing concentrations of H⁺. The concentration of H⁺ in each compartment can be determined either from the resonance position relative to the external standard or by comparing the separation of the central resonances to a standard curve (10, 11). When such measurements were carried out at various external pH values, the data shown in Fig. 2 were obtained. When two different probes were used (i.e. the di- and trifluorocompounds) the agreement between the measurements was excellent. The figure shows that the internal pH of the hepatocytes was more alkaline than the external one when pH was less than approximately 7.1 but was more acid when pH was greater than 7.1. Thus, in the pH range from 6.1 to 8.0, the intracellular pH changed less than the extracellular pH, on the average by 0.45 pH units for each pH unit change in external pH.

The pH Dependence of Glucose Synthesis—The rates of glucose synthesis from 2 mM lactate were measured in cells incubated for 30 min at either 25 or 37 °C in media adjusted to different pH values by addition of appropriate concentrations of bicarbonate (see "Materials and Methods" for details). (Preliminary experiments established that glucose synthesis was linear for at least 60 min at pH 7.45 and 45 min at pH 6.45 and 8.05.) The results (Fig. 3, A and B) show that the rate of glucose production was markedly dependent on pH at both temperatures. Moreover, at 25 °C (the temperature at which the intracellular pH was measured), the profile of activity against [H⁺] was much steeper when related to intracellular pH than when referred to pHi. The general characteristics of the curves were, however, the same; the maximum of activity was in the pH range 7.2-7.4 (equivalent to pH of 7.3-7.7), and the rate declined much more precipitously at the acid than at the alkaline side of the maximum. When pHi was lowered from 7.1 to 6.9, glucose production decreased by about 80%, whereas it was reduced by only 35% when pHi was raised from 7.4 to 7.6.

The dependence of the rate of glucose synthesis on pH was similar to that at 25 °C except that it was more pronounced; the maximum rate was shifted from a pH of 7.45 at 25 °C to 7.2 at 37 °C. The maximal rate at the latter temperature was greater than the corresponding value at 25 °C by approximately 3.2-fold.

The pH Dependence of Urea Synthesis by Isolated Hepatocytes—The synthesis of urea from NH₄⁺ was measured under the same conditions as those used for measuring glucose production (Fig. 4, A and B). Cells were incubated for 30 min at either 25 or 37 °C at various [HCO₃⁻] and the concentration of urea was determined as the difference between the 30 and 0 min values. (It was established in preliminary experiments that urea synthesis was linear for at least 60 min at pH, 7.45 and 45 min at pH, 6.45 and 8.05.) The rate of urea synthesis was pH-dependent at both temperatures, and similar to glucose production, much more dependent on pHi than on pH. At 25 °C, maximal rates were seen at pH, 7.25-7.46 (pH, 7.4-7.9) and the activity declined at both the acid and alkaline sides of the maximum. The decline in the activity, especially at the acid side of pH 7.1, was much less abrupt than that seen in glucose production. The pH profiles for 25 and 37 °C were similar except that maximal rates were shifted from pH, 7.7 at the former temperature to 7.45 at the latter temperature. The maximal rates measured at 37 exceeded those at 25 °C by approximately 3.6-fold.

Dependence of Hepatocyte Energy Metabolism on pH—The dependence of cellular energy metabolism on [H⁺] was inves-
tigated by following the behavior of intracellular [ATP]/[ADP][P], [acetoacetate]/[3-OH-butyrate], the respiratory rate, and the reduction state of cytochrome c in hepatocytes incubated for 15-30 min at different pH values at either 25 or 37 °C. The inorganic phosphate was found to be about 3.2 nmol/g, wet weight, and essentially independent of pH, from 6.5 to 8.0 (Fig. 5). Slight decreases were observed at pH 6.1 and 8.0, which suggests that intracellular P may have been lost at both extremes of pH, perhaps by leakage from the cell to the external medium.

The state of reduction of cytochrome c increased with increasing pH; this ratio was 0.5 at pH, 6.85 and 3.4 at pH, 7.55. The Eₙ for the [acetoacetate]/[3-OH-butyrate] couple (and thereby for the intramitochondrial NAD⁺/NADH couple) calculated from these results was approximately −0.266 V at pH, 6.8 and became more negative as pH was raised, attaining a value of −0.280 V at pH, 7.5.

The respiratory rate was much more dependent on pH at 37 than at 25 °C although the general characteristics of the pH profiles were very similar (Fig. 6). At 37 °C, the maximum oxygen consumption rate, and the reduction state of cytochrome c couples on intracellular pH. Hepatocytes were suspended as described in the legend to Fig. 3. Measurements of acetoacetate and 3-OH-butyrate were made in quenched samples while the reduction of cytochrome c was measured spectrophotometrically in parallel experiments. The oxidation-reduction potentials for each redox couple were calculated as described under "Materials and Methods" and both the metabolite ratios and Eₙ values were plotted against the intracellular pH calculated from the linear regression best fit to the data in Fig. 2.

The state of reduction of cytochrome c increased with increasing pH, from a [cytochrome c⁺]/[cytochrome c⁻] ratio of 8 at pH, 6.8 to a ratio of 3.0 at pH, 7.6 (Fig. 7). The Eₙ value for this redox couple became correspondingly more negative as pH became more alkaline, changing from 0.295 V at pH, 6.8 to 0.260 V at pH, 7.7.

**DISCUSSION**

Isolated hepatocytes are capable of maintaining an intracellular pH which is different from that of the external environment. This observation confirms the results of Baron et al. (4) and Cohen et al. (5) who showed that in perfused livers the intracellular pH was a nearly linear function of Pco₂ (when [HCO₃⁻] in the perfusate was maintained constant) with a ΔpH/apH, ratio of 0.54 and pH, equal to pH, at approximately 7.1. In our studies, carried out over a wider range of external pH (6.0-8.2), achieved by varying the [HCO₃⁻] in the external medium (while Pco₂ was kept constant), pH, of the hepatocytes was also a nearly linear function of external pH with a slope of 0.45 and pH, = pH, at 7.09. Thus, there is a similar resistance to changes in intracellular pH to alterations in external H⁺ concentration in both isolated hepatocytes and perfused liver.

The hepatocytes have a transmembrane electrical potential of about −30 mV at pH 7.4 (6); hence, if the H⁺ were in electrochemical equilibrium with the membrane potential, the...
intracellular pH would be 0.5 pH unit more acid than the external value. It was observed, however, (Fig. 2) that at this pH the actual ΔpH was much smaller, 0.15 pH unit, which means that protons must be actively extruded from the cell. Moreover, at pH, less than 7.1, the intracellular [H+] was smaller than the extracellular value which suggests that the extrusion of protons occurs against both a concentration gradient and an electrical potential gradient. Although the mechanism(s) responsible for the maintenance of this displacement from equilibrium has not been explored in this work, evidence has been presented that acid extrusion in snail neurons (20, 21) and barnacle muscle (22, 23) is electroneutral, and it has been proposed that exchange of one Na+ moving in for one H+ moving out is responsible for regulation of pH. If such a H+/Na+ exchange were the only mechanism responsible for maintaining intracellular pH in hepatocytes, and assuming that 50% of the oxygen consumed was converted to CO₂ (and thereby to H+) one could calculate that for each O₂ utilized, 0.5 Na+ is moved across the membrane or 3 Na+/36 ATP formed. Since 3 Na+ are transported for each ATP hydrolyzed by the Na+/K+ transport system (24), this mechanism would suggest that approximately 3% of the total ATP synthesized is consumed for the regulation of internal pH. Any leakage of protons through the membrane into the cell (which is probably more active at lower pH) would increase the rate of ATP consumption to maintain intracellular pH.

It should also be pointed out that the maintenance of pH, different from that of the external environment, is a measure of the ability of the cell to regulate its internal concentration of hydrogen ions actively (i.e. energy-dependent). This regulation is in marked contrast to the intracellular buffering power (defined as the amount of H+ required to change the pH by one unit at equilibrium) which is an energy-independent process.

It is interesting to note that not all cells regulate pH, equally stringently. It appears, for example, that human peripheral lymphocytes (10) maintain almost constant intracellular pH of 7.15 in the pH, range from about 6.8 to 7.4 but change it in parallel with [H⁺] when the pH is more acid than 6.8 and more alkaline than 7.4. By contrast, intracellular pH of the kidney was reported to be 7.19 when the extracellular pH was 7.4, but to be equal to the extracellular pH when the latter was lowered to 6.85 (25). Both behavior patterns are very different from that of hepatocytes (this paper and Baron et al. (4) and Cohen et al. (5)) and suggest that regulation of intracellular pH is tissue-specific and designed to meet the needs of specialized organ functions.

The pH Dependence of Gluconeogenesis and Urea Synthesis—The maximal rate of glucose synthesis from 2 mM lactate by hepatocytes was found to be 0.23 μmol/min/g, wet weight, at 25 °C and 0.78 μmol/min/g at 37 °C. This latter value compares very favorably with the results of Krebs et al. (26) for hepatocytes incubated with the same lactate concentration and at the same temperature and pH. Liver perfused with 2 mM lactate at 35 °C produces glucose at very similar rates (1.15 μmol/min/g (27), 1.0 μmol/min/g (28), 0.77 μmol/min/g (29)).

Production of glucose is markedly pH-dependent in both isolated hepatocytes and perfused liver and the dependence is similar in the two systems. Hems et al. (27) found that in liver perfused at 35 °C, glucose synthesis was nearly constant for pH, 7-1-7.7 and declined to one-half of the maximal value at pH, 8.2 and to one-tenth value at pH, of 7.0. Iles et al. (30) confirmed this observation, although in their experiments the decrease on the acid side of pH 7.2 was less dramatic. Our results on isolated hepatocytes at 37 °C show steep pH dependence with a relatively rapid fall in the rate at both the acid and alkaline sides of pH 7.2-7.4. As pointed out by Iles et al. (31) from their measurements of intermediates in the gluconeogenic pathway, the changes in the rate of glucose synthesis with pH on the acid side of the pH optimum may be caused by the H+-dependent alterations in the activity of pyruvate carboxylase.

The production of urea by hepatocytes is also pH-dependent although somewhat less than is glucose synthesis. The maximum activity is somewhat broader and the rate declines more at acid than alkaline pH values.

The inhibition of both gluconeogenesis and urea synthesis by intracellular acidification has important physiological consequences. Acidosis severely impairs the ability of the liver to produce glucose by gluconeogenesis, making glycogenolysis the principal source of blood glucose. Therefore, acidosis, after exhaustion of the liver and kidney glycogen stores, would result in an inability of an organism to maintain normal blood glucose levels. Acidotic decrease in urea synthesis would tend to increase blood levels of NH₃, which is toxic in concentrations of 70 μM and higher. The effect of acidosis on nitrogen balance is, however, somewhat alleviated by increased renal excretion of ammonium ions.

The pH Dependence of Cellular Energy Metabolism—Our results show that the cellular [ATP]/[ADP][P₁] is markedly dependent on pH due to a decline in the [ATP]/[ADP] when pH, is raised from 6.5 to 8.0. This behavior would suggest that the amount of available energy declines at alkaline pH. There are, however, two terms which contribute to the free energy of hydrolysis of ATP (ΔGₜₚₚ): ΔGₜₚₚ is the standard free energy change at 1 M concentrations at a given pH, and the concentration-dependent term which for 25 °C is 1.36 log [ATP]/[ADP][P₁] in kilocalories/mol. Both of these terms and ΔGₜₚₚ depend on pH, and

![Fig. 8. Dependence of the cellular energy state on the intracellular pH. The lines drawn as "best fit" to the data in Figs. 5 and 7 were used to calculate -1.36 log [ATP]/[ADP][P₁] and -ΔGₚₚ for hepatocytes incubated with the same lactate concentration and at the same temperature and pH. Liver perfused with 2 mM lactate at 35 °C produces glucose at very similar rates (1.15 μmol/min/g (27), 1.0 μmol/min/g (28), 0.77 μmol/min/g (29)).](http://www.jbc.org/Downloaded from)
are plotted as a function of pH, in Fig. 8. The value of $-1.36 \log [ATP]/[ADP][P_i]$ decreases by approximately 0.8 kcal/mol as pH increases from 6.7 to 7.6, the change being due almost entirely to decrease in $[ATP]/[ADP]$ (Fig. 5). The $\Delta G_{ATP}$ is also pH-dependent (32, 33) but its value becomes more negative by approximately 0.8 kcal/mol as pH increases from 6.7 to 7.6. Since the increase in $\Delta G_{ATP}$ is a mirror image of the decrease in $-1.36 \log [ATP]/[ADP][P_i]$, $\Delta G_{ATP}$ remains essentially constant at $-12.05$ kcal/mol, changing by only $\pm 0.6\%$ from pH 6.7 to 7.5 (At pH values more acid than 6.7, $\Delta G_{ATP}$ decreases sharply and there is evidence for a slight decrease at pH more alkaline than 7.5.) These considerations allow a conclusion that the behavior of the $[ATP]/[ADP]$ ratios described in this work is consistent with homeostatic regulation of cellular energy production to provide ATP under conditions of nearly constant free energy of hydrolysis (18, 19, 34).

The observation that the free energy of hydrolysis of ATP remains high and constant under conditions when the rates of gluconeogenesis and urea synthesis are markedly inhibited means that the pH dependence of these two synthetic pathways arises from the activities of the enzymes and not from a decrease in available ATP.

One interesting observation is that cytochrome c is more reduced at alkaline pH than at acid pH, despite the fact that $\Delta G_{ATP}$ is relatively unchanged and the respiratory rate is decreased. Similar findings have previously been reported in suspensions of isolated mitochondria (35) and of Paracoccus denitrificans cells (36). Such behavior is characteristic of the pH dependence of cytochrome c oxidase in coupled mitochondria and is independent of the first two sites of oxidative phosphorylation (35).

The respiratory rate was found to be much less pH-dependent than the glucose and urea-synthesizing pathways. It can be calculated (Fig. 9) that, at pH 7.3 and 37 °C, glucose synthesis from lactate utilized 0.72 µmol/min/g, wet weight, × 6 mol of ATP/mmol of glucose, i.e. 1.38 µmol of ATP/min/g, whereas urea synthesis from ammonia consumed 3.26 µmol/min/g × 4 mol of ATP/mmol of urea, i.e. 13.0 µmol/min/g. Thus, the combined ATP requirement for urea synthesis and gluconeogenesis is 17.4 µmol/min/g. Assuming that one O$_2$ yields 6 ATP, the ATP consumed by the two pathways at pH 7.3 accounts for 37.5 µmol/min/g of oxygen utilized, i.e. about 50% of the total oxygen taken up under these conditions. A similar pattern was seen at 25 °C (Fig. 9) except that maximal utilization of ATP for glucose and urea synthesis occurred at pH 7.5 to 7.7 and at this pH only 31% of the total respiration was utilized to provide ATP for these reactions. Since at 37 °C the rates of urea production and of gluconeogenesis declined rapidly at both the acid and alkaline sides of pH 7.2–7.4, one would expect that the rate of oxygen utilization would decrease to reflect the decreased rate of ATP utilization. However, when the rate of ATP synthesis required to support the rates of glucose and urea synthesis is subtracted from that which is generated by the respiration (assuming all of the respiration was mitochondrial (Fig. 9)), the remaining rate of ATP synthesis is found to increase linearly from pH 8.3 to pH 6.8. It is possible that this increase in respiration and presumably in ATP synthesis with rising external acidity reflects, in part, the higher energy requirements for regulating intracellular H$^+$ at more acid pH$_a$.

Previous work, both with intact cells (18, 19, 34) and suspensions of isolated mitochondria (37, 38), indicated that the first two sites of energy coupling, for which the overall reaction is

$$\text{NADH}_a + 2 \text{cyt} c^{\text{red}} + 2 \text{ADP} + P_i \rightleftharpoons \text{NADH}^{\text{ox}} + \text{cyt} c^{\text{ox}} + 2 \text{ATP},$$

are near equilibrium. (In Equation 5 the subscripts $m$ and $c$ are used to designate the mitochondrial and cytosolic compartments.) This means that the calculated $\Delta G_{ATP}$ should be, within the limits of experimental error, equal to the amount of energy available in the redox reactions between the NAD and cytochrome c couples ($\Delta G_{ATP} = -23.06(E_{be} - E_{bo_{NAD}})$ where $E_{be}$ and $E_{bo_{NAD}}$ are the oxidation-reduction potentials of the cytochrome c and NAD couples). As may be seen from Fig. 8, $-\Delta G_{ATP}$ shows the same pH dependence as $-\Delta G_{ATP}$ calculated from the total cellular concentration of ATP, ADP, and P, but is greater than the latter by about 0.8 kcal/mol of ATP, i.e. equivalent to an $[ATP]/[ADP][P_i]$ which is 4-fold greater than the measured value.

There are several lines of evidence indicating that the measured total $[ATP]/[ADP][P_i]$ is less than the thermodynamically relevant ratio of the free reactants in the cytosol ($[ATP]/[ADP][P_i]$).

1) Liver cells incubated with adenosine increase their cy-
toplamic adenine nucleotides by 2-4-fold and the total cellular [ATP]/[ADP] rises nearly 2-fold (18, 39). This is consistent with the increase in total ADP in the cytosol which saturates cytoplasmic binding sites for this nucleotide so that the total [ATP]/[ADP] is closer to the cytosolic [ATP]/[ADP].

2) Cellular fractionation techniques indicate that the cytoplasmic [ATP]/[ADP] may exceed the total cell ratio by as much as 2-fold, due to the intramitochondrial [ATP]/[ADP] being less than the cytoplasmic value (40) (see Ref. 41 for review).

3) Measurements of binding of adenine nucleotides to the cell contents of hepatocytes whose plasma membranes had been made permeable to small molecules by treatment with filipin indicate that selective binding of ADP could account for the cytoplasmic [ATP]/[ADP]/Pi being greater than the total (measured) value by 30 to 50% (42).

4) Calculations of the cytoplasmic [ATP]/[ADP]/Pi from the equilibrium of the glyceraldehyde-3-phosphate dehydrogenase and 3-phosphoglycerate kinase reactions give numbers calculated from near equilibrium of Reaction 5.

All of these approaches place the value of the cytosolic [ATP]/[ADP]/Pi at between 2 and 10-fold greater than the experimentally obtained values.

REFERENCES

1. Krebs, H. A., Woods, H. F., and Alberti, K. G. M. M. (1975) Essays Med. Biochem. 1, 81-103
2. Atkinson, D. E., and Camien, M. N. (1982) Curr. Top. Cell. Regul. 21, 261-302
3. Lloyd, M. H., Iles, R. A., Simpson, B. R., Strunin, J. M., Layton, J. M., and Cohen, R. D. (1973) Clin. Sci. Mol. Med. 45, 543-549
4. Baron, P. G., Iles, R. A., and Cohen, R. D. (1978) Clin. Sci. Mol. Med. 55, 175-181
5. Cohen, R. D., Henderson, R. M., Iles, R. A., and Smith, J. A. (1982) J. Physiol. (Lond.) 330, 89-90
6. Berry, M. N., and Friend, D. S. (1969) J. Biol. Chem. 244, 6538-6547
7. Cornell, N. W., Lund, P., Hems, R., and Krebs, H. A. (1973) Biochem. J. 134, 671-672
8. Krebs, H. A., Cornell, N. W., Lund, P., and Hems, R. (1974) in Alfred Benzon Symposium VI, pp. 718-743, Munksgaard, Copenhagen
9. Krebs, H. A., and Henseleit, K. (1932) Hoppe-Seyler's Z. Physiol. Chem. 210, 33-36
10. Deutsch, C., Taylor, J. S., and Wilson, D. F. (1982) Proc. Natl. Acad. Sci. U.S.A. 79, 7944-7948
11. Taylor, J. S., and Deutsch, C. (1983) Biophys. J., in press
12. Lamprecht, W., and Trautschold, I. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 4, pp. 2101-2110, Academic Press, New York
13. Jaworek, D., Gruber, W., and Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 4, pp. 2127-2131, Academic Press, New York
14. Gutmann, I., and Bergmeyer, H. U. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 4, pp. 1791-1798, Academic Press, New York
15. Gawehn, K. (1974) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) Vol. 4, pp. 2234-2238, Academic Press, New York
16. Williamson, D. H., Mellanby, J., and Krebs, H. A. (1962) Biochem. J. 82, 90-96
17. Dutton, P. L., Wilson, D. F., and Lee, C. P. (1970) Biochemistry 9, 5077-5084
18. Wilson, D. F., Stubbs, M., Veech, R. L., Erecinska, M., and Krebs, H. A. (1974) Biochem. J. 140, 57-64
19. Wilson, D. F., Stubbs, M., Oshino, N., and Erecinska, M. (1974) Biochemistry 13, 5305-5311
20. Thomas, R. C. (1977) J. Physiol. (Lond.) 273, 217-338
21. Thomas, R. C., and Meech, R. W. (1982) Nature (Lond.) 299, 826-828
22. Boron, W. F., McCormick, W. C., and Roos, A. (1981) Am. J. Physiol. 240, C80-C89
23. Boron, W. F., and Russell, J. M. (1983) J. Gen. Physiol. 81, 373-399
24. Glynn, I. M., and Karlish, S. J. D. (1975) Annu. Rev. Physiol. 37, 13-55
25. Ackerman, J. J. H., Lowry, M., Radda, G. K., Ross, B. D., and Wong, G. G. (1981) J. Physiol. (Lond.) 319, 65-79
26. Krebs, H. A., Lund, P., and Stubbs, M. (1976) in Gluconeogenesis: Its Regulation in Mammalian Species (Hanson, R. W., and Mehlman, M. A., eds) pp. 269-291, Wiley-Interscience, New York
27. Hems, R., Ross, B. D., Berry, M. N., and Krebs, H. A. (1966) Biochem. J. 101, 284-292
28. Exton, J. H., and Park, C. R. (1967) J. Biol. Chem. 242, 2622-2630
29. Schnitger, H., Scholz, R., Bucher, T., and Lobber, D. W. (1965) Biochem. Z. 341, 334-339
30. Iles, R. A., Cohen, R. D., Rist, A. H., and Baron, P. G. (1977) Biochem. J. 164, 185-191
31. Iles, R. A., Baron, P. G., and Cohen, R. D. (1978) Clin. Sci. Mol. Med. 55, 183-188
32. Rosing, J., and Slater, E. C. (1972) Biochim. Biophys. Acta 267, 275-290
33. Guyon, R. W., and Veech, R. L. (1973) J. Biol. Chem. 248, 6966-6972
34. Erecinska, M., Wilson, D. F., and Nishiki, K. (1978) Am. J. Physiol. 234, C82-C89
35. Wilson, D. F., Owen, C. S., and Holian, A. (1977) Arch. Biochem. Biophys. 182, 749-762
36. Erecinska, M., Davis, J. S., and Wilson, D. F. (1979) Arch. Biochem. Biophys. 197, 463-469
37. Erecinska, M., Veech, R. L., and Wilson, D. F. (1974) Arch. Biochem. Biophys. 160, 412-421
38. Forman, N. G., and Wilson, D. F. (1982) J. Biol. Chem. 257, 12908-12915
39. Chaguva de Sanchez, V., Brunner, A., and Pina, E. (1972) Biochem. Biophys. Res. Commun. 46, 1441-1445
40. Soboll, S., Scholz, R., and Heidl, H. W. (1978) Eur. J. Biochem. 87, 377-390
41. Erecinska, M., and Wilson, D. F. (1982) J. Membr. Biol. 70, 1-14
42. Gankema, H. S., Groen, A. K., Wanders, R. J. A., and Tager, J. M. (1983) Eur. J. Biochem. 131, 447-451
43. Groen, A. K., Vervoorn, R. C., Wanders, R. J. A., van der Meer, R., and Tager, J. M. (1982) Biochim. Biophys. Acta 721, 172-177
44. Veech, R. L., Randolph Lawson, J. W., Cornell, N. W., and Krebs, H. A. (1979) J. Biol. Chem. 254, 6538-6547.
Dependence of gluconeogenesis, urea synthesis, and energy metabolism of hepatocytes on intracellular pH.
T Kashiwagura, C J Deutsch, J Taylor, M Erecinska and D F Wilson

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