Facilitating an understanding of integrative physiology: emphasis on the composition of body fluid compartments

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Abstract: As a teaching exercise, we used deductive reasoning and a quantitative analysis to convert a number of facts into a series of concepts to facilitate an understanding of integrative physiology and shed light on the composition of the different body fluid compartments. The starting point was the central need to regenerate ATP to perform biologic work. Because a large quantity of O_2 must be delivered to cells at a sufficiently high concentration to aid its diffusion into mitochondria, approximately one third of the O_2 in inspired air was extracted; this led to a P_{CO_2} in arterial blood of 40 mmHg (1 mmHg = 133.322 Pa). Blood flow to individual organs must be adjusted precisely to avoid having too low or too high a P_{O_2} in mitochondria — the latter augments the formation of reactive O_2 species. The extracellular fluid (ECF) bicarbonate concentration ($E_{HCO_3^-}$) must be high to minimize H^+ buffering by proteins. This high $E_{HCO_3^-}$ sets the ECF concentrations of ionized calcium (Ca^{2+}) and inorganic phosphate (HPO_4^{2-}) because of solubility issues. Three features defined the intracellular fluid (ICF) volume and composition. First, expelling monovalent anions minimized its mass (volume). Second, controlling the tissue P_{CO_2} ensured a relatively constant net valence on intracellular proteins. Third, the range of ICF Ca^{2+} concentrations must both induce regulatory signals and avoid $Ca_3(PO_4)_2$ formation. All the above were incorporated into the integrated response that optimized the capacity for vigorous exercise.

Key words: acid–base, bicarbonate, calcium, carbon dioxide, exercise, integrative physiology, intracellular fluid pH, oxygen, water.

Résumé : Comme exercice pédagogique, nous avons utilisé le raisonnement déductif et une analyse quantitative pour convertir des faits en une série de concepts afin de faciliter la compréhension de la physiologie intégrative et de jeter une lumière sur la composition des différents compartiments des liquides corporels. Notre point de départ a été la nécessité de régénérer l'ATP pour effectuer un travail biologique. Étant donné qu'une grande quantité d'oxygène doit être distribuée aux cellules à une concentration suffisamment élevée pour faciliter sa diffusion dans les mitochondries, environ 1/3 de l'oxygène de l'air inspiré a été extrait, ce qui a donné une P_{CO_2} dans le sang artériel de 40 mm Hg (1 mmHg = 133.322 Pa). Le débit sanguin aux divers organes doit être ajusté de manière à équilibrer la quantité de P_{O_2} dans les mitochondries; une trop forte concentration favorise la formation des espèces réactives de l'oxygène. La concentration de bicarbonate extracellulaire (LEC) ($E_{HCO_3^-}$) doit être élevée pour atténuer le tamponnage des ions H^+ par les protéines. Cette forte concentration de $E_{HCO_3^-}$ règle les concentrations de calcium ionisé (Ca^{2+}) et de phosphate inorganique (HPO_4^{2-}) du LEC pour des raisons de solubilité. Trois caractéristiques ont défini la composition et le volume du liquide intracellulaire (LIC). Premièrement, l'expulsion des anions monovalents a diminué sa masse (volume). Deuxièmement, la régulation de la P_{CO_2} tissulaire a maintenu une valence nette relativement constante sur les protéines intracellulaires. Troisièmement, la plage de concentrations du Ca^{2+} du LIC doit à la fois induire des signaux régulateurs et éviter la formation de $Ca_3(PO_4)_2$. Toutes ces données ont été incorporées dans la réponse intégrée qui a optimisé la capacité à effectuer un exercice vigoureux.

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Introduction

Normal values for the volumes and concentrations of ions in the extracellular fluid (ECF) and intracellular fluid (ICF) compartments have been clearly defined. We ask: Why are these values the “normal” ones? With such a broad question, where should we begin? Which subject deserves more emphasis? How deeply should each subject be explored? Because of a need to be succinct, only selected subjects can be considered. The topics we shall focus on in a sequential fashion are energy metabolism, oxygen, carbon dioxide, acid–base, calcium, phosphate, water, cell voltage, and the integrative physiology of vigorous exercise. Because our aim is to focus on a breadth of ideas, no subject will be covered in depth and the literature will not be referenced in detail.

Each section begins with a broad yet simple question to focus attention on critical issues. We relied heavily on what we call a function/control analysis (Halperin and Rolleston 1993) — once the function becomes clear, one can deduce how that function is likely to be controlled. Subject matter quickly crosses traditional subspecialty boundaries and a quantitative analysis is an essential tool. The discussion of a particular line of inquiry leads ultimately to a crossroad. Only the path that is consistent with our objective of defining why we have the specific composition of the major body compartments will be followed.

I. The central importance of energy

This section begins with a consideration of work and energy. The correct amount of O_2 must be delivered to tissues to permit the performance of biological work while not elevating the tissue concentration of O_2 (P_{O_2}) unduly. An obligatory link becomes evident between the P_{O_2} and the concentration of CO_2 (P_{CO_2}) in the blood and interstitial compartments. A list of the questions to be considered in this section is given in Table 1.

Question 1: What is the most important requirement for survival in an adult?

Define function

While a supply of water, food, and O_2 and the elimination of waste products are essential for survival, a source of energy for muscle contraction is needed for all. The ability to escape from danger (fight and flight response) and to perform other biological work such as ion pumping and biosynthesis all help to focus on the central importance of a source of energy. Adenosine triphosphate (ATP) is the body's energy currency and ensuring its continuous availability is the most important requirement for survival.

Deduce controls

Once the function is clear, its regulation can be deduced. Figure 1 is provided to illustrate where the metabolic process that leads to regeneration of ATP should be regulated. Because one cannot run faster simply because more food is

consumed, the conclusion is obvious — performing work generates a signal (a rise in the concentration of adenosine diphosphate (ADP)) to stimulate energy metabolism and ATP regeneration. At this point, the emphasis switches to metabolic regulation in the setting of the physiology of vigorous exercise (the hunt for food).

(a) Control of energy metabolism during vigorous exercise

Question 2: Will the supply of O_2 or fuel limit the rate of ATP formation during intense exercise?

Define function

A much more rapid rate of fuel oxidation and O_2 consumption is needed to perform vigorous work.

Deduce controls

Both O_2 and fuel are supplied ultimately from the blood. If their entry into cells were not the rate-limiting step, a quantitative analysis emphasizing the stoichiometry of fuel oxidation (eqs. 1a and 1b) could help identify the likely limiting factor. To completely oxidize the 5 mmol of glucose in 1 L of blood, 30 mmol of O_2 must be consumed (6 O_2 /glucose, eq. 1b). Nevertheless, 1 L of blood contains only 8 mmol of O_2 (Appendix A). Therefore, the supply of O_2 and (or) the accumulation of the product CO_2 are the most likely factors that limit energy metabolism in steady state and thereby the capture of useable energy in the form of ATP. Of interest, much of the energy from fuel oxidation is released as heat in a relatively few nonequilibrium reactions. These irreversible reactions are the major sites where metabolic pathways are regulated.

where P_i is inorganic phosphate.

(b) The supply of O_2

O_2 is now the central issue. Again, quantities and chemistry provide the basis to deduce answers to our questions. As background, the following facts help identify critical issues. Air is 21% O_2 and the balance is inert nitrogen gas; air has only trace amounts of the other important gas, CO_2 . Because atmospheric pressure is 760 mmHg (1 mmHg = 133.322 Pa) and water vapor pressure of fully humidified air at 37°C (body temperature) is 47 mmHg, air entering the lungs has a P_{O_2} of 21% (760–47 mmHg) or close to 150 mmHg (Schmidt-Nielsen 1997). Since each millimole of O_2 occupies 25.4 mL at body temperature, there is close to 8 mmol O_2 /L inspired air at sea level. As we shall soon see, the percentage of O_2 extracted from inspired air will dictate the ionic composition of the ECF compartment.

Table 1. Questions concerning the importance of energy.

1. What is the most important requirement for survival in an adult?
2. Will the supply of O_2 or fuel limit the rate of ATP formation during intense exercise?
3. What proportion of O_2 should be extracted from each litre of inspired air?
4. What are the normal stimuli for ventilation?
5. What is the optimal relationship between the P_{O_2} and the binding of O_2 to hemoglobin so that more O_2 can be extracted from blood at a high P_{O_2} during exercise?
6. What should increase the blood flow rate to a specific organ?
7. Is there a danger if the mitochondrial P_{O_2} becomes too high?
8. What might the biological role of UCP-3 be in skeletal muscle during fasting?

Fig. 1. Function and control of metabolism. To perform work, ATP must be hydrolyzed (bottom arrow). Therefore, the central need of metabolism is to reconvert ADP to ATP (top arrow) at a rate to match the rate of use of ATP. Control of this process is initially by the rate of biological work. At higher rates of work, the supply of O_2 becomes a rate-limiting step.

(c) Pulmonary extraction of O_2

Question 3: What proportion of O_2 should be extracted from each litre of inspired air?

Define function

Enough O_2 should be extracted from inspired air to meet the metabolic demands of the body both at rest and during intense exercise.

Deduce controls

The circulatory system must deliver as much O_2 as needed and at a sufficiently high P_{O_2} to optimize diffusion of O_2 into mitochondria in steady state (Fig. 2). The fact that air is mainly nitrogen plays an important role in answering question 3. For example, if a person inspired 1 L of pure O_2 , and if one third of this O_2 were extracted, the remaining gas would still have virtually the same P_{O_2} , but its volume would now be two thirds of a litre. In contrast, when O_2 is consumed from nitrogen-rich air, there is little change in total volume, so the P_{O_2} must decline appreciably. To select the optimum strategy for O_2 extraction, three options will be considered.

(i) *Extract most of the O_2 in inspired air* — While the work of breathing would be lower because a smaller volume of air needs to be inspired, the disadvantages are overwhelming. First, the P_{O_2} in alveolar air and arterial blood could become so low that rapid diffusion of O_2 into mitochondria would be limited (Fig. 2). Second, there would be little reserve of O_2 available in the lungs for immediate use during the fight or flight response. Third, the P_{CO_2} of alveolar air and body fluids would be very high — why this would be a fatal flaw

Fig. 2. Supply of O_2 . The rectangle represents a mitochondrion. Diffusion of O_2 is needed, so a high P_{O_2} is required (site 1). For metabolism, the essential feature is the absolute quantity of O_2 delivered to tissues (site 2). This in turn sets the requirement for a bound form of O_2 (hemoglobin) with unique kinetics for O_2 binding (see Fig. 3).

will be considered in more depth in Section II (control of the composition of the ECF compartment).

(ii) *Extract only a tiny amount of O_2 from each litre of inspired air* — This strategy would result in both a high P_{O_2} and a maximum O_2 reserve in alveolar air. The major disadvantage would be the very low P_{CO_2} in alveolar air and in blood; a minor disadvantage would be that the work of breathing would have to increase many fold.

(iii) *Extract close to one third of the O_2 from inspired air* — With this strategy, the P_{O_2} in the circulatory system can be high enough to support diffusion of O_2 and it will set the P_{CO_2} in arterial blood and body fluids at close to 40 mmHg.

(d) Control of ventilation

Question 4: What are the normal stimuli for ventilation?

Define function

If O_2 provided the stimulus for ventilation, the sensor could detect either the O_2 content or the P_{O_2} of arterial blood.

Deduce controls

The P_{O_2} should become the major control of ventilation only when the content of O_2 in arterial blood is clearly low. By examining Fig. 3, the P_{O_2} should stimulate ventilation when the arterial P_{O_2} has declined to the steep portion of the hemoglobin- O_2 dissociation curve. This would not occur in everyday life, so other options will be explored.

Fig. 3. Content and concentration of O_2 in air. The quantity of O_2 per litre of air is directly proportional to the P_{O_2} (top panel) whereas the quantity of O_2 per litre of blood is a sigmoid function of the P_{O_2} (bottom panel). When tissues need more O_2 at a high P_{O_2} , this curve must shift to the right as illustrated by the vertical dashed line.

Redefine function

The consumption of O_2 leads to the production of CO_2 (eq. 1b). Hence, it is possible that P_{CO_2} could be the major signal to drive ventilation.

Deduce controls

Chemistry permits one to understand how ventilation may be controlled by CO_2 . First, CO_2 is very soluble in water and it will be converted into carbonic acid (H_2CO_3) (eq. 2). Although the speed of this reaction is not instantaneous, the reaction rate becomes very rapid because carbonic anhydrase (CA) is present. Second, H_2CO_3 dissociates very rapidly into H^+ and HCO_3^- . The position of the equilibrium for the bicarbonate buffer system (BBS) is such that the ratio of the concentration of HCO_3^- ($P_{HCO_3^-}$) to that of H^+ is close to 1 million to 1 in plasma. Since a small change in P_{CO_2} will generate one H^+ molecule and one HCO_3^- molecule (eq. 2), it will induce a much larger percent change in the concentra-

tion of H^+ than in that of HCO_3^- . Accordingly, it is not surprising that the ultimate stimulus for ventilation might be H^+ with or without a direct role for P_{CO_2} . The compartment where this change in H^+ concentration is sensed will be one where there is the maximum change in H^+ concentration per rise in P_{CO_2} — i.e., CA is present with very few non- HCO_3^- buffers (proteins). Moreover, the metabolic production of CO_2 and the blood flow rate to the site of regulation should not be variables that could control its P_{CO_2} and thereby ventilation. One site that meets these requirements is adjacent to the cerebrospinal fluid in the brain.

(e) Delivery of O_2 to tissues

Because O_2 is not very soluble in water and a large amount must be transported in blood, a bound form of O_2 is needed (hemoglobin-bound O_2) (Fig. 3). Hemoglobin must be able to donate the majority of its O_2 to tissues at a high P_{O_2} to aid the diffusion process — this is especially important when the workload increases.

Question 5: What is the optimal relationship between the P_{O_2} and the binding of O_2 to hemoglobin so that more O_2 can be extracted from blood at a high P_{O_2} during exercise?

Define function

The release of virtually all of the O_2 in blood at the tissue level must occur while its P_{O_2} is sufficiently high to permit adequate diffusion of O_2 into mitochondria.

Deduce controls

To release O_2 at a high P_{O_2} , the kinetics of O_2 binding to hemoglobin must be nonlinear (Fig. 3). Were this the only adaptation, the O_2 delivery system would not be optimal. Therefore, modifiers that permit the downloading of O_2 at a higher P_{O_2} should be present when work increases. The products of work, CO_2 (Fig. 1) and heat, are two of the major causes of a rightward shift of this O_2 content – P_{O_2} curve for hemoglobin (Fig. 3). Hydrogen ions are another modifier when not enough O_2 is delivered to meet energy needs of an organ because, with a deficit of O_2 , ATP and H^+ are produced by anaerobic glycolysis. Moreover, a rise in P_{CO_2} also increases the H^+ concentration (eq. 2), so this H^+ signal is an important one. The BBS (eq. 2) will be considered in more detail when we discuss H^+ buffering in Section II.

(f) Adjusting the blood flow rate to individual organs

Question 6: What should increase the blood flow rate to a specific organ?

Define function

The delivery of O_2 to tissues is directly related to their blood flow rate. Both blood flow rate and metabolic rate should influence the P_{O_2} and P_{CO_2} in tissues.

Deduce controls

When an organ consumes more O_2 , signals are required to dilate its small arteries. Since O_2 delivery is such an important function, one can anticipate redundant control mechanisms.

Fig. 4. Uncoupling of oxidative phosphorylation. The horizontal structure represents the inner mitochondrial membrane. At the far left, the diffusion of H^+ into mitochondria drives ATP regeneration (coupled oxidative phosphorylation). In the central part of the figure, only complexes I and II are shown; they pump out H^+ and are linked by the diffusion of CoQ. Reduced CoQ will react with O_2 to produce ROS. The UCP-3 is shown at the right. When ROS rise and fatty acids are present, flux of H^+ through UCP-3 rises and CoQ becomes less reduced but ATP is not regenerated.

Two major signals become obvious candidates for regulation of blood flow rate, a fall in P_{O_2} and a rise in P_{CO_2} .

Individual organs have unique needs in terms of speed and sensitivity of response for regulation of their blood flow. The heart, for example, must augment its blood flow rate immediately when its work increases. Thus, the heart might be expected to have high local concentrations of vasodilators. At this point, an important measurement becomes relevant. The heart normally extracts 70% of its O_2 delivery (Goodale et al. 1959). This ensures the needed low P_{O_2} and high P_{CO_2} to favor vasodilation. Moreover, a low local P_{O_2} could stimulate new blood vessel formation. There are other possible signals for control of blood flow. First, a link between a higher P_{CO_2} and insufficient O_2 is a rise in the concentration of H^+ due to anaerobic glycolysis or possibly a product of ATP hydrolysis (called adenosine) (Lutz 1992). Therefore, a rise in concentration of H^+ and (or) adenosine are likely vasodilators. While these controls do operate, the need for additional and more sensitive ones will be illustrated in the response to the next question.

Question 7: Is there a danger if the mitochondrial P_{O_2} becomes too high?

Define function

A lipid-soluble factor, coenzyme Q (CoQ), is a key component of the electron transport system that diffuses through the inner mitochondrial membrane when ATP is regenerated. CoQ links the first two complexes of the electron transport system (Fig. 4) (Mitchell 1961). The reduced form of CoQ will react with molecular O_2 and form reactive oxygen species (ROS) (eq. 3). This is a nonenzymatic reaction, so it depends only on local concentrations of O_2 and reduced CoQ (Beckman and Ames 1998). Therefore, if the P_{O_2} rises excessively near mitochondria, more ROS will be formed. The heart, for example, might be less likely to form ROS because it has a low interstitial P_{O_2} in vivo.

Deduce controls

Regulation of the concentration of ROS occurs by lowering their rate of formation or by increasing their rate of removal.

(i) *Decrease the formation of ROS by lowering the P_{O_2} near mitochondria* — Regulation of the P_{O_2} at the mitochondrial level requires that there be a system to match the blood flow rate to the rate of O_2 consumption. During exercise, the blood flow rate to skeletal muscle must rise. The first step that permits this blood flow rise is the binding of adrenaline to one of its receptors in vascular smooth muscle cells. Vasodilation is the response in skeletal muscle because β -receptors are the predominant ones in this organ. In contrast, in other organs, vasoconstriction is required, so α -adrenergic receptors are their predominant receptors. Thus, blood can be diverted to supply skeletal muscle in high adrenergic states such as the flight or fight response — a necessary response because of the limited capacity to increase cardiac output. Nevertheless, this form of regulation lacks sensitivity because blood flow may have to increase immensely, but only to those muscles that are participating actively in the exercise. Hence, one can anticipate that local factors related to work load will be needed to adjust regional blood flow to individual skeletal muscles.

To understand how regional blood flow is regulated, the control of blood flow must be examined at a molecular level. Vasoconstriction requires that the concentration of Ca^{2+} in vascular smooth muscle cells must rise. Hence, one can anticipate that the ICF Ca^{2+} concentration ($I_{Ca^{2+}}$) will increase in these cells when vasoconstriction is the desired effect whereas this $I_{Ca^{2+}}$ will fall when vasodilation is required (Fig. 5). To have a sustained rise in $I_{Ca^{2+}}$ when an α -adrenergic stimulus is present, voltage-gated Ca^{2+} channels in the plasma membrane of vascular smooth muscle cells must remain in an open configuration (for a review, see Landry and Oliver 2001). Regulation of the open-probability of these Ca^{2+} channels is via modulation of the magnitude of the negative voltage in these cells — Ca^{2+} channels will open in response to a less negative voltage and they will be closed when the intracellular voltage is more negative.

Fig. 5. Vasoconstrictor tone in vascular smooth muscle cells. The circles represent a vascular smooth muscle cell. When the ICF has a less negative voltage because its K_{ATP} and K_{Ca} channels are closed, the voltage-gated Ca^{2+} channel can be maintained in an open configuration, permitting a sustained rise in the $I_{Ca^{2+}}$. Hence, vasoconstriction will be the dominant response (left of the dashed line). In contrast, when K_{ATP} channels are opened by $Mg\cdot ADP$, L -lactate anions (L^-), and H^+ or the K_{Ca} channels are open secondary to an excessively high $I_{Ca^{2+}}$, this leads to a more negative ICF voltage and closure of their voltage-gated Ca^{2+} channels. Nitric oxide (NO) causes both of these K^+ channels to be open and leads to vasodilation (right of the dashed line).

A negative intracellular voltage is created firstly by actions of Na^+/K^+ ATPase because it pumps positive voltage out of cells — it exports three Na^+ in conjunction with the entry of two K^+ into cells (Russell 2000) — and secondly by K^+ diffusion out of cells down their concentration difference. This latter component requires that there be K^+ channels in the plasma membrane that are in an open configuration (Miller 2000). Hence, one could anticipate that factors that cause vasoconstriction close K^+ channels whereas stimuli for vasodilation will open K^+ channels and cause the more negative ICF voltage to reduce Ca^{2+} entry into cells.

There are two members of the K^+ channel family that participate in an important way in the regulation of negative voltage in vascular smooth muscle cells. First, there is the so-called ATP-gated K^+ channels (K_{ATP}) — these K_{ATP} channels would always be closed because the concentration of ATP is high enough in cells to permit constant binding to the K_{ATP} cytoplasmic binding site (Quayle et al. 1997). Therefore, the regulatory unit of the K_{ATP} channel must have a physiological regulatory site that binds monovalent ions such as $Mg\cdot ADP$ and L -lactate (Fig. 5). When vasodilation is needed, the K_{ATP} channels should be open. The factors that open these K_{ATP} channels and lead to a more negative ICF voltage become obvious from an analysis of the physiology of intense exercise (see Section IV). Anaerobic metabolism leads to the formation of ADP and L -lactic acid — the latter enters vascular smooth muscle cells via a monocarboxylic acid cotransporter (Juel and Halestrap 1999). Magnesium-bound ADP, H^+ , and L -lactate anions act as ligands to open K_{ATP} channels. As a result, the ICF voltage becomes more negative, voltage-gated Ca^{2+} channels close, and the $I_{Ca^{2+}}$ concentration falls — this reduces vasoconstrictor tone and blood flow to working muscle rises as long as the exercise persists. This regulatory system is also important in β -cells of the pancreas, for example, but here the emphasis is on a

fall in ADP (Wollheim 2000). Glucose can stimulate the release of insulin by diminishing intracellular ADP levels, thereby closing β -cell K_{ATP} channels, which lowers the intracellular negative voltage and results in more Ca^{2+} entry.

There must also be a redundancy of controls that control the $I_{Ca^{2+}}$ because regulation of blood flow is needed in the absence of a rise in H^+ and L -lactate $^-$. For example, there are other signal systems that operate to open K_{ATP} channels in vascular smooth muscle cells — the best-known one is nitric oxide (Taylor and Geller 2000).

There is a second type of K^+ channel, a Ca^{2+} -gated K^+ channel (K_{Ca}), that has a different function. These channels open when the $I_{Ca^{2+}}$ rises to dangerously high levels — this limits the rise in the $I_{Ca^{2+}}$ (see Section III for more discussion). These K_{Ca} channels are also placed in a more open configuration by the vasodilator nitric oxide.

(ii) *Decrease the quantity of ROS by increasing the removal of reduced CoQ* — One can anticipate that there should be a system to remove ROS before they cause tissue damage. As background, the concentrations of the reduced forms of CoQ are usually high in mitochondria for two reasons. First, this allows mitochondria to regenerate ATP rapidly when ADP is formed as a result of performing biologic work. Second, high levels of reduced cofactors related to complex I of the electron transport system in mitochondria (NADH) are part of the essential components of the negative feedback signal system that inhibits key regulatory enzymes in energy metabolism (e.g., dehydrogenases in the Krebs cycle and of greatest importance, pyruvate dehydrogenase (Denton and Halestrap 1979; Randle 1986)).

There are two ways to lower the level of reduced forms of CoQ if the level of ROS were to rise. First, the tight linkage between mitochondrial O_2 consumption and the availability of ADP can be uncoupled (Mitchell 1961). This uncoupling of oxidative phosphorylation is achieved by permitting H^+ to

Fig. 6. Role of tissue P_{CO_2} in the selection of intracellular buffer systems. The aim of buffering is to prevent H^+ from binding to proteins in cells; this can occur if the tissue P_{CO_2} falls. For this to happen, the arterial P_{CO_2} must fall due to stimulation of ventilation. There must also be a fall in the venous P_{CO_2} . The latter will be present if the blood flow rate rises. When the tissue P_{CO_2} falls, the H^+ concentration in cells will fall so intracellular proteins ($\text{H}\cdot\text{PTN}^+$) will not buffer H^+ ; rather, H^+ will be removed solely by the BBS.

enter mitochondria by a pathway distinct from their usual entry pathway that regenerates ATP (Fig. 4). This alternate H^+ entry pathway is catalyzed by proteins called uncoupler proteins (UCP) (Lowell and Spiegelman 2000) that are activated by an increased concentration of ROS. Therefore, with a faster flux through the electron transport system, both the local P_{O_2} and the level of ROS decline, lowering ROS formation (eq. 3). Second, ROS can be removed by catabolism catalyzed by superoxide dismutase (SOD and catalase). In fact, when mitochondria are incubated in the presence of SOD, O_2^- levels decline and this inhibits uncoupled respiration because of a decreased flux through UCP-3 (Echtay and Brand 2001).

Question 8: What might the biological role of UCP-3 be in skeletal muscle during fasting?

A puzzling feature of a member of the UCP family, UCP-3 in skeletal muscle, is that it is induced by 5- to 10-fold during starvation (Boss et al. 1997). Hence, energy appears to be wasted when it is most needed.

Define function

It is clear that thermogenesis and wasting of energy stores are not the primary function of UCP-3 in skeletal muscle. Some claim that UCP provides advantages for metabolic regulation. An alternative speculation is that UCP-3 provides a means to lower the concentration of ROS (Brand 2000; Harper et al. 2001).

Deduce controls

If the rate of mitochondrial respiration were to increase, the steady-state concentration of the reduced forms of CoQ would decline (eq. 3). Hence, inducing UCP-3 in starvation could keep the level of ROS from rising excessively when flux through the electron transport system is low. This uncoupled flux of H^+ into mitochondria via UCP-3 will cause an energy drain, so the control system should be extended to consider which fuel should be oxidized. There are limited stores of carbohydrate and the levels of ketoacids must be maintained because they are the primary fuel for the brain in prolonged starvation (Owen et al. 1967). Therefore, the obvious fuel to oxidize in skeletal muscle for its uncoupled ox-

idation is fatty acids. In fact, ROS stimulate UCP-3 flux if fatty acids are available (Rial et al. 1983).

In summary, the feedback loop is complete because the reduced forms of CoQ activate UCP-3 in skeletal muscle. Flux through UCP-3 lowers the reduced forms of CoQ levels and a new steady state is produced. This system requires a high level of fatty acids so oxidation of protein (potential glucose) can be inhibited, thereby conserving lean body mass — the price to pay is a small increase in the catabolism of stored triglycerides. As we shall see when intense exercise is discussed (Section V), when exercise stops, there will be a period when blood flow to working muscles is high, yet demand for O_2 is now much less. At this time, there will be a high P_{O_2} and a high concentration of the reduced forms of CoQ. Therefore, if UCP-3 flux rose, less ROS would be formed.

II. Composition of the ECF compartment

The composition of the ECF compartment will be dictated by the fact that its P_{CO_2} is close to 40 mmHg. Although a number of facts suggest that the concentration of H^+ should be regulated as a primary process, an argument will be put forward that it may be more important to control the ECF concentration of HCO_3^- ($E_{\text{HCO}_3^-}$). Two facts then act in concert to select the optimal $E_{\text{HCO}_3^-}$, a high value to buffer H^+ in a sprint, yet not too high to maintain an optimal ECF Ca^{2+} concentration ($E_{\text{Ca}^{2+}}$). This optimal $E_{\text{HCO}_3^-}$ should set the $E_{\text{Ca}^{2+}}$ and divalent inorganic phosphate (HPO_4^{2-}) concentrations so that they would be virtually equal and that their ion product would be very close to the solubility product constant (K_{sp}) of $\text{Ca}_3(\text{PO}_4)_2$. The questions to be addressed in this section are summarized in Table 2.

(a) CO₂ and buffering of H⁺

There are two ways to think about H^+ buffering. First, the traditional view is that buffering can be thought of as simply a means to prevent a large rise in the H^+ concentration in the body. From a slightly different perspective, the function of the BBS is to remove H^+ without permitting a significant rise in the H^+ concentration. CO_2 is central to the control of

Table 2. Questions concerning the ECF compartment.

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9. Is buffering of H^+ always a beneficial process in physiology?
 10. Which ion, H^+ or HCO_3^- should dictate the concentration of the other one?
 11. Why might Ca^{2+} salts precipitate in only some regions of the ECF compartment?
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the H^+ concentration (eq. 2; Fig. 6). To remove a big H^+ load, there must be a very large amount of HCO_3^- and this HCO_3^- must in some way have a high likelihood of binding H^+ at their concentration in body fluid compartments.

Question 9: Is buffering of H^+ always a beneficial process in physiology?

Define function

If the H^+ concentration were to rise, H^+ would bind to all H^+ acceptors including proteins in cells. When H^+ bind to proteins, their charge, shape, and possibly their function will be altered (eq. 4).

Deduce controls

Because our major enzymes, transporters, and contractile elements are proteins, only few H^+ should bind to them. Nevertheless, if the H^+ concentration were to rise, simple chemistry mandates that H^+ would bind to proteins. This dichotomy of removing a H^+ load while preserving protein structure can be resolved by having a unique system that will remove H^+ before the H^+ concentration rises, forcing H^+ to bind to E_{HCO_3} (Fig. 6). The mechanism becomes obvious when CO_2 is the focus of attention. Reducing the CO_2 concentration will drive eq. 2 to the left. While this lowers both the HCO_3^- and H^+ concentrations, the effect on the H^+ concentration is relatively much larger because the E_{HCO_3} is almost 10^6 -fold larger than that of H^+ .

Question 10: Which ion, H^+ or HCO_3^- , should dictate the concentration of the other one?

Because the arterial P_{CO_2} was fixed by the amount of O_2 extracted from each litre of inspired air, the body can "choose" to have a given concentration of H^+ or HCO_3^- . Once one is selected, the other becomes the dependent variable (eq. 2). Although one could mount an argument for regulating the concentration of either H^+ or HCO_3^- , a compelling reason to choose one versus the other is elusive. Therefore, we shall seek a specific property of H^+ or HCO_3^- that could help with this decision.

(b) Regulate the H^+ or the HCO_3^- concentration

(i) The body should regulate the concentration of H^+ —
 Let us begin with a bias: H^+ are much more important than HCO_3^- because they may bind to proteins changing their charge, shape, and possibly their function (eq. 4). The next step is to select the most common acid that accumulates in a large amount in physiologic settings, L-lactic acid (Cohen and Woods 1976) (See Appendix C). Consider cardiogenic shock where L-lactic acid accumulates due to accelerated anaerobic glycolysis secondary to poor delivery of O_2 to tissues. Analysis of data from these patients provides a statistical association for the primacy of H^+ (i.e., the higher the H^+ concentration, the higher the mortality); however, all that was established was an association, not a causal rela-

tionship. Other data could be cited to support the view that H^+ are more important than HCO_3^- . Studies performed in vitro indicate that when the H^+ concentration rises, less adrenaline binds to its receptors in the heart (Nahas et al. 1967). The heart rate and the strength of contraction decline when there is less adrenergic activity.

It is important to recognize that one never proves that a hypothesis is correct (Beveridge 1951). Rather, if one fails to disprove it, that hypothesis maintains its credibility. Therefore, it is important to seek a "flawless" experiment to disprove the hypothesis concerning the primacy of H^+ . The most common physiological basis for L-lactic acidosis is a sprint (Cheetham et al. 1986). In fact, after 10 s, the L-lactic acidosis may be more severe than during cardiac arrest, but the sprinter survives and has an excellent cardiac output. Therefore, it is difficult to attribute the poor cardiac output simply to a rise in H^+ and (or) L-lactate anion concentrations. Moreover, failure to deliver O_2 at an adequate rate for ATP regeneration might explain the mortality in cardiac arrest and the accumulation of L-lactic acid could be a secondary event. Alternatively, the high P_{CO_2} in tissues due to a very slow blood flow rate could have led to diminished buffering of H^+ by the BBS and more H^+ binding to proteins (Fig. 6). Therefore, the primary importance of H^+ was not established.

(ii) The body should regulate the concentration of HCO_3^- —
 The E_{HCO_3} should be as high as possible to permit the maximum number of H^+ to be eliminated by the BBS during a sprint. Nevertheless, there is an upper limit for this E_{HCO_3} because HCO_3^- is involved in another reaction, its conversion to carbonate ions (eq. 5). The carbonate so-formed could react with ionized Ca^{2+} forming insoluble calcium carbonate ($CaCO_3$) (eq. 6). The requirement for an ionized $E_{Ca^{2+}}$ that is close to 1 mmol/L will be discussed in the next section. Independent forms of regulation of the E_{HCO_3} (plus carbonate) and $E_{Ca^{2+}}$ can be anticipated.

(c) Integrative physiology of calcium

Question 11: Why might Ca^{2+} salts precipitate in only some regions of the ECF compartment?

Ca^{2+} salts that form a precipitate provide the rigid structure of bone. Bone formation demands that the substrates in eqs. 6 and 7 be close to their K_{sp} . Because bone formation is important for growth, the product of the concentrations of carbonate and Ca^{2+} as well as HPO_4^{2-} and Ca^{2+} should be close to their respective K_{sp} . Moreover, it should not be a surprise that the plasma HPO_4^{2-} concentration is higher in children because $Ca_3(PO_4)_2$ is the major form of Ca precipitate in bone and bone accretion occurs in children. Because it would be very disadvantageous to form Ca^{2+} precipitates in soft tissues, other factors in addition to the K_{sp} for Ca^{2+}

Table 3. Questions concerning the ICF compartment.

12. What accounts for the majority of body mass?
13. What features would be needed to keep anions or cations outside cells?
14. Why is the ICF volume so large (40% of body mass)?
15. What would be needed to prevent the negative voltage in cells from expelling HCO_3^- from cells?
16. What is the consequence of raising the concentration of HPO_4^{2-} in the ICF compartment during intense exercise?
17. In thermodynamic terms, how many fold must the $I_{\text{Ca}^{2+}}$ rise so that after binding to its receptor on a regulatory signal protein, there will be enough energy to induce the desired protein conformational change?

Fig. 7. Minimizing the volume of water in the body. The circles represent the size of the ICF compartment. The osmolalities of the ECF and ICF are equal because cell membranes are permeable to water. The volume of the ICF compartment will be twice as large if it contains an equal number of monovalent cations (K^+) and anions (Cl^-), as shown in the left portion of the figure. In contrast, if the electrical force (negative voltage) expels monovalent Cl^- through ion-specific Cl^- channels and the ICF has polyvalent macromolecular phosphate anions for electroneutrality, the volume of the ICF compartment will halve at the same total osmolality (right portion of the figure).

salts must be considered. Precipitation reactions can be favored or retarded depending on the presence of stimulators or inhibitors of precipitation. Therefore, one can anticipate that the chemistry of Ca^{2+} and HPO_4^{2-} will permit supersaturated solutions and that a compound that initiates precipitation from a supersaturated solution is ideal in bone but could be harmful elsewhere.

(d) Selection of the optimal ionized Ca^{2+} concentration

When eq. 7 is examined, one should consider both the K_{sp} and the ratio of Ca^{2+} to HPO_4^{2-} in the $\text{Ca}_3(\text{PO}_4)_2$ precipitate. Therefore, the appropriate $E_{\text{HCO}_3^-}$ to select should depend both on buffering of H^+ (high $E_{\text{HCO}_3^-}$) and in selecting an $E_{\text{Ca}^{2+}}$ that results in near-equal concentration of ionized Ca^{2+} and HPO_4^{2-} in the ECF compartment for more efficient mineralization of bone.

III. Intracellular fluid compartment

The ICF compartment is the largest one in the body; it contains our vital enzyme systems. Precipitation of Ca^{2+} should never occur in cells. The key issues for the ICF are to limit its size (mass) and to provide an optimal environment that permits $I_{\text{Ca}^{2+}}$ to be a metabolic regulator. The questions to be addressed in this section are summarized in Table 3.

(a) Efficiency of movement: the need to minimize body mass

Question 12: What accounts for the majority of body mass?

Define function

Should a subject have a smaller mass, less energy need be expended during intense exercise. To decrease mass, the quantity of the major constituent of the body, water, must be as small as possible. The number of particles dissolved in water determines body water content because there is a constant effective osmolality of body fluids. The upper limit on the effective osmolality is one that prevents the effects of a high ionic strength on protein structure.

Deduce controls

To understand how a minimum water content can be achieved, the concept of osmolality in body fluid compartments becomes paramount (Fig. 7). There are two components that dictate how much water exists in cells: the total osmotic pressure and the number of dissolved solute molecules in the ICF compartment.

Question 13: What features would be needed to keep anions or cations outside cells?

Define function

To have less body water, one needs a smaller number of solute molecules and (or) a high value for osmolality of body fluids.

Deduce controls

Applying these concepts to the largest water compartment (ICF), the majority of ICF osmoles are ions. The number of

positively and negatively charged ions must be virtually equal in all body compartments (very tiny differences actually occur to induce an electrical force (Giebisch 1998)). A smaller number of molecules would be present in cells if many of its ions were present as polyvalent macromolecules instead of monovalent ions. In fact, the majority of anions in the ICF compartment are polyvalent organic phosphate anions (Halperin and Jungas 1983). To maintain a low quantity of monovalent anions in cells, there must be a force to export them from cells (negative voltage in the ICF) along with open ion channels that permit them to exit from cells (Fig. 7). This negative voltage must be large enough to expel most of these monovalent anions, i.e., be greater than -60 mV (create a 10-fold concentration difference). Because the most abundant monovalent anion is Cl^- , cell membranes should (and do) have specific Cl^- channels (Jentsch 2000).

A quantitative analysis emphasizes the importance of ensuring that there are few monovalent anions in the ICF compartment. Water accounts for two thirds of body mass (45 L or 45 kg in a 70-kg adult) and two thirds of this water is in cells (30 L or 30 kg) because of the large number of ICF osmoles that are predominantly K^+ . If ICF anions were all monovalent, the number of osmoles in the ICF compartment would also double (Fig. 7). Hence, at the same osmolality, the ICF volume would double. Therefore, if Cl^- remained in the ICF compartment, body mass in a 70-kg adult would rise by close to 30 kg! To complete this thought process, a negative voltage in the ICF is needed to expel Cl^- .

Question 14: Why is the ICF volume so large (40% of body mass)?

Define function

The number of solute molecules at a given effective osmolality determines the volume of the ICF compartment.

Deduce controls

The vast majority of ICF solutes are K^+ and their number is determined by the number of macromolecular anions because of the requirement for electroneutrality. Because of biological complexity, a large amount of the nuclear macromolecule DNA is needed (Alberts et al. 1989). Organic phosphate is a major constituent of DNA. Another nucleic acid, RNA, is needed for the dynamic process of protein synthesis, and phospholipids serve many functions. Part of the economy of volume is achieved by having these organic phosphates in a monovalent, diester form. Therefore, the need for organic phosphates dictates the ICF K^+ content (electroneutrality) and thereby the ICF volume.

It is also reasonable to ask why the major cations in the ICF compartment are not divalent because this would decrease the number of effective solutes in the ICF compartment. This would be a poor strategy because divalent cations such as Ca^{2+} and Mg^{2+} would form precipitates with HPO_4^{2-} if their concentrations exceeded a few millimoles per litre.

To have the smallest mass, most of our stored energy should be insoluble in water (fat). Nevertheless, this solution creates problems for the transport of energy as fat in an aqueous environment, but this topic will not be developed in this article.

(b) Control of the H^+ concentration in the ICF compartment

Since our vital enzymes and transporters are proteins, their structure must be maintained. This in turn demands a specific H^+ concentration in the ICF compartment to minimize H^+ binding to proteins (eq. 4). Since O_2 consumption and the need for a high P_{O_2} fix the P_{CO_2} , one could have a near-constant H^+ concentration by regulating the concentration of H^+ or HCO_3^- in the ICF compartment (eq. 2). It is also advantageous to have a relatively high amount of HCO_3^- available for buffering of H^+ inside cells. If, as with Cl^- , the negative voltage in cells were able to expel HCO_3^- from or attract H^+ into cells, we could not have an adequate amount of HCO_3^- inside cells for "good" buffering (protecting the electrical charge on proteins).

Question 15: What would be needed to prevent the negative voltage in cells from expelling HCO_3^- from cells?

Define function

To maintain a high ICF HCO_3^- concentration ($I_{\text{HCO}_3^-}$), a negative voltage should not cause HCO_3^- to be exported or H^+ to be imported across cell membranes.

Deduce controls

HCO_3^- and H^+ will not be driven across cells by a negative voltage if there are no ion channels for HCO_3^- or H^+ in their membranes. This implies that the concentration of H^+ inside and outside cells will be regulated independently.

Because some organs produce and others use organic acids, H^+ and (or) HCO_3^- must cross cell membranes electro-neutrally. There are three electroneutral H^+ or HCO_3^- ion transport systems. First, there are cotransporters for H^+ and the major biological monocarboxylate anions (L-lactate and ketoacid anions (Juel and Halestrap 1999)). Second, an equal number of Na^+ and H^+ could move in opposite directions (an electroneutral Na^+/H^+ exchanger (NHE) (Solemani and Burham 2000)). Third, an equal number of Cl^- and HCO_3^- could move in opposite directions (an electroneutral $\text{Cl}^-/\text{HCO}_3^-$ anion exchanger (AE) (Alper 1991)). Following their stoichiometries a little further, NHE must normally be an inactive state because the product of the concentrations of its substrates (ECF Na^+ and ICF H^+) is more than an order of magnitude higher than the product of the concentrations of ICF Na^+ and ECF H^+ in steady state. When the NHE is activated, Na^+ enters cells down a concentration difference and H^+ exits for a similar reason (Fig. 8). On the one hand, this will alkalize the cell; NHE flux cannot cause H^+ entry into normal cells. On the other hand, subsequent export of these intracellular Na^+ will occur by the Na^+/K^+ ATPase that causes the interior of cells to become more electronegative (Moore 1983). Therefore, it is not surprising that the activator of NHE is insulin (Moore 1983), the signal of food ($+\text{K}^+$) intake, so that much of this ingested K^+ can be translocated into the ICF compartment to avoid a higher plasma K^+ concentration (P_{K}).

Again, because of steady-state Cl^- and HCO_3^- concentrations in the ECF and ICF compartments, we know that the AE is also in an inactive state in cell membranes. When this AE becomes active, $I_{\text{HCO}_3^-}$ exits from cells because of the large transmembrane Cl^- concentration difference (greater than an order of magnitude higher in the ECF compartment).

Fig. 8. Effect of the transport of H^+ or HCO_3^- on the resting membrane potential. The circle represents the cell membrane. Both NHE (left) and AE (right) are inactive at rest as evidenced by the concentration differences for the substrates and products of these electroneutral transporters (small shaded circles). After NHE is activated, Na^+ will be exported via Na^+/K^+ ATPase. This electrogenic export of Na^+ makes the interior voltage more negative. In contrast, after AE is activated, Cl^- accumulates in cells. This leads to the electrogenic export of Cl^- through its ion channel making the inside of cells less negative.

Looking at overall stoichiometry, because flux through the AE raises the concentration of Cl^- in the ICF compartment, Cl^- is driven out of cells by the negative intracellular voltage because there are Cl^- channels in cell membranes (Jentsch 2000); this leads to a less negative ICF voltage, and secondary to this voltage change, K^+ exits from cells (DeMars et al. 2001). Hence, by selecting either NHE or the AE to influence the ICF HCO_3^- or H^+ concentration, the resting membrane potential and K^+ movement will change in opposite directions.

(c) Avoiding precipitation of $\text{Ca}_3(\text{PO}_4)_2$ in cells

Question 16: What is the consequence of raising the concentration of HPO_4^{2-} in the ICF compartment during intense exercise?

Define function

When a sprint is initiated, ATP is hydrolyzed, yielding ADP (eq. 8a). In response to this rise in ADP, phosphocreatine is hydrolyzed and this leads to high levels of HPO_4^{2-} and an alkali load in cells (eq. 8). This rise in HPO_4^{2-} is very large in skeletal muscle cells because they have a large amount of phosphocreatine (~25 mmol/L ICF). Overall, the HCO_3^- load from the complete hydrolysis of phosphocreatine in skeletal muscle (25 mmol/L $\times$ 20 L = ~500 mmol) is greater than the total ECF content of HCO_3^- .

Events differ a few seconds later in the sprint; ATP is formed predominantly by anaerobic glycolysis leading to high rates of L-lactic acid production (Cheetham et al. 1986). In this setting, the ICF H^+ concentration may rise by two to threefold, shifting eq. 9 to the right, converting HPO_4^{2-} to its

monovalent form (H_2PO_4^-) that does not react with $\text{I}_{\text{Ca}^{2+}}$. This H^+ concentration rise is particularly important because the pK for eq. 9 is only somewhat lower than the pH of the ICF compartment (Schwartz et al. 1959). It is the HPO_4^{2-} that is critical for the formation of the $\text{Ca}_3(\text{PO}_4)_2$ precipitate (Parks et al. 1997).

Deduce controls

Three facts can be deduced. First, cell membranes will restrict the movement of inorganic phosphate to avoid causing a rise in the $\text{Ca}^{2+} \times \text{HPO}_4^{2-}$ ion product in the ECF compartment. Second, the $\text{I}_{\text{Ca}^{2+}}$ must always be low enough in cells to avoid unwanted precipitation reactions. Third, when intense exercise stops, phosphocreatine is synthesized rapidly, yielding a very large H^+ load. This must occur when the muscle P_{CO_2} can fall to have the H^+ buffered primarily by its BBS.

(d) Importance of the ICF Ca^{2+} concentration for metabolic regulation

The $\text{I}_{\text{Ca}^{2+}}$ is an important component of ICF regulatory systems. Based on an estimate of the highest HPO_4^{2-} concentration in skeletal muscle (a HPO_4^{2-} concentration greater than one order of magnitude higher than its concentration in the ECF compartment), one could avoid $\text{Ca}_3(\text{PO}_4)_2$ precipitation if the $\text{I}_{\text{Ca}^{2+}}$ were always two orders of magnitude lower than its concentration in the ECF compartment (~1000 μM). In fact, the $\text{I}_{\text{Ca}^{2+}}$ is 0.1 μM at rest and it reaches a peak value of 10 μM , fulfilling this prophecy (Alberts et al. 1989). The requirement for having a Ca^{2+} signal system with an optimal range of $\text{I}_{\text{Ca}^{2+}}$ will be examined in the discussion of question 17.

Question 17: In thermodynamic terms, how many fold must the $\text{I}_{\text{Ca}^{2+}}$ rise so that after binding to its receptor on a regulatory signal protein, there will be enough energy to induce the desired protein conformational change?

Define function

At equilibrium with no ligand present, almost 100% of the regulatory signal protein must be in its inactive (or "off") form.

Deduce controls

The following thermodynamic analysis illustrates how a regulatory protein may use changes in the $I_{Ca^{2+}}$ as its signal switch mechanism. Four factors need to be considered to explain the energy requirements for $I_{Ca^{2+}}$ to elicit a signal that is strong enough to switch an ICF regulatory protein from its "off" to an "on" configuration.

(i) *Energy difference needed between two protein conformations to ensure that only a tiny proportion will be in the active form in the absence of a rise in the $I_{Ca^{2+}}$* — For a regulatory signal to be elicited only when there is a rise in the $I_{Ca^{2+}}$, virtually 100% of that regulatory protein must exist in its "off" conformation at rest. The needed quantitative information concerning the energy difference between these two conformations can be obtained from the Boltzmann formula (eq. 10) where ΔG represents this energy difference between the two conformations of a regulatory protein (Tinoco et al. 1985). Close to 1420 cal/mol (1 cal = 4.184 J) is needed to have a 10-fold preference in distribution between its stable ("off") and high-energy ("on") configurations at body temperature, i.e., 10% of its molecules will be in the "on" position without binding of $I_{Ca^{2+}}$. Because too much of this protein is still in the "on" conformation in the absence of Ca^{2+} binding, the energy difference between the stable and the high-energy states should be even higher to make this signal switch biologically useful. If this energy difference were 2840 cal/mol, 1% of the switch molecules would be in the "on" position in the absence of ligand binding. This is still not good enough for most cell signaling purposes, which require that the switch be designed to be "off" at the 99.9% level with a need for an energy difference of close to 4260 cal.

$$[10] \quad \Delta G = -RT \ln X$$

where $X = [Ca^{2+}]_{ICF}/\text{affinity for } Ca^{2+}$.

(ii) *How much energy is available when Ca^{2+} binds to our regulatory protein?* — The $I_{Ca^{2+}}$ in the unstimulated state is 0.1 μM (Alberts et al. 1989). Probably this nadir of 0.1 μM was selected because a significantly lower $I_{Ca^{2+}}$ in the basal state would slow binding of Ca^{2+} due to kinetic problems. When the $I_{Ca^{2+}}$ is 0.1 μM and Ca^{2+} binds to our signal protein, there are zero calories per mole of energy (i.e., $-RT \ln 1$ is zero). At 1 μM $I_{Ca^{2+}}$, binding of Ca^{2+} to our signal protein would generate 1420 cal/mol, which is not enough energy to turn the switch "on" if we must overcome the close to 4260 cal/mol energy barrier. The calculation illustrates the thermodynamic argument why the basal $I_{Ca^{2+}}$ must be quite low so that the $I_{Ca^{2+}}$ can rise by much greater than 100-fold to have a signal system with less than 1% erroneous signal.

(iii) *Range of $I_{Ca^{2+}}$ that would provide an optimal signal system* — A 1% random "on" position of our signal switch is probably too high for biological specificity. Therefore, another 1420 cal/mol would be needed for a mechanism with only a 0.1 erroneous "on" signal, bringing the energy difference to 4260 cal/mol. This means that a 1000-fold rise in the $I_{Ca^{2+}}$ would be needed for full turn-"on", which is rather too much because it brings the $I_{Ca^{2+}}$ to a range where there may be a $Ca_3(PO_4)_2$ problem in skeletal muscle early during a sprint.

In fact, the rise in the $I_{Ca^{2+}}$ concentration should be closer to 100- than 1000-fold when Ca^{2+} signals are induced — how can this dilemma of kinetics versus solubility be resolved? One answer might be to use two switches in series. Now if both are 99% "off", the two in tandem are 99.99% "off" and that would make a good switch. In addition, these two switches must function independently and cannot be just two proteins in a cascade arrangement where, once one is signaled "on", this leads to both proteins being turned "on". A possible solution is to have the signal switches in a dimer conformation and require that Ca^{2+} bind to both to initiate activity. Thus, if thermodynamics were the only consideration, one could predict that the ICF receptor or signal switch will be a dimeric protein with no interaction between its units. Ca^{2+} must bind to each unit independently while they turn "on" separately. In this way, a 100-fold rise in $I_{Ca^{2+}}$ would suffice to turn the switch "on" with only a 0.01% erroneous signal without ligand binding. In fact, calmodulin has two separate binding domains for Ca^{2+} on each half of this protein (Alberts et al. 1989) (see Appendix B). Of interest, two of the four binding sites for Ca^{2+} have a much lower affinity for Ca^{2+} (Alberts et al. 1989). The importance of these sites with a lower affinity for Ca^{2+} is considered in the following paragraph.

(iv) *Prevent too large a rise in the $I_{Ca^{2+}}$ concentration* — If the process that permits the $I_{Ca^{2+}}$ to rise is activation of a Ca^{2+} channel, there needs to be a set of signals that decrease this channel activity and stimulate the Ca^{2+} removal mechanism when the $I_{Ca^{2+}}$ rises more than 100-fold. Perhaps this might also explain the requirement for two types of binding sites for Ca^{2+} on calmodulin with a high affinity and two others with a lower affinity. These lower affinity sites should inhibit the Ca^{2+} channel activity (Alberts et al. 1989). In addition, as the $I_{Ca^{2+}}$ rises, the K_{Ca} channels become active (Fig. 5). When K^+ exits from cells, the ICF voltage becomes more negative and this closes the voltage-gated Ca^{2+} channels in the plasma membrane. This is, in fact, consistent with the bell-shaped curve depicting the Ca^{2+} concentration and channel activity (Bezprozvanny et al. 1991). In addition, the higher $I_{Ca^{2+}}$ will activate the Ca^{2+} ATPase in plasma membranes as well as in endoplasmic reticulum membranes as well as an Na^+/Ca^{2+} exchanger with a relatively lower affinity for Ca^{2+} . These systems limit the peak $I_{Ca^{2+}}$ to avoid both precipitation of $Ca_3(PO_4)_2$ and uncoupling oxidative phosphorylation.

IV. Composition of the urine

Define function

All of the water-soluble waste products and all of the ions and water ingested in excess of needs must be excreted in the urine without forming precipitates.

Deduce controls

Excreting urine with a composition that makes ions or organic materials that are sparingly soluble in water more soluble is an important physiological response. Key to this aim is to have independent regulation of the urine pH: select a value that is close to 6.0 to achieve this aim (Coe and Parks 2000) (Fig. 9). Excreting urine at this pH must not sacrifice acid-base balance. This in turn means that with a large,

Fig. 9. Urine pH and kidney stones. The safest urine pH to avoid kidney stones is close to 6. Below this value, uric acid stones are most likely to form. By driving NH_4^+ excretion with a high NH_3 rather than a high H^+ concentration, a considerable quantity of H^+ can be eliminated at a urine pH close to 6.0. By excreting organic anions rather than HCO_3^- , a considerable quantity of HCO_3^- can also be eliminated at a urine pH close to 6.0 (see Fig. 10).

chronic acid load, the excretion of NH_4^+ should be maximally high at a urine pH of close to 6 (for further discussion, see Kamel et al. 1998). Similarly, when an alkali load is ingested, it must be excreted without obligating a large excretion of HCO_3^- and thereby a high urine pH (Cheema-Dhadli et al. 2002; Lin et al. 1998); this latter topic will be discussed in more detail when $\text{Ca}_3(\text{PO}_4)_2$ stones are considered below.

(a) Avoiding uric acid kidney stones

Uric acid is the waste product of purine metabolism (Asplin 1996). The free acid form, uric acid, rather than total urates is the critical component for kidney stone formation because uric acid is sparingly soluble in water (eq. 11). Because the pK of uric acid in the urine at 37°C is close to 5.3 (Asplin 1996), precipitation of uric acid can be avoided without increasing the urine volume by raising the urine pH to 6 at the same total urate excretion rate (Kamel and Halperin 2002).

(b) Avoiding $\text{Ca}_3(\text{PO}_4)_2$ kidney stones

All of the Ca^{2+} absorbed from the GI tract of an adult is excreted in the urine in steady state (Bushinsky 1999). The chemistry of Ca^{2+} and HPO_4^{2-} in the urine is the same as in the body (Parks et al. 1997), but with one exception. A “metabolic equivalent” of HCO_3^- , called citrate (Simpson 1983), can chelate Ca^{2+} in the urine, forming a soluble ion complex and thereby minimize the risk of $\text{Ca}_3(\text{PO}_4)_2$ kidney stone formation. This excretion of citrate rises with an alkali load (Simpson 1983), a time when urine H_2PO_4^- is converted to HPO_4^{2-} , i.e., when the urine pH rises towards the pK of the phosphate buffer system (pH 6.8). In more detail,

Fig. 10. Elimination of alkali of dietary origin. As a result of consuming fruits and vegetables, there is an alkali load ($\text{K}^+ + \text{HCO}_3^-$, step 1). HCO_3^- is removed by reacting with H^+ formed when organic acids including citric acid are produced during the metabolism of neutral precursors (step 2). Citrate anions are made into end-products of metabolism by being excreted in the urine because the HCO_3^- load inhibits the renal reabsorption of citrate (site 3).

the body disposes of the usual alkali load of the diet by forming an organic acid such as citric acid (Cheema-Dhadli et al. 2002; Lin et al. 1998). The H^+ of citric acid is used to titrate HCO_3^- . The citrate anions rather than an appreciable amount of HCO_3^- are excreted in the urine so that the urine pH remains close to 6.0 without sacrificing acid-base balance (Kamel and Halperin 2002) (Figs. 9 and 10). Excreting citrate rather than HCO_3^- when there is a dietary alkali load chelates Ca^{2+} in the urine, lessening the risk of Ca-containing kidney stones in alkaline urine (Coe and Parks 1980).

V. Integrative physiology of vigorous exercise

We shall now utilize the concepts developed in earlier sections to describe the integrative physiology of vigorous exercise. This topic will be divided into two parts; the first focuses on alkalinization of the ICF compartment and the second deals with direct and indirect effects of this alkalinization.

(a) Alkalinization of the ICF compartment of skeletal muscle

There are two processes that cause the ICF pH to rise, a fall in P_{CO_2} (respiratory alkalosis) and a rise in $I_{\text{HCO}_3^-}$ (metabolic alkalosis). The resultant fall in the ICF H^+ concentration causes proteins to become less cationic (or more

Fig. 11. Integrative physiology of vigorous exercise (see text for details). The oval represents a muscle cell. The respiratory and metabolic processes that alkalinize its ICF compartment are shown in the left portion of the figure. The effects of that alkalization are shown in the middle portion of the figure whereas the indirect response to the alkaline cell pH, a less negative voltage of the ICF compartment (exit of K^+ from cells), is shown in the right portion of the figure.

anionic) because they release some of their bound H^+ in response to the rise in ICF pH (Fig. 6; eq. 4) (Halperin and Goldstein 1998). This, in effect, permits a person to have the beneficial effects of the BBS before the sprint because H^+ and CO_2 will be produced in large quantities in this burst of exercise. By reducing the quantity of H^+ bound to proteins in skeletal muscle before the intense exercise occurs, there are more potential binding sites for H^+ on ICF proteins that are now in a more anionic form (Fig. 11). In contrast, after a second or two of intense exercise, CO_2 production will rise enormously due to L-lactic acid generation (H^+ reacts with $I_{HCO_3^-}$) causing a rise in the tissue P_{CO_2} . Hence, beginning the race with a tissue P_{CO_2} that is distinctly less than 40 mmHg better defends protein structure.

(i) *Respiratory alkalosis* — The low arterial P_{CO_2} is due to a high adrenergic activity that leads to stimulation of alveolar ventilation. In the first second or two before the metabolic acidosis, release of K^+ from muscle cells seems to provide an additional stimulus to ventilation (see below).

(ii) *Metabolic alkalosis* — The anionic valence on phosphocreatine³⁻ reflects the fact that its phosphate is a relatively strong acid (pK 2.7 and 4.6 (Dawson et al. 1969)) and it has a carboxyl group (Fig. 11). The initiation of work leads to a transient fall in ATP and rise in ADP. Because phosphocreatine plus ADP are converted to ATP and creatine⁰ by a near-equilibrium reaction, phosphocreatine is hydrolyzed promptly in response to a small rise in ADP, yielding less anionic products (eq. 8). For electroneutrality, HCO_3^- is formed when H^+ is generated to create the cationic NH_3^+ group on creatine⁰ (eq. 8b). At the ambient cell pH,

most of the HPO_4^{2-} will be converted to $H_2PO_4^-$ (eq. 9), requiring the generation of more HCO_3^- via eq. 2.

(b) Effect of alkalization of this ICF compartment

There are three major effects of alkalization of the ICF compartment. The two more direct ones are, first, a rise in the ICF pH to stimulate anaerobic glycolysis at PFK-1 (Halperin et al. 1969), and, second, to have a less negative transmembrane voltage. The indirect effect is to release K^+ from cells to stimulate ventilation.

(i) *Stimulation of anaerobic glycolysis* — Alkalization of the ICF compartment, when accompanied by a rise in adenosinemonophosphate (AMP) levels, could provide the initial signal to stimulate glycolysis by activating PFK-1 (Halperin et al. 1969). If this did not occur, a much larger fall in ATP and rise in AMP would be needed to activate PFK-1.

(ii) *Export of alkali from cells* — There is a net release of alkali from cells early in a sprint because the pH and $P_{HCO_3^-}$ both rise in venous blood draining exercising muscles in the first 3 s of intense exercise (Wasserman et al. 1997). Because HCO_3^- and (or) H^+ must be exported from cells via electroneutral cotransporters (Fig. 8), either NHE or AE could be a component of this process. Nevertheless, NHE cannot be involved in this process because it causes the export and not the entry of H^+ into cells owing to the Na^+ concentration difference between the ECF and ICF compartments. Thus, this export of alkali likely occurs via the AE cotransporter. Activation of the electroneutral AE will cause HCO_3^- exit along with a stoichiometric gain of

Cl^- in the ICF compartment. The resultant higher ICF Cl^- concentration obligates Cl^- exit from cells via Cl^- channels, lessening the net negative voltage in these cells (Fig. 8).

(iii) *Release of K^+ from muscle cells* — The indirect effect of alkalization of the ICF compartment is a less negative resting membrane potential. As a result, K^+ will move from the ICF to the ECF compartment, leading to a rise in the P_K early in vigorous exercise. There are two important potential roles of this rise in P_K . First, hyperkalemia will stimulate alveolar ventilation provided that the arterial P_{CO_2} does not fall (Patterson 1992). Therefore, alveolar ventilation can be augmented without requiring its usual stimulators, a low P_{O_2} , metabolic acidosis, or a high arterial P_{CO_2} . Alveolar ventilation could also be stimulated by nervous impulses from exercising muscles and this might be facilitated by a high K^+ concentration around these nerve endings. The net result is having an arterial P_{CO_2} distinctly less than 40 mmHg. Second, an elevated P_K can cause local vasodilation and thereby have a circulatory benefit during intense exercise.

(c) Increase in blood flow

Control of blood flow was discussed in Section II. The essential element is that local release of L-lactate⁻ and H^+ causes vasodilation because these ions lead to a more open configuration of the K_{ATP} channels in vascular smooth muscle cells (Fig. 5). When K^+ diffuses out of cells, the ICF voltage becomes more negative, voltage-gated Ca^{2+} channels close, the $I_{\text{Ca}^{2+}}$ falls, and as a result, there is less vasoconstriction.

Concluding remarks

Integrative physiology is a critically important subject for the clinician and student. Because it involves a series of interconnected events, it is a particularly difficult subject to examine. Thinking by subspecialty hinders a broad understanding in this area. An appreciation of basic sciences such as chemistry and physics helps provide answers to important questions, but interpretations will need to undergo modification with insights gained from molecular biology. By starting with a very important question concerning survival, the trail followed involved a quantitative analysis of work, energy, O_2 , CO_2 , H^+ , HCO_3^- , carbonate, ionized calcium, inorganic phosphate, metabolic regulation by Ca^{2+} , and avoiding kidney stones. The outcome permitted us to deduce the basis for the normal ionic composition and volume of our ECF and ICF compartments. All of these features were illustrated by considering the integrative physiology of vigorous exercise.

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Appendix A. Concentration of O_2 in blood

Each millimole of hemoglobin binds 4 mmol of O_2 . Because the molecular mass of hemoglobin is close to 70 000 and that each litre of blood has 140 000 mg of hemoglobin, there are 8 mmol of O_2 bound to hemoglobin per litre of blood.

Appendix B. Importance of dimers

Dimers might have broader biological implications. For example, hormone receptors often function when in a dimer conformation on the cell surface (Czech et al. 1988). This makes the need for circulating hormone concentrations to be 10-fold lower while having a reliable switch mechanism. Hence, their renal clearance is not important at these low concentrations.

Appendix C. H^+ production and glycolysis

With a deficit of O_2 , glucose must be broken down to L-lactate by anaerobic glycolysis to reconvert ADP to ATP. However, the proton released when ATP was broken down to ADP cannot be buffered by the L-lactate so it will accumulate. The complete equation for anaerobic glycolysis is

Note that there is no production of protons in this process, but glycolysis cannot occur unless work is first performed. This will cause the following:

Thus when work and glycolysis both occur the result is the sum of the above two equations:

In other words, glycolysis cannot produce both ATP and H^+ ; it can produce ATP (eq. C1) or it can produce H^+ (eq. C3) when coupled with work. It is the work that produces the H^+ . Note that we treated all changes as whole integers; they are really fractional, of course, but giving the exact changes does not alter the argument.