Energy Metabolism and Effects of Energy Depletion or Exposure to Glutamate[1]

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The entire program of the first day of the IBRO satellite meeting entitled Ions, Water, and Energy in Brain Cells was devoted to the subject of energy. There were three sessions on the topics of energy metabolism, activation, and development and pathological conditions, followed by a final general discussion on the contents of the day's topics. During this general discussion there were spirited exchanges on the role of glycogen in the energy metabolism of the brain, on the metabolic source of the energy consumed by functional activity, e.g., glycolytic or oxidative energy metabolism, and on the sources of the acid-equivalents that are responsible for the tissue acidosis accompanying cerebral hypoxia. Despite the arguments pro and con presented on all of the issues that were discussed, it is doubtful that a consensus was achieved on most of the issues.

Key words: glycogen, glycolysis, oxidative metabolism, acidosis, energy metabolism.

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Le programme de la première journée de la rencontre satellite de l'OIRC sur les ions, l'eau et l'énergie dans les cellules du cerveau a été consacré à l'énergie. Trois sessions ont porté sur les thèmes suivants : métabolisme énergétique, activation, développement et conditions pathologiques; elles ont été suivies d'une discussion générale sur les thèmes de la journée. Cette discussion a donné lieu à des échanges animés sur le rôle du glycogène dans le métabolisme énergétique du cerveau, sur la source métabolique de l'énergie consommée par l'activité fonctionnelle, par ex., le métabolisme énergétique oxydatif et glycolytique, et sur les sources d'équivalents acides responsables de l'acidose tissulaire accompagnant l'hypoxie cérébrale. Les arguments présentés en faveur ou contre les hypothèses avancées n'ont généralement pas suffi à établir un concensus.

Mots clés : glycogène, glycolyse, métabolisme oxydatif, acidose, métabolisme énergétique.

[Traduit par la rédaction]

The entire program of the first day was devoted to the subject of *energy*. There were three sessions, on (*i*) energy metabolism, (*ii*) activation, and (*iii*) development and pathological conditions, and they were followed by a general discussion that dealt mainly with issues raised or suggested in the formal presentations and their associated discussions during these sessions. Most of the general discussion revolved around three issues, not all mutually independent and unrelated.

Role of glycogen in the energy metabolism of the brain

It was generally accepted that glycogen in brain is almost exclusively confined to astroglia and that it is metabolically active (i.e., it is constantly being turned over). There was less agreement on what its role might be. J. LaManna pointed out that glycogen can be rapidly mobilized during increases in cellular activity, conditions in which energy metabolism would be expected to be increased. There was further discussion on whether energy derived from glycogen metabolism served some special function different from that of energy derived from ordinary glucose utilization. For example, R. Swanson raised the question of whether the mobilization of glycogen during functional activation might not be evidence that glycolytic energy is used to support the functional activity rather than oxidative energy. L. Sokoloff questioned whether or not too much was being made of the issue of a unique role for glycogen, distinct from that of glucose, in the energy metabolism of the brain. He argued that glycogen should not be viewed as anything other than a reservoir of glucose that was in dynamic equilibrium with glucose and its immediate phosphorylated products. As such, it could serve as a buffer for temporary imbalances between glucose utilization and glucose resupply by the cerebral circulation. It could only be temporary because the cerebral glycogen content, which consists only of glucose residues, is very low compared with the rate of glucose utilization. For example, in normal rat brain the equivalent glucose contained in the total glycogen content is only about 3 μmol/g (Nelson et al. 1968; Siesjö 1978; Clarke et al. 1989), just about enough to support the normal rate of glucose utilization in the brain for approximately 3 min. It would last for even less time in conditions in which energy metabolism is activated by increased cellular activity.

To assess glycogen's role in cerebral energy metabolism, it is helpful to examine the intermediary metabolism of glycogen and glucose, which are in a sort of dynamic equilibrium (Clarke et al. 1989). Glycogen is constantly being turned over; it is hydrolyzed to glucose-1-phosphate (G1P) and resynthesized from G1P via uridine 5′-diphosphoglucose (UDPglucose). The glycogen content of the tissue is determined by the balance between its hydrolysis to G1P and its resynthesis from G1P, which is derived mainly from glucose metabolism. Glucose is delivered by the circulation to the pool of glucose in brain from which it is transported back from brain to blood or phosphorylated by hexokinase to glucose-6-phosphate (G6P).

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The concentration of glucose in brain, which is normally considerable, i.e., about 2–2.5 μmol/g (Dienel et al. 1991), is determined by the balance between its net delivery from the blood and its rate of phosphorylation. G6P is metabolized mainly via the Embden–Meyerhof (glycolytic) pathway to pyruvate, lactate, and aerobically eventually to CO_2 and H_2O. Some of the G6P is converted to G1P, the precursor of glycogen; in fact, the two phosphorylated derivatives of glucose are in rapid equilibrium. If the G6P concentration falls, then so does the G1P concentration, and glycogen synthesis is decreased. Conversely, if the G6P concentration rises, the G1P concentration also rises, and glycogen synthesis is increased. The G6P concentration is influenced by the rate of glucose utilization via the Embden–Meyerhof pathway, the higher the rate the lower the G6P concentration and, therefore, also the G1P concentration. Glycogen synthesis is then reduced relative to its rate of hydrolysis, and glycogen content is decreased; glycogen is essentially "mobilized" to provide more G1P and thus also more G6P to the glycolytic pathway. When the G6P concentration is increased during reduced rates of glycolysis, glycogen synthesis exceeds glycogenolysis, and the glycogen content of the tissue is increased (Nelson et al. 1968). Ancillary factors, such as neurotransmitter effects on adenylylcyclase activity and the level of cyclic AMP (cAMP), which stimulates phosphorylase and glycogenolysis, may further amplify these effects, but in the same directions. The fact that the glycogen content of brain changes with altered functional and metabolic activities does not, therefore, indicate that glycogen serves any function qualitatively different from that of glucose; it is exactly what would be expected from a reserve of glucose equivalents that are stored to buffer transiently in times of need the phosphorylated derivatives of glucose that are the intermediates in the glycolytic pathway. Whether the phosphorylated glucose intermediates come from glycogen or directly from glucose has nothing to do with the ultimate fate of the glucose carbon, i.e., whether it goes into the tricarboxylic acid pathway for oxidation to CO_2 and H_2O or ends up in lactate.

There is still the question about the relationship between the function of glycogen in brain and its localization in astroglia. Glycogenolysis produces G1P, which proceeds further to G6P and other phosphorylated intermediates of the glycolytic pathway, ending eventually in pyruvate and lactate. The phosphorylated intermediates cannot get out of the cell and are retained in the astroglia. Because glucose-6-phosphatase (G6Pase) activity in brain is very low, the hexosephosphates cannot be hydrolyzed to free glucose, which can get out of the cells. This is how glycogen serves as a reservoir for free glucose in liver, which does contain very high G6Pase activity and secretes the released glucose into the blood. If the astroglia cannot release them into the extracellular space, then are the phosphorylated hexoses produced by glycogenolysis consumed only in the astroglia themselves? If so, then the glycogen exists to serve only the energy requirements of the astroglia. J. Edmond suggested an alternative. The products of glycogen hydrolysis are metabolized eventually to pyruvate and lactate, and these compounds can get out of the cells and be readily oxidized by the neuronal elements. In that case, the astroglia would be the sites of storage of fuel for the energy metabolism of the neuronal elements of brain. This is a reasonable hypothesis, but further work on this question is needed.

Source of energy supporting functional activity

Another controversial issue that was discussed was the source of the energy used to support normal functional activity of brain. Except in ketotic states, glucose is practically the exclusive substrate for the brain's energy metabolism (Sokoloff 1989). It is metabolized first via the Embden–Meyerhof (glycolytic) pathway to pyruvate, which in the presence of adequate oxygen enters the tricarboxylic acid (TCA) cycle and is oxidized to CO_2 and H_2O. Both the glycolytic pathway and the TCA cycle produce ATP, the former by glycolytic phosphorylation and the latter by oxidative phosphorylation. Glycolysis leads to a net production of 2 mol of ATP and oxidative phosphorylation to 36 mol for each mole of glucose consumed. Therefore, only 2 of the possible 38 moles of ATP derived from the oxidative metabolism of 1 mol of glucose come from glycolysis (Krebs 1972; Lehninger 1975). A flurry of excitement has recently permeated the field of neuroscience in response to claims that the energy, i.e., ATP, derived from these two pathways has different functions, and that the ATP needed to support functional activity is mainly, if not exclusively, of glycolytic origin. The question raised by R. Swanson about the possibility that glycogen is mobilized to provide specifically glycolytic energy to support functional activation is related to this issue. L. Sokoloff addressed this issue and argued that the evidence to support this provocative claim is based on questionable and unverified methods. He cited the findings accumulated from many laboratories over much of this century demonstrating that average glucose utilization (CMR_{glc}) and oxygen consumption ($CMRO_2$) in the brain as a whole are stoichiometrically related: 5–6 mol of O_2 are consumed per mole of glucose utilized. The median of the values reported in the literature for normal young men under steady-state condition is 5.5 mol of O_2 per mole of glucose (Sokoloff 1960, 1989). The theoretical ratio for exact equivalence for the complete oxidation of glucose to CO_2 and H_2O is 6.0. Only about 20% of the glucose consumed is unaccounted for by the oxygen consumption, suggesting that the rate of glycolysis is normally about 20% greater than the rate of oxidation of the products of glycolysis. Some of this excess glucose consumption is undoubtedly due to loss of some of the numerous intermediary metabolites between glucose and the sites in the TCA cycle where the O_2 is used, mainly lactate, some pyruvate, possibly citrate, etc., and also to incorporation of carbon derived from glucose into other chemical constituents of brain, such as acetylcholine, lipids, phospholipids, amino acids, proteins, nucleic acids, etc. The stoichiometric ratio of 5.0–6.0 has been found under steady-state conditions in all kinds of physiological and pathological states, whether the brain is functionally more or less active, i.e., sleep, wakefulness, sedation, presence or absence of visual or auditory stimulation, anxiety, performance of mental arithmetic, and a variety of disease states, including even seizures (Sokoloff 1960, 1989). The only exceptions have been ketotic states, in which some of the O_2 is used to oxidize ketone bodies in place of glucose (Owen et al. 1967); hypoglycemia (Kety et al. 1948), in which the glucose supply is limiting and the brain uses O_2 to oxidize endogenous carbohydrates, at least temporarily; hypoxia and (or) ischemia when glycolysis is activated by lack of O_2, resulting in a reversal of the Pasteur effect; aging for a variety of reasons, including cerebral vascular insufficiency with consequent relative ischemia and hypoxia; and, occasionally, convulsions in which steady states may not

exist and (or) O_2 resupply to the brain by the blood flow cannot keep pace with the enhanced demand, with resulting tissue hypoxia. These results were obtained by measurements of the O_2 and glucose concentrations in the arterial and cerebral venous blood; the molar ratio of their arteriovenous differences is equal to the ratio of their rates of utilization by the brain. The results are not, therefore, dependent on the uncertain validity of model-dependent assumptions and (or) measuring techniques; they are based on direct chemical measurements by established, highly precise and accurate analytical techniques.

Recent studies with ^{15}O and positron emission tomography (PET) at Washington University (Fox and Raichle 1986; Fox et al. 1988) have provided results that appear to disagree with the established doctrine. Activation of sensory or visual pathways in normal man was reported to raise local blood flow and CMR_{glc} almost proportionately in the appropriate cerebral cortical regions, with little increase in O_2 consumption. These results were interpreted as evidence that normal functional activity in the nervous system is supported by energy derived almost entirely from glycolysis and little, if at all, from the TCA cycle and oxidative phosphorylation. This conclusion, however, makes no biochemical sense. For example, it is not clear how, in the presence of adequate O_2, glycolysis can be selectively stimulated without O_2 consumption also being increased, inasmuch as the same intracellular changes associated with increased functional activity, e.g., increased levels of both phosphate acceptor (ADP) and inorganic phosphate activate both phosphofructokinase, the enzyme catalyzing the rate-limiting step in glycolysis (Passonneau and Lowry 1964; Krebs 1972), and electron transport in the mitochondrial respiratory chain (Krebs 1972; Lehninger 1975). In biochemical parlance the mitochondrial state would pass from state 4 to state 3 respiration in response to the same intracellular changes that activate glycolysis (Lehninger 1975). It is true that the sensitivity of phosphofructokinase to these intracellular changes and the maximal capacity of the glycolytic pathway are probably greater than those of the respiratory chain, and there may be dissociations between the time courses of the glycolytic and respiratory responses, e.g., the glycolytic preceding the oxidative response. The findings, however, of an almost constant stoichiometry of 5.0–6.0 between oxygen consumption and glucose utilization under steady-state conditions in a variety of functional states of the brain suggest that any dissociation between the two processes, if it does indeed occur, is generally temporary. Also, an increase restricted only to glycolysis would be very inefficient and would provide little additional energy compared with that which would be obtained if oxidative metabolism were proportionately increased; a 50% increase in glucose utilization restricted only to glycolysis would add only a 3% increase in ATP generation whereas, if oxidative metabolism were also increased, ATP generation would also be increased by 50%. Finally, acute complete hypoxia results in loss of consciousness almost immediately. Is consciousness not a reflection of functional activities in the brain? If it is, why is the O_2 essential and why cannot glycolysis support consciousness if it is the main energy source for neural functional activity?

Despite these disturbing questions, the findings reported from Washington University have made a major impact that was manifested in some of the comments made in the formal presentations and in the general discussion. What should be considered, however, before discarding the old established doctrine is the validity of the methods used to obtain these provocative new results. L. Sokoloff presented a detailed analysis of the basic principles of the ^{15}O-PET method for determination of regional $CMRO_2$ and pointed out some potential sources of error. This method (Mintun et al. 1984) is based on a very complicated theoretical model that itself is not unreasonable, but, like all models, is almost certainly an incomplete and oversimplified facsimile of the true biological system. Its implementation is difficult and dependent on a number of assumptions that have never been validated and are probably not appropriate for all, if any, experimental conditions, and at least one of the assumptions has not been adequately met. The method is based on the Fick principle, which in this context states that in a steady state the rate of oxygen consumption by the brain tissue is equal to the difference between the rates at which O_2 is delivered to and removed from the tissue by the cerebral circulation. This principle can be expressed mathematically as follows:

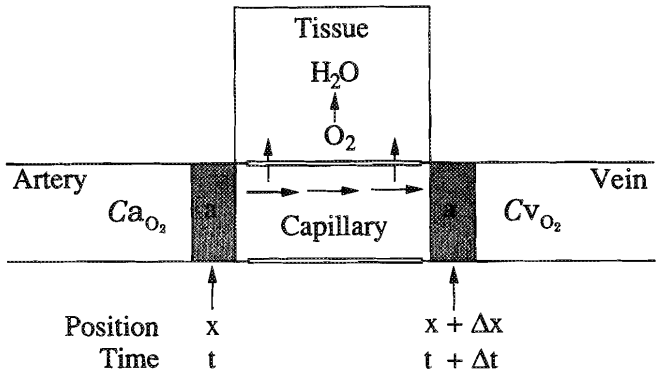

FIG. 1. Diagrammatic representation of O_2 exchange between tissue and blood during oxygen consumption by the tissue. The speckled areas marked with *a* represent the same element of blood as it enters the tissue at time t and leaves the tissue at time $t + \Delta t$, where Δt is the transit time through the capillary. The other symbols are defined in the text following eq. 1.

$$[1]\quad R_i = F\,Ca_{O_2}(t) - F\,Cv_{O_2}(t+\Delta t) = F\,(Ca_{O_2}(t) - Cv_{O_2}(t+\Delta t))$$

where R_i is the rate of oxygen consumption by tissue i in mL $O_2 \cdot g^{-1}$ tissue $\cdot$ min^{-1}; F is the rate of blood flow in mL blood $\cdot$ g^{-1} tissue $\cdot$ min^{-1}; $Ca_{O_2}(t)$ is the O_2 content in the arterial blood entering the tissue at time t in mL O_2/mL; $Cv_{O_2}(t + \Delta t)$ is the O_2 content in the same element of blood in mL O_2/mL at time $t + \Delta t$ min when it reaches the vein after traversing the capillaries; and Δt is the transit time for the element of blood to pass through the capillary from the arterial to venous end. These relationships are diagrammatically represented in Fig. 1.

Multiplying the right side of eq. 1 by $Ca_{O_2}(t)/Ca_{O_2}(t)$,

$$[2]\quad R_i = F\,Ca_{O_2}(t)\left(\frac{Ca_{O_2}(t) - Cv_{O_2}(t+\Delta t)}{Ca_{O_2}(t)}\right) = F\,Ca_{O_2}(t)\,E$$

where the oxygen extraction fraction =

$$E = \left(\frac{Ca_{O_2}(t) - Cv_{O_2}(t+\Delta t)}{Ca_{O_2}(t)}\right)$$

In a steady state Cv_{O_2} remains constant with time, and therefore, $Cv_{O_2}(t)$ becomes equal to $Cv_{O_2}(t + \Delta t)$. Therefore, in a steady state

$$[3] \quad E(\text{steady state}) = E(\text{ss}) = \left(\frac{Ca_{O_2}(t) - Cv_{O_2}(t)}{Ca_{O_2}(t)}\right)$$

and eq. 2 becomes

$$[4] \quad R_i = F\ Ca_{O_2}(t)\ E(\text{ss})$$

To calculate oxygen consumption by eq. 4, it is necessary to determine the steady-state oxygen extraction fraction, E(ss). In the absence of a steady state, the oxygen extraction fraction can vary because of the capillary transit time, Δt, as well as the fact that some of the O_2 taken up or released by the tissue may not be related to its utilization but rather to changes in the tissue pool of molecular O_2. In man the mean transit time in the brain is about 6 s; during this interval the oxygen extraction fraction would appear to be 100% before it declines to its true steady-state value.

The method for determination of $CMRO_2$ at Washington University is based on this valid principle, but there is a definite source of error introduced in their implementation of the determination of the E(ss). They fail to determine the oxygen extraction fraction in a steady state. Their procedure is essentially as follows. Regional cerebral blood flow is measured with $H_2{}^{15}O$ and PET by an established technique, and the oxygen extraction fraction is determined with $^{15}O_2$ and PET. $^{15}O_2$ used for energy metabolism is incorporated into $H_2{}^{15}O$; the total amount of $H_2{}^{15}O$ produced in a region of interest over an interval of time, therefore, equals the total amount of $^{15}O_2$ extracted from the blood and used for energy metabolism during that interval. From eq. 2

$$[5] \quad d(H_2{}^{15}O)(t)/dt = R_i = F\ Ca(^{15}O_2)(t)\ E$$

where $d(H_2{}^{15}O)(t)/dt$ is the rate of formation of $H_2{}^{15}O$ in region i at time t.

Integrating eq. 5 and transposing,

$$[6] \quad E = \frac{(H_2{}^{15}O)(T)}{F \int_0^T Ca(^{15}O_2)(t)dt}$$

where $(H_2{}^{15}O)(T)$ is the total amount of $H_2{}^{15}O$ that had been formed in region i in the interval from time zero to the end of the measurement at time T.

From the measured regional value of F, the measured time course of the $^{15}O_2$ concentration in arterial blood, and the value determined for $(H_2{}^{15}O)(T)$ with PET, E for region i is calculated. R_i is then calculated as the product of this value of E, the measured blood flow, F, and the measured concentration of O_2 in the arterial blood according to eq. 1. The E, however, is determined with a PET scan of 40-s duration following a pulsed input of $^{15}O_2$. This is much too short a time for a steady state for E to be reached. First of all, the mean capillary transit time, during which the E would appear to be 100%, alone takes up about 6 s of the total 40 s of the scan. Secondly, the $^{15}O_2$ content in the arterial blood is changing rapidly during the 40 s, preventing the establishment of a steady state. The errors introduced by these factors are variable with the functional state. For example, during functional activation blood flow is increased, and the capillary transit time is therefore decreased. This would reduce the error owing to the transit time effect, lower the value for E, and operate against the finding of an increased $CMRO_2$ during functional activation.

There are other less obvious problems associated with the method for the determination of regional $CMRO_2$ with PET. The method is based on a model that requires a number of assumptions that may appear to be reasonable but are difficult to validate under all conditions. No practical model is comprehensive enough to account for all the vagaries of a complex biological system, and even if it is adequate for one set of conditions it may not be for altered functional states. Furthermore, the determination of $CMRO_2$ requires computations based on three separate PET scans: (*i*) an $H_2{}^{15}O$ scan to determine blood flow, (*ii*) a $^{15}O_2$ scan to determine the $H_2{}^{15}O$ formed in the tissue from the $^{15}O_2$, and (*iii*) a $C^{15}O$ scan to determine regional hemoglobin contents, which are used to calculate the amount of ^{15}O contained in the blood in the regions of interest. The ^{15}O bound to the hemoglobin is subtracted from the total measured amount of ^{15}O to derive the amount of $H_2{}^{15}O$ in the region of interest, and this correction is substantial and based on as yet unverified assumptions about the arteriovenous gradient for $^{15}O_2$ down the capillary and the relative proportions of arterial, venous, and capillary blood in the region of interest. These are relationships that are certain to change from one functional state to another. Finally, the amount of $H_2{}^{15}O$ in the tissue region of interest, calculated by subtracting from the total measured ^{15}O the contaminating amount of hemoglobin-bound ^{15}O in the blood in the tissue, is still not the total amount of $H_2{}^{15}O$ that was formed during the 40-s scan; it must be corrected for both the amounts of $H_2{}^{15}O$ produced by $^{15}O_2$ metabolism elsewhere in the body and delivered to the brain by the cerebral circulation and for the $H_2{}^{15}O$ that was removed from the brain by the circulation over the course of the experimental period. These corrections are computed from the blood flow determined with the $H_2{}^{15}O$ scan and the measured time courses of the arterial and tissue $H_2{}^{15}O$ concentration during the $^{15}O_2$ scan. The final value for $CMRO_2$ is computed from the results of the three scans, each with its own peculiar errors that can be propagated and compounded, and various corrections based on a variety of untested and hypothetical assumptions. It would seem that the provocative conclusion that energy derived from oxidative metabolism contributes little, if anything, to the support of functional activity in nervous tissue that is based on results obtained with such a method and is at variance with years of prior experience is, at the very least, premature.

Not all were convinced by these arguments, and the question was raised by several whether the finding of increased lactate levels during functional activation was not, in fact, evidence that glycolysis was enhanced without a concomitant proportionate increase in oxidative metabolism. L. Sokoloff replied in the negative and pointed out that the rates of both glycolysis and oxidative metabolism were represented by the fluxes through these pathways and that the concentrations of intermediates in these pathways were not sufficient to describe the rates of flux. In fact, Sokoloff argued, a rise in lactate levels was to be expected. It was the obvious consequence of the location of the sites of the regulated rate-determining steps in the pathways. Because glucose is the substrate for the brain's oxidative metabolism, energy and oxidative metabolism cannot be increased without there first being an increase in glycolysis and more rapid production of pyruvate, the product of glucose metabolism that enters the TCA cycle. The pyruvate concentration then rises to a new steady-state level before

oxidative metabolism can rise proportionately to the increased rate of glycolysis. The rate-limiting step in glycolysis is the phosphofructokinase-catalyzed phosphorylation of fructose-6-phosphate. This is a so-called "crossover point" in the glycolytic pathway, and when this step is accelerated (which must occur for glycolysis to be increased) all intermediary metabolites preceding this step are decreased and all intermediates following this step are increased. It is analogous to the opening of the sluices in a dam; the water levels ahead of the dam recede, and the water levels downstream rise. Pyruvate levels must, therefore, rise when glycolysis is increased, and so then must also the lactate levels because lactic dehydrogenase activity in brain is great enough to produce very rapid equilibration between lactate and pyruvate, with the equilibrium constant favoring lactate. Furthermore, the rises in lactate level associated with physiological activation of functional activity are far too low to be accounted for by the magnitude of the rises in glycolysis associated with the observed increases in glucose utilization without invoking either increased oxidation of pyruvate and lactate or else their rapid removal from the brain by the circulation. The former possibility is incompatible with the claim of little if any rise in oxygen consumption associated with functional activation (Fox and Raichle 1986; Fox et al. 1988); the latter is unlikely in view of the limited blood–brain barrier transport of these compounds (Pardridge and Oldendorf 1977).

Sources of acid-equivalents responsible for the acidosis in hypoxia

There was a spirited discussion concerning the chemical pathogenesis of the acidosis in cerebral tissue that develops during hypoxia. M. Ereciñska and B. Siesjö expressed contrary opinions. The former argued that the acidosis was due to protons liberated by ATP hydrolysis and not lactic acid formation. The latter attributed the acidosis to the increased anaerobic glycolysis and lactate production. The issue was debated at length, but there was no apparent resolution of the difference during the discussion. On further thought, however, it seems that the difference may be more apparent and semantic than real. Glucose is a neutral compound, and during glycolysis one molecule of glucose is converted to two molecules of pyruvate or lactate, a process that produces and adds two acid-equivalents to the H^+ pool in the tissue. As Ereciñska pointed out, however, glycolysis also generates two molecules of ATP from two molecules each of ADP and inorganic phosphate, a process that withdraws two acid-equivalents from the H^+ pool. The net result is, in effect, that only the anions of pyruvate and lactate are produced without any corresponding net production of H^+. Under normal aerobic conditions the pyruvate and lactate are oxidized eventually to CO_2 and H_2O by the TCA cycle. The ATP is constantly being hydrolyzed by energy-requiring processes but replenished and maintained by both glycolysis and oxidative phosphorylation, mainly the latter. Therefore, as long as the ATP level is maintained in a steady state, glycolysis per se does not cause a net change in H^+ concentration and is neutral with respect to pH (Krebs et al. 1975; Alberti and Cuthbert 1982; Hochachka and Mommsen 1983; Busa and Nuccitelli 1984). In hypoxia, however, oxidative phosphorylation is depressed, glycolysis cannot itself fully satisfy the energy demands, and ADP phosphorylation cannot keep pace with the rate of ATP utilization. There is a net conversion of ATP to ADP that releases one H^+ ion per ATP converted to ADP. In fact, it is because the ATP level cannot be maintained that the rate-limiting phosphofructose kinase step is activated and the rate of glycolysis increased in hypoxia. The glycolytic pathway then becomes essentially free running because the normal inhibition of phosphofructose kinase activity by rising ATP/ADP ratios is lost. ADP may also be further degraded to AMP by the action of adenylic kinase, and this hydrolysis releases an additional H^+ ion into the pool. In anaerobic glycolysis two H^+ ions are still added to the H^+ pool in the tissue by the conversion of one molecule of glucose to two molecules of lactate, and two H^+ ions are still removed from the pool by ATP formation, but the latter two are returned to the pool because the ATP level is not maintained. Therefore, as long as the anaerobic glycolysis continues, there is net accumulation of both H^+ and lactate as though both were produced together, and tissue acidosis ensues. It is the combination of the two, anaerobic glycolysis and failure to maintain the ATP level, that is responsible for the tissue acidosis. Whether the H^+ ions come from ATP hydrolysis or lactic acid formation may be moot.

Summary

The general discussion was spirited and addressed the issues in depth. Issues given most time and attention were properly those that were most controversial, at least among the participants at the meeting. Some of the controversy may have been a consequence of the multidisciplinary composition of the interests of the participants, and some of the controversial issues themselves may be less so in their own particular fields and disciplines. Some of the differences may have been resolved; most were probably not. Nevertheless, the educational value of the discussions, particularly for those not directly in the discipline or expert in the subject of discussion, was enormous. All gained knowledge from the experience.

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