Review Article

Mechanisms of Glycolysis and Fermentation: A Non-Equilibrium Thermodynamics Perspective

Dirk Roosterman[1,2], Graeme S. Cottrell[3]

1. Ruhr Universität Bochum, Germany; 2. Independent Scholar, Germany; 3. School of Pharmacy, University of Reading, United Kingdom

Every single chemical formula of modern models of glycolysis violates two laws of nature. Yet, the formulae of the pioneers who investigated metabolism did not violate the laws of nature. Recently, the well-established models of metabolism have collapsed by re-introducing hydrogen as the energy entity driving biological processes. This review builds on a scientific concept of metabolism by introducing that glycolytically generated energy is either transformed into ATP or drives a biological process. The dynamic production and utilization of lactate (lactate flow non-equilibrium) is introduced as a central ATP-driven process and the first step of biosynthesis. A metabolism model based on non-equilibrium thermodynamics replaces the current understanding that one end product of glycolysis is consumed by mitochondria with two intermediates of the two-cell model of metabolism that are consumed by mitochondria. The pyruvate dehydrogenase complex, consuming pyruvic acid, saves one redox unit (2H) for storage as lipid or glycogen, whereas mitochondrial consumption of lactic acid enhances ATP recovery. An uncounted number of signalling pathways temporarily regulate the distribution of this single redox unit. Glycogenolysis massively impacts the flow non-equilibrium, an event permanently memorized by cells. The two-cell model of metabolism starts to functionally unite fields such as memory formation, obesity, exercise, schizophrenia, cancer, and inflammation by the common denominator: metabolism.

Introduction

Equilibrium thermodynamics, the 2nd law of thermodynamics, provides the understanding that an apple falls on Earth and Earth falls on an apple[1]. Non-equilibrium thermodynamics, the 4th law of

 doi.org/10.32388/Y9K21X

thermodynamics, has to provide an understanding of how the apple has reached flow non-equilibrium with Earth in order to fall down. Non-equilibrium thermodynamics is the Physics of Biology.

Maxwell's observation of the dissipation of energy in nature guided Thomson to the formulation of the thermodynamic demon hypothesis in 1879[2]. The hypothesis implies that thermodynamic demons carry apples on trees to let them drop on physicists' heat. Physicists have hunted the demons for more than a century, in vain. Recently, the demon hypothesis was updated to the zombie-hypothesis: Maxwell's zombies mulling and mauling the 2nd law of thermodynamics[3]. The undead are open systems; they eat and drink. Open systems do not fall under the jurisdiction of the 2nd law of thermodynamics. The undead metabolize and temporarily retain the gained energy by transferring the energy to a more stable energy particle. A living organism is an open system in steady state flow non-equilibrium with the environment and falls under the jurisdiction of the 4th law of thermodynamics.

Physicists have developed a comprehensive working plan to understand life. The aim of biochemistry is to provide physicists with the name of the energy particle (demon, zombie).

Today, in Biology, it is known that apple trees transform sunshine into the fuel glucose and glycose is burned to water, carbon dioxide, and apple. The chemistry of the process burning to water, carbon dioxide, and ~ $-\Delta G$ 2800 kJ/mol is known. Apple trees do not emit the energy of ~ $-\Delta G$ 2800 kJ/mol. The biochemistry of metabolic burning of water, carbon dioxide, and apple is unknown. The historical name of the thermodynamic demon is *Phlogiston*, which is emitted when substances are burned[4]. The Phlogiston cult was replaced by the formulation of the law of the conservation of mass. Thus, if the demon has a molecular mass, then *Phlogistron* is hidden in the chemical formulae of glycolysis and fermentation. In open systems, *Phlogistron* turns into a zombie. In vivo, *Phlogistron* is generated during the metabolic burning or oxidation ($NAD^+ \rightarrow$ NADH-H^+-system) of glucose and moves to a defined coupled biological process. The coupled biologic process retained energy from emitting as heat by transforming energy to a temporarily more stable energy particle. The thermodynamic zombie vanishes from chemical formulae during transformation.

Chemical formulae of the metabolic burning of glucose show carboxylic acids (R-COOH), even if it is known that acids promptly dissociate in water to anion ($R\text{-}COO^-$), proton (H^+), and heat[5]. Now consider the kinetics of a proton-linked Monocarboxylate Transporter (proton-linked MCT): 1st, a proton (H^+) binds; 2nd, an anion (R-COO-) binds; and the charge-neutral acid (R-COOH) moves through the membrane[6]. The kinetics is in line with the 3rd law of thermodynamics, the law of

motion. First energy, second reaction. Thus, H^+ is the energy particle freed during burning (NADH-H^+), the energy particle that freely dissociates (emits) in water, and the energy particle that initiates and guides unidirectional movement in biological processes.

Enzymes catalysing the burning of glucose are organized in complexes or metabolons[7][8]. Enzyme complexes, such as the pyruvic acid dehydrogenase complex (PDHc), lactic acid consuming Citric Acid Cycle complex, and the mitochondrial ATP-synthase complex, have in common that the intermediates are acids (figure 1).

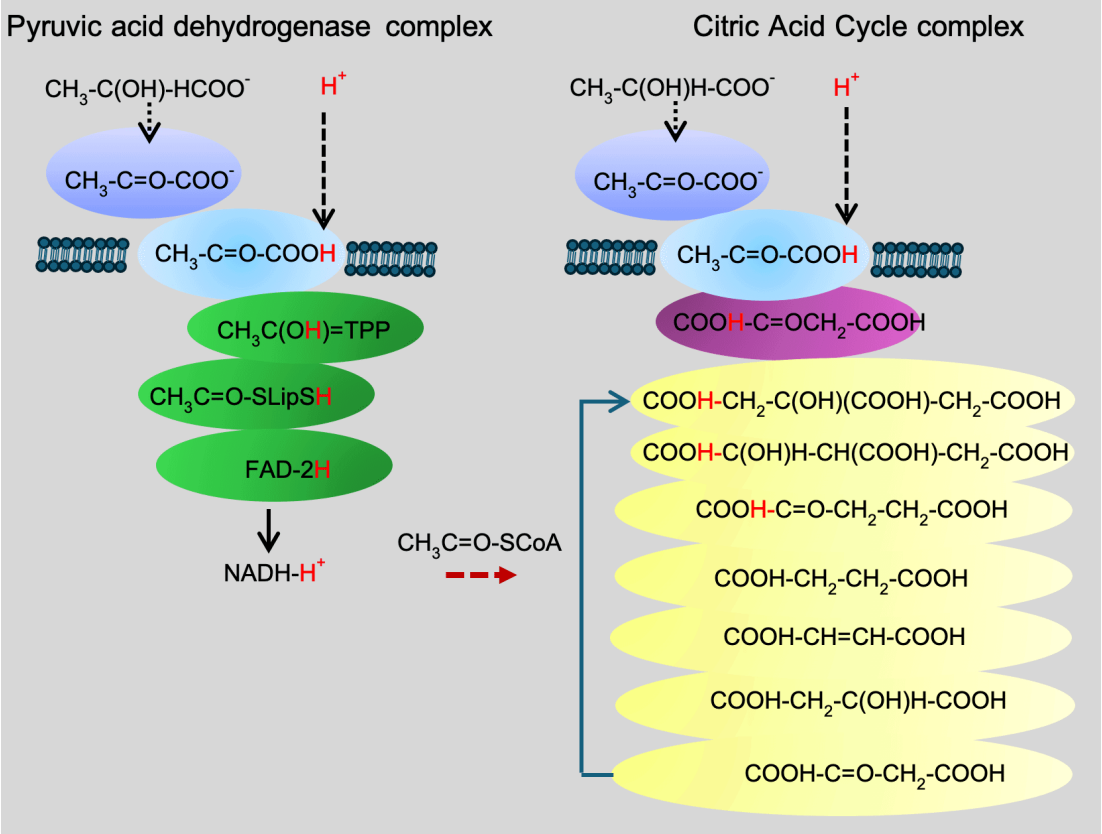


Figure 1. Mitochondrial consumption of lactic acid. Mitochondrial lactate dehydrogenase metabolically burns lactate (CH_3-C(OH)H -COO^-) to pyruvate (CH_3-C=O-COO^-). Proton-linked monocarboxylate transporter 1 transfers pyruvic acid (CH_3-C=O-COOH) to (left) Pyruvic Acid Dehydrogenase complex or (right) pyruvic acid carboxylase. Pyruvic Acid Dehydrogenase complex metabolically burns pyruvic acid to acetyl-SCoA and NADH-H^+[9][10]. Pyruvic acid carboxylase refiles the attached Citric Acid Cycle complex with oxaloacetic acid (COOH-C=O-CH_2-COOH)[11][12][13][7].

doi.org/10.32388/Y9K21X

This review introduces that the first intermediates of the glycolytic metabolon are acids. The Proton Transport Chain hypothesis states that intermediates are directly or water-free transferred within complexes to save dissociation heat for the coupled biological process[14]. First, the proton is transferred to the coupled enzymatic process; 2nd, the anion follows. In 1966, the flow of the energy particle proton (H^+) through mitochondrial ATP synthase was named protecity[15]. The biochemistry of enzyme complexes indicates that enzyme complexes are bio-wires (figure 2).

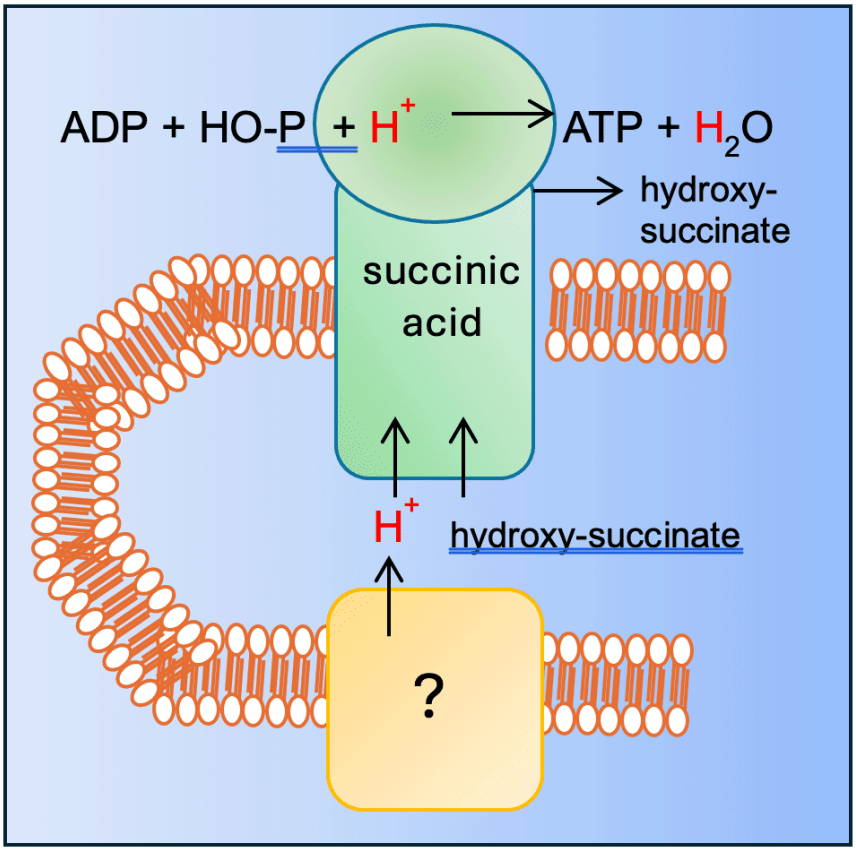


Figure 2. Mitochondrial ATP synthase. Proton (H^+) and hydroxy succinate form succinic acid. Charge-neutral succinic acid is wired through the enzyme complex. ATP-synthase catalyses the transfer of energy from unstable succinic acid to the temporarily more stable *tri*-phosphoric acid anhydride group of ATP. The proton (H^+) is discharged to water[15].

Enzymes stabilize their substrate water-free within the active site. The substrate is solved by the enzyme complex, not by water. The physical quantity [mol/L] is applied to calculate an enzymatic

catalysed reaction of a thermodynamic equilibrium. Direct or water-free transfer of an intermediate within an enzyme complex is mathematically one molecule in zero Litre of water. 1 molecule of acid divided by 0 L of water is an infinite molarity. An infinite molarity of one product changes the understanding (Physics and Mathematics) of a chemical process into the understanding of the biochemical process of an irreversible metabolic flow.

One enzyme catalyzes 1 molecule at a time. Molecule/time is the physical quantity of a flow. Acid/time is the physical quantity of the flow of the energy entity H^+ and material (anion) through enzyme complexes.

In ecology, the tentative 4^{th} law of thermodynamics states: a flow of energy and material is sufficient to form ordered structures[16]. In this case, we suggest calling the thermodynamic zombie, proton (H^+).

The biochemistry of the proton-linked MCT_1-Carbonic Anhydrase II complex, located at the cell membrane, is best visualized as an 'ordered structure' in a state of flow non-equilibrium. Carbonic Anhydrase II activity is located outside the cell (Figure 3). At time point 0, the environment and the cell are in lactate equilibrium. Carbonic Anhydrases contain zinc (Zn^{2+}), which is a Lewis acid in their active sites. Zn^{2+} reacts with environmental water to initiate the Proton Transport Chain: $Zn^{2+} + H_2O \diamond Zn^{2+}[OH^-] + H^+$[17].

Thus, firstly, the energy particle H^+ taken from water is water-free transferred to the proton-linked MCT_1; secondly, lactate binds and lactic acid moves into the cell.

The cell continuously emits carbon dioxide (the acid anhydride of carbonic acid). This emission restores the activity of Carbonic Anhydrase II, following the reaction: $Zn^{2+}[OH^-] + CO_2 \rightarrow Zn^{2+} + HCO_3^-$. The flow of CO_2 permanently restores Carbonic Anhydrase II activity. This process generates a protecity that pumps lactic acid from the environment into the cell, causing the cell to enter a state of lactate flow non-equilibrium with its surroundings.

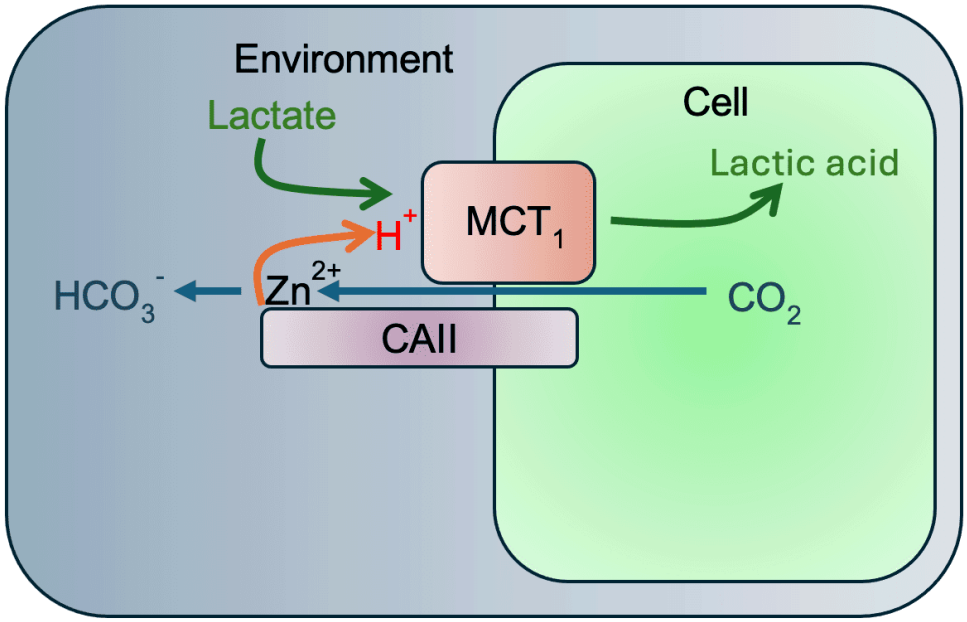


Figure 3. Biochemistry of the proton-linked MCT_1 (MCT_1) Carbonic Anhydrase II (CAII)-complex. In chemistry, CAII catalyzes the hydration of carbonic dioxide (CO_2) to carbonic acid (H_2CO_3)[18]. In biochemistry, CAII participates in the flow of energy and material. The proton-linked MCT_1-CAII complex transfers the power of permanently emitting CO_2 to generate a cellular lactic acid flow non-equilibrium.

The path the authors have taken to this review started with the formulation of the Proton Transport Chain hypothesis and is documented in five reviews[19][7][20][21][14]. This review discusses the chemical formulae of glycolysis and fermentation published by Meyerhof[5]. Meyerhof's review is the most recently published concept of glycolysis and fermentation not violating the law of the conservation of mass that we have found.

The understanding of Chemistry, the 2nd law of thermodynamics, guided scientists to define lactic acid as the exported end product of fermentation and lactate as the end product of glycolysis[22][23]. This review updates the chemistry of glycolysis and fermentation to the biochemistry of glycolysis and fermentation. In line with the 4th law of thermodynamics, the path of the energy entity proton within the glycolytic metabolon was followed.

We assume this review presents the first scientific (in line with the laws of nature and published pioneer work) integration of the NAD^+/NADH-H^+ system and ATP in glycolysis and fermentation[5].

doi.org/10.32388/Y9K21X

Understanding the *tri*-phosphoric acid anhydride group of ATP as the storage of the energy of a proton allowed us to introduce lactate as the first ATP-driven biosynthesis product. The glycolytic flow of energy and material generates a cytosolic lactate flow non-equilibrium. The generation of this cytosolic lactate concentration gradient enables the mitochondrial lactate dehydrogenase-proton-linked MCT_1 complex (figure 1) to catalyse the metabolic burning of lactate to pyruvate and $NADH-H^+$ and the membrane transfer of pyruvic acid to the membrane-associated PDHc and Citric Acid Cycle complex.

Finally, the first part of the apple is on the tree. The energy particle (H^+) freed during the hydrolysis of ATP: ATP + H_2O ♢ $ADP-H^+$ + HO-P does not chaotically emit, heat up, and acidify the cell but is wired from hexokinase to lactate dehydrogenase to generate a cytosolic lactate flow non-equilibrium. Lactate, understood as the first biosynthesis product, falls into mitochondrial degradation to be metabolically burned to pyruvic acid and $NADH-H^+$. Thus, 85 years after the formulation of the lactic acid-consuming Citric Acid Cycle, Krebs' work is rationally linked with glycolysis[11][12][24]. A century after the discovery of the $NAD^+/NADH-H^+$ system and ATP, the co-enzymes are finally scientifically (in line with the laws) integrated into glycolysis and fermentation.

History of the Two-Cell Model of Metabolism

F. Cori: "Some 50 years ago - in 1929, to be exact - we proposed a cycle of the glucose molecule which could be in turn liver glycogen, blood glucose, muscle glycogen. The conversion of lactic acid to liver glycogen would complete the cycle"[25][26]. The Cori cycle was formulated in the year K. Lohmann discovered ATP[27]. At this time, the citric acid cycles and thereby mitochondrial participation in biosynthesis and ATP recovery was undiscovered. F. and G. Cori determined that insulin accelerates the Cori cycle from liver to muscle glycogen, while epinephrine accelerates the cycle in the opposite direction[25]. Today, it is known that other organs such as blood (thrombocytes, macrophages, T-cells) or brain (astrocytes) also contain glycogen. The Cori cycle developed into the two-cell model of metabolism[20].

Cells memorize the event of glycogenolysis[28][29][30]. Astrocytic glycogenolysis, or more specifically the astrocyte neuron lactate shuttle hypothesis (ANLS), was introduced as energy on demand. Briefly, memory formation is triggered by a boost of fuels directed from astrocytes to neurons. More recently, the ANLS hypothesis was interpreted as astrocyte neuron communication (ANC)[19]. ANC shifted the

biological function of glycogenolysis from fuel to a signalling pathway and added pyruvic acid as a metabolic messenger to the two-cell model of metabolism. Glycogen was introduced as storage of the metabolic signalling molecules glucose, lactic acid, and pyruvic acid. Transmitters, such as insulin and epinephrine, temporarily adapt the steady-state flow non-equilibrium between two cells according to environmental changes[25][26]. The steady-state lactate/pyruvate flow ratio in the liver is approximately 7/1, whereas the steady-state lactate/pyruvate flow ratio in resting muscle is approximately 12/1 and 159/1 in working muscles[31][32]. Transmitters triggering glycogenolysis massively impact the cytosolic lactate/pyruvate flow ratio. The temporary imbalance is memorized by changes in expression levels of enzyme isoforms and enzyme subtypes in a way leading to a permanent acceleration of the flow of metabolic messengers[33].

The two-cell model of metabolism also comprises cell adhesion proteins physically stabilizing cell-cell communication[20]. The two-cell model of metabolism has given D.O. Hebb`s `reverberatory activity´ the molecular background of glucose metabolism[34]. Our approach has opened avenues by linking the scientific fields of memory formation and schizophrenia via a functional concept of metabolism. Genetic and environmental factors statistically associated with schizophrenia are candidates to be sorted into the two-cell model of metabolism. Predisposition to schizophrenia was functionally linked to instability of the physical interaction between astrocytes and neurons. Pronounced leakage of fuels into the interstitial fluid during stress-triggered astrocytic glycogenolysis was linked to acute schizophrenia[35]. Leaked glucose is a signalling molecule of inflammation, an activator of microglia cells, and triggers the release of inflammatory messengers[20]. Thus, glucose metabolism is the common denominator of all biological processes.

Unfortunately, all of the above cannot be rationally followed on a molecular level. As already stated at the beginning of the review, the synthesis, export, import, and mitochondrial consumption of lactic acid have been under investigation for more than a century. Today, however, the well-established chemical formulae of glucose metabolism violate the law of the conservation of mass, as well as published pioneer work. The chemical formulae no longer provide the obligatory proton (H^+) to synthesize lactate and the obligatory proton (H^+) to export lactic acid (lactate-H^+). Whereas the synthesis, export, and import of lactic acid have been under investigation for more than a century[36][37], the molecular mechanisms behind “energy for storage as glycogen” and “energy on demand” lie far outside common understanding. *In vivo*, a single cell simultaneously synthesizes lactate from

pyruvate and metabolically burns lactate to pyruvate. Mitochondrial consumption of lactate entails that the mitochondrial LDH-proton-linked MCT_1 complex unidirectionally catalyses the reaction aiming to equalize a cytosolic lactate flow non-equilibrium[38]. Whereas LDH anchored with the glycolytic metabolon must unidirectionally generate a cytosolic lactate flow non-equilibrium (biosynthesis) to enable mitochondrial consumption of lactate.

Two LDH isoforms simultaneously acting in opposite directions are introduced in the context of memory formation and formulated in the ANLS hypothesis. Nevertheless, the understanding behind the ANLS hypotheses/*in vivo* metabolism lies far outside common understanding because it is well-established that the isoforms of LDH catalyse the identical thermodynamic equilibrium[39]. Therefore, it is well-established that we do not understand biosynthesis or the generation of a cytosolic lactate flow non-equilibrium on the basis of equilibrium thermodynamics (enzyme kinetics). Nevertheless, *in vivo*, lactate is simultaneously synthesized, exported, imported, and degraded[37][23]. Biology is obsessed with thermodynamic demons. These demons are known to have the ability to stop, strike, push, or pull any lactate molecule at will, thereby altering its natural course of motion[2].

This review continues to challenge an understanding based on equilibrium thermodynamics and enzyme kinetics by developing a flow non-equilibrium thermodynamics-based concept of metabolism. "Equilibrium is an inert state of death; here the flow of matter and energy through a biological system stops, and the system reaches a lifeless state of thermodynamic equilibrium"

[40]. In other words, equilibrium thermodynamics and enzyme kinetics are the path to understand lifeless material. The mathematics of equilibration thermodynamics is well-established from basic enzyme kinetics; isolated enzymes in homogenous and closed systems catalysing the identical lifeless state of a thermodynamic equilibrium. Isolated enzymes are dead material acting in line with the 2^{nd} law of thermodynamics.

Our approach to beginning to understand the dissipation of the energy particles in nature[2] is to open the reference of glycolysis/fermentation, published by O.F. Meyerhof in 1951[5].

Methods

Meyerhof's Scheme 1 is the peak of knowledge and understanding of the chemistry of glycolysis and fermentation and the scientific reference for glycolysis and fermentation[5]. Meyerhof understood that the 2^{nd} law of thermodynamics excludes the storage of energy as ATP; he also understood that the

law of the conservation of mass excludes that 1 H^+ is both used to generate ATP ($(ADP + P\text{-}OH)^- + H^+ \rightarrow ATP + H_2O$) and exported as lactic acid ($lactate\text{-}H^+$). Meyerhof's understanding of science prevented him from integrating ATP (discovered 1929) and NAD^+ (discovered 1906) in Scheme 1[41][27][15]. Neither ATP nor the $NAD^+/NADH\text{-}H^+$ system can be scientifically integrated into a concept based on equilibrium thermodynamics. Consequently, all models of glycolysis showing ATP are alchemistic inventions not supported by the law of the conservation of mass, the 2nd law of thermodynamics, and published pioneer work on glycolysis and fermentation.

Set theory: the one and only common intersection of the chemical formulae presented by Meyerhof and actual glycolysis inventions is glucose. Subduction of the atoms of glycolytic intermediates from the scientific reference indicates the atoms not currently accounted for or recognized in the current models of glycolysis and fermentation. In modern models of glycolysis, all protons (H^+) have been deleted from chemical formulae.

Some readers will encounter problems following scientific publications. Nomenclature clearly differentiates between carboxylate ($R\text{-}COO^-$) and carboxylic acid (R-COOH). Deleting all protons from chemical formulae entails that the nomenclature of basic molecules has turned into a matter of discussion. For example, illustrations of the Citric Acid Cycle commonly show citrate as an intermediate. Common understanding usually uses carboxylate as a synonym of carboxylic acid. The difference between citric acid and citrate, pyruvate and lactic acid, as well as gold and lead, are three protons. Moreover, didactic models of glucose metabolism shorten Scheme 1 by setting pyruvate as the end product. Deleting synthesis, export, import, and mitochondrial consumption of lactic acid from common understanding deletes the key molecule of the Cori cycle and the Citric Acid Cycle complex. This review re-introduces lactate and lactic acid in so-called aerobic glycolysis.

Changes to the original concept, such as replacing phosphoric acid[5] with ATP, entail that 1 glycolytically generated H^+ must be removed from the chemical formula of the metabolic pathway. Meyerhof understood that the integration of ATP necessitates changing the end product from lactic acid to lactate. Stoichiometry dictates that 1 H^+ is either exported as lactic acid or stored as ATP. Pioneers were aware that the chemical mechanisms of fermentation are nearly completely understood and just the fate of some protons remained unexplainable[42].

Notably, pioneers knew that acids rapidly dissociate in water, but Meyerhof's Scheme 1 shows a Proton Transport Chain starting with undissociated 3-(dihydroxy)-phosphoglyceric acid and ending

with the export of lactic acid[5]. The law of the conservation of mass dictates that the hydrolysis of the acid anhydride 1,3-diphosphoglyceric acid provides the acid 3-(dihydroxy)-phosphoglyceric acid. Pioneers experimentally demonstrated that the glycolytic metabolon protects the acidic intermediates from dissociation. Pioneers chemically blocked the glycolytic flow to determine the glycolysis rate via the acidification of the environment by the released 3-phosphoglyceric acid[43].

Let us consider the state of knowledge and understanding in 1951[5] and the current state of knowledge and understanding[44].

Meyerhof understood:

$$H_2PO_3\text{-O-CO-C(OH)H-CH}_2\text{-O-PO}_3H_2 + H_2O \rightleftharpoons H_3PO_4 + \text{COOH-C(OH)H-CH}_2\text{-PO}_3H_2$$

1,3-(dihydroxy)-diphosphoglyceric and water are in equilibrium with phosphoric acid and 3-(dihydroxy)-phosphoglyceric acid.

Today:

$$\text{P-O-CO-C(OH)H-CH}_2\text{-O-P} + \text{ADP} \rightleftharpoons \text{COO}^-\text{-C(OH)H-CH}_2\text{-O-P} + \text{ATP}$$

1,3-bisphosphoglycerate and ADP are in equilibrium with 3-phosphoglycerate and ATP.

Our approach glycolysis:

$$^-HPO_3\text{-O-CO-C(OH)H-CH}_2\text{-O-PO}_3H_2 + \text{ADP} \longrightarrow \text{ATP} + \text{COOH-C(OH)H-CH}_2\text{-PO}_3H_2$$

1-(monohydroxy), 3-(dihydroxy)- diphophoglyceric acid and ADP are unidirectionally metabolized to ATP and 3-(monohydroxy)-phosphoglyceric acid.

Our approach biosynthesis:

$$^-HPO_3\text{-O-CO-C(OH)H-CH}_2\text{-O-PO}_3H^- + \text{ADP} \longleftarrow \text{ATP} + \text{COO}^-\text{-C(OH)H-CH}_2\text{-PO}_3H^-$$

1,3-(monohydroxy)-diphophoglyceric acid and ADP are unidirectionally synthesised from ATP and 3-(monohydroxy)-glycerate.

Depending on the individual point of view, the modern formula is either illustrated as an equilibrium or a unidirectional process. The phospho-groups are more commonly illustrated as two-times negatively charged (all protons are deleted) than yellow dots. A common feature of modern models is the change in nomenclature of 1,3-diphosphoglyceric acid.

Our approach physically separates the pathways of glycolysis from cytosolic biosynthesis and chemically differentiates between the acidic glycolytic intermediates and the pH-neutral cytosolic pool of carboxylates.

The aims of this review are to (i) determine the glycolytic reactions generating the energy particle proton (H^+), (ii) trace the flow of protons driving the reduction of pyruvate to generate a lactate flow non-equilibrium, (iii) trace the proton flow initiating monocarboxylic acid export (fermentation), and

(iv) trace the flow of energy and material storing the energy of the particle (H^+) as a temporarily stable glycolytic steady state ATP flow non-equilibrium (glycolysis).

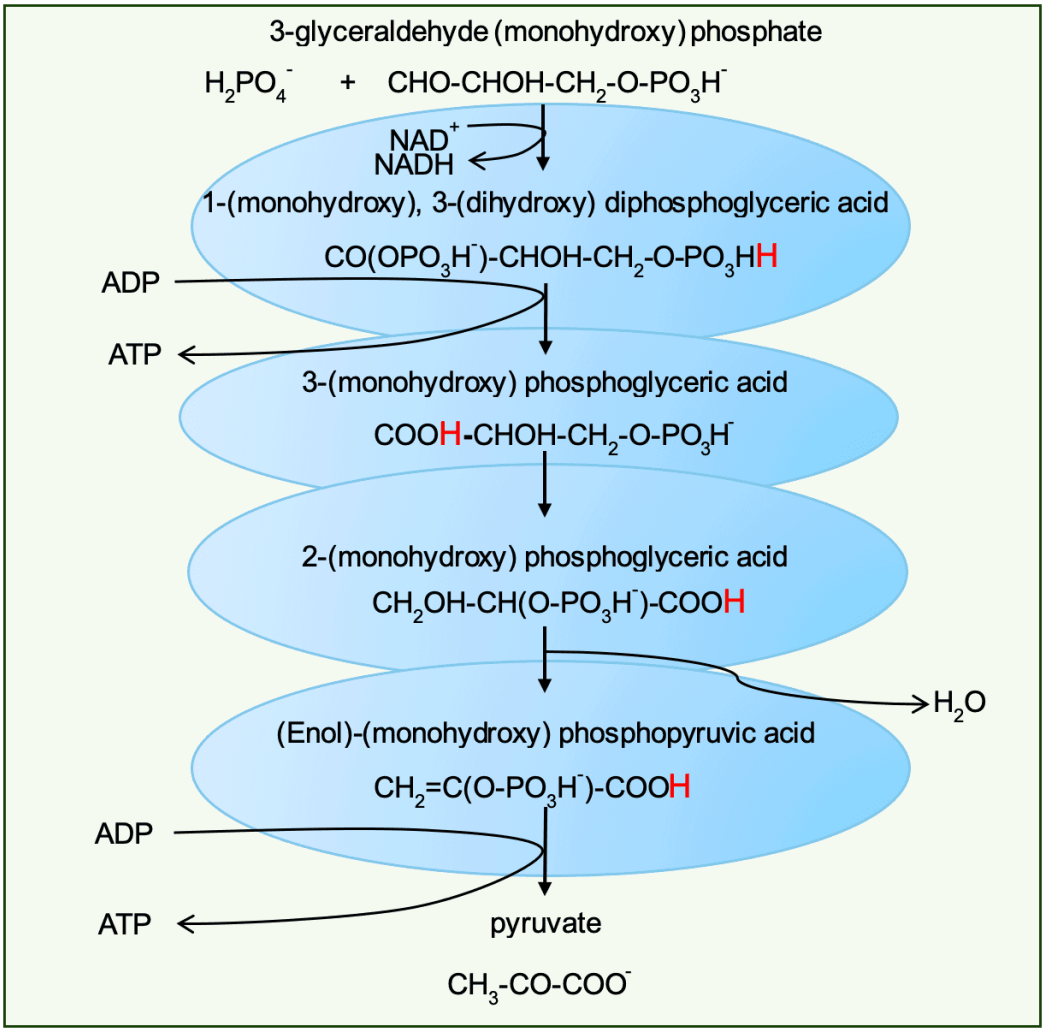
S.E. Jørgensen formulated the tentative 4^{th} law of thermodynamics as: a flow of energy and material is sufficient to form ordered structures[45][16]. The 4^{th} law of thermodynamics is best understood in its negation: the energy entity H^+ generated in a homogenous solution chaotically emits. Chaotically emitted protons (H^+) acidify the cytosol, maximize reaction heat, and maximize chaos, but not one synthesis step is driven by wasted energy. Tracing the flow of the energy particle H^+ from generating to consuming enzymatic reactions must reveal evolutionarily formed ordered structures, such as the glycolytic metabolon and the cytosolic lactate flow concentration gradient (biosynthesis).

The amalgamation of the law of the conservation of mass and the 4^{th} law of thermodynamics constitutes the principles guiding the scientific domain of biochemistry. The authors' interpretation and nature's application of natural laws may vary. Hence, we recommend seizing the opportunity to align the laws of nature with the individual well-established model of metabolism. Do all chemical formulas of well-established glycolysis genuinely fail to adhere to stoichiometry? Does glycolysis generate 2 protons twice, aiming to store the energy as a steady state ATP flow non-equilibrium?

Integration of ATP into the original Embden-Meyerhof-Parnas pathway

Glycolysis produces 2 H^+ twice for storage as two sets of 2 ATP. The initial H^+ generated by the glycolytic flow is stored as ATP through the reaction catalysed by a phosphoglycerate kinase isoform (PGK) unidirectionally acting as 1-(monohydroxy), 3-(dihydroxy)-diphosphoglyceric acid ATP synthase when integrated into the glycolytic metabolon. Meyerhof depicted 3-glyceraldehyde-(dihydroxy)-phosphate as the substrate of oxidative phosphorylation. We suggest cytosolic 3-glyceraldehyde-(monohydroxy)-phosphate as the primary substrate of the enzyme complex and to replace -2H by integrating NADH-H^+ into glycolysis (pathway 1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyses the oxidative phosphorylation (metabolic burning) of 3-glyceraldehyde-(monohydroxy)-phosphate to 1-(monohydroxy), 3-(dihydroxy)-diphosphoglyceric acid. The glycolytically formed energy particle (H^+) is suggested to be attached at the 3-(monohydroxy)-phosphate group. A second energy particle is hidden in the acid anhydride group of 1,3-diphosphoglyceric acid. 1-(monohydroxy), 3-(dihydroxy)-diphosphoglyceric acid moves to the coupled enzymatic process. A PGK isoform catalyses energy transfer from H^+ (dihydroxy-phosphate

group) to the more stable adenosine *tri*-phosphoric acid anhydride (ATP) and the hydrolysis of 1,3-diphosphoglyceric acid to 3-(monohydroxy)-phoshoglyceric acid.



Pathway 1. Oxidative branch of the glycolytic flow. Meyerhof's Scheme 1 presents the chemistry of glycolysis and fermentation[5]. We divided the glycolytic metabolon into oxidative and reductive branches. Here, all reversible reactions have been changed into a unidirectional flow. The proton (H^+) of the acidic intermediates protected from dissociation by the metabolon (blue) is illustrated in red. Phosphoric acid is represented by ATP, and the +/-2H transfer is depicted as the NAD^+/NADH-H^+ system. The metabolic burning of 3-glyceraldehyde (monohydroxy)-phosphate generates two H^+ to be stored as two ATP. The first H^+ (NADH-H^+) attaches at the (monohydroxy)-phosphate group to form the acidic (dihydroxy)-phosphate group and initiate the Proton Transport Chain. The next catalytic reaction stores one glycolytically generated H^+ as ATP and hydrolyses the acid anhydride group of 1,3-diphosphoglyceric acid. Hydrolysis frees the second H^+ as 3-phosphoglyceric acid. Enol(monohydroxy)-phophopyruvic acid is the last intermediate of the glycolytic metabolon. The energy of the carboxylic acid is transferred to ATP, and pyruvate is given into the cytosol of carboxylates.

doi.org/10.32388/Y9K21X

The glycolytic flow of the proton (H^+) and material (glycolytic intermediates) is unidirectional. Therefore, all reaction arrows Meyerhof indicated as reversible processes (2^{nd} law of thermodynamic dynamics) are changed into a unidirectional or irreversible flow. Flow-generated energy is stored as cytosolic ATP flow non-equilibrium. *In vivo*, energy does not chaotically emit; energy flow has formed the glycolytic metabolon. The glycolytic flow of energy particle H^+ ends with the storage of energy as temporarily more stable cytosolic ATP flow non-equilibrium catalysed by (Enol)-(monohydroxy)-phosphopyruvic acid ATP synthase (or a specific isoform of pyruvate kinase)[46]. Pyruvate, the end product of the glycolytic flow, is given into the cytosolic pool of carboxylates.

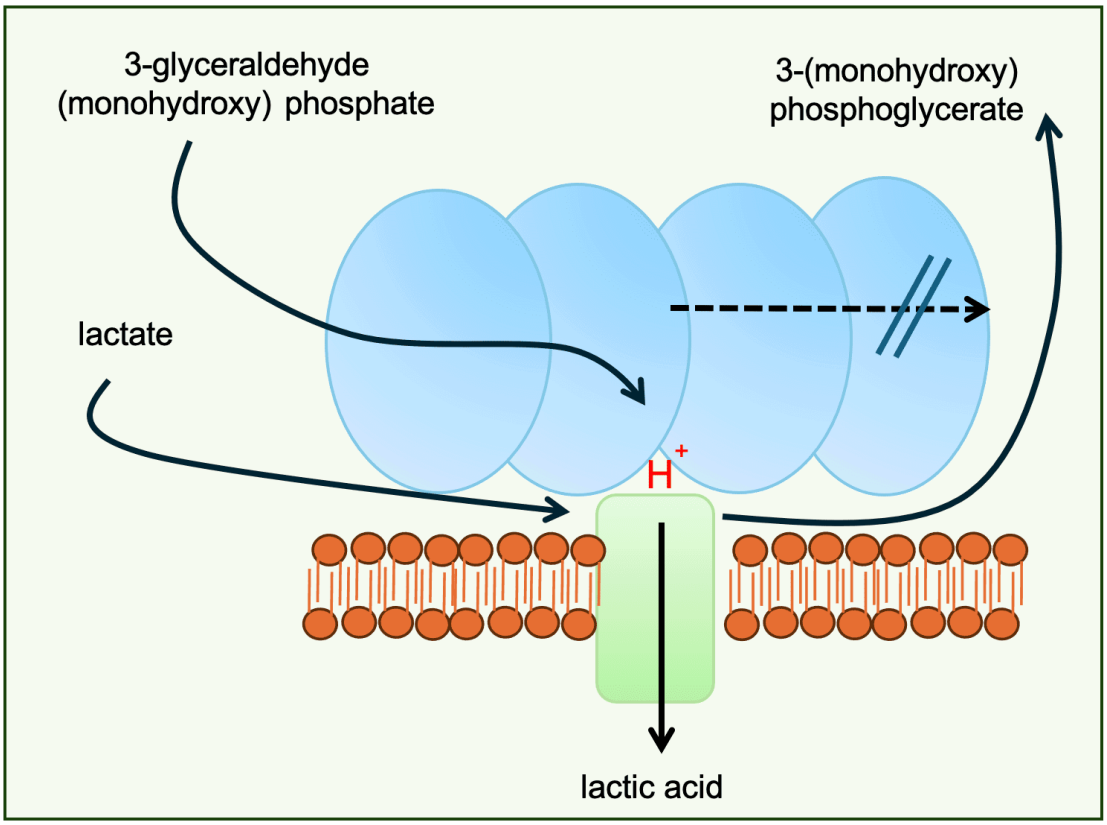
The integration of ATP and NADH-H^+ into the chemistry of glycolysis and fermentation challenges stoichiometry by opening two questions: firstly, fermentation: how can lactic acid (lactate-H^+) be exported when the proton of lactic acid already vanished from the chemical formula during discharging to water and storage of the energy as ATP? Secondly, regarding the recovery of the co-enzyme NADH or the cytosolic lactate flow concentration gradient; what is the origin of the obligatory proton (H^+) driving biosynthesis of lactate when glycolytically generated energy (NADH-H^+) is stored as cytosolic ATP flow non-equilibrium?

The fermentation pathway

Experimental work suggests that the association of GAPDH and PGK at the plasma membrane is a regulated process[47]. An acid, such as 3-(monohydroxy)-phosphoglyceric acid, released in the immediate proximity of proton-linked MCT initiates the export of monocarboxylic acid[6]. Close proximity can be interpreted as creating an acidic micro-environment, encouraging the transporter to equalize the generated membrane pH gradient. In this scenario, 3-(monohydroxy)-phosphoglyceric acid reacts with the bicarbonate-buffered micro-environment of the glycolytic metabolon, and hydration energy is emitted as heat and carbon dioxide. Nevertheless, an acidic micro-environment is sufficient to explain the characterization of proton-linked MCT_4 as an exporting carboxylic acid transporter[48][49].

Evolution surely optimizes the efficiency of proton (H^+) flow by transitioning from the creation of an acidic flow micro-environment (pH) to the direct transfer of the energy particle H^+ from unstable 3-(monohydroxy)-phosphoglyceric acid to proton-linked MCT. It is rational to postulate that the glycolytic metabolon releases 3-(monohydroxy)-phosphoglyceric acid if the flow of energy and

material is blocked[43]. The flow can be blocked by low cytosolic ADP. Thus, fermentation is introduced as regulated incomplete glycolysis (Pathway 2).



Pathway 2. Fermentation. The oxidative branch of the glycolytic metabolon (blue) is attached at the plasma membrane. Quenched glycolytic flow (dashed line) triggers the release of 3-phosphoplyceric acid[43]. 3-phosphoglyceric acid transfers the proton (H^+) to proton-linked MCT (green), initiates binding of lactate and export of lactic acid. Released 3-phosphoglycerate is given into the cytosolic pool of carboxylates.

Proton-linked MCT-glycolytic metabolon unidirectionally catalyses:

3-(monohydroxy)-phosphoglyceric acid (glycolytic metabolon) + lactate (cytosolic pool) → lactic acid (environment) + 3-(monohydroxy)-phosphoglycerate (cytosol)

Altering the historical perspective from the gradual degradation of the carbon backbone to the flow of the energy particle can be a somewhat perplexing concept. Notably, the exported monocarboxylate is not synthesized during fermentation but rather sourced from the cytosolic pool. The ATP balance of fermentation is +/- 0. These findings prompt the question: What purpose does fermentation serve?

Fermentation is the biosynthesis of 3-(monohydroxy)-phosphoglycerate. For the price of one monocarboxylate taken from the cytosolic pool and one H^+ not given into storage as ATP, a cytosolic 3-(monohydroxy)-phosphoglycerate non-equilibrium is generated. Similar to the recently reintroduced term `incomplete burning´, fermentation can be understood as incomplete glycolysis[50].

Equilibrium thermodynamics and enzyme kinetics guided the understanding of synthesis as the reverse reaction of degradation. A unidirectional flow allows us to understand synthesis as incomplete degradation. The mechanism of incomplete burning was introduced in the context of the regulation of the Citric Acid Cycle complexes: an imbalance of the fuel/oxygen ratio in favour of the fuel quenches the recovery of the co-enzymes. Quenched recovery of the activity of mitochondrial malic acid dehydrogenase (NAD^+) blocks metabolic burning, and malic acid is NAD^+-regulated and pushed into the pool of carboxylates.

Generating a 3-phosphoglycerate flow non-equilibrium is a rational prerequisite for serine biosynthesis. Proliferation of some cancer cells depends on serine biosynthesis; 3-phosphoglycerate dehydrogenase and proton-linked MCT_4 are targets for anti-cancer treatment. 3-phosphoglycerate levels seem to be directly involved in p53 activation to control cell fate[51][52][53][54]. Thus, we suggest that obligate aerobe cells can regulate the switch to (aerobe) fermentation at a high ATP/ADP ratio to pronounce biosynthesis and proliferation.

We generalized the observation that enzyme complexes protect acidic intermediates from dissociation. Dissociation is the exothermic reaction of an acid with water. Pioneers experimentally demonstrated that the glycolytic metabolon physically separates intrinsically formed 3-phosphoglyceric acid from the cytosolic pool of 3-phosphoglycerate[43]. In 1966, the flow of protons through enzyme complexes was named protecity[15]. Mitchell considered the direct chemical involvement of the proton in ATP recovery: $(ADP + HO\text{-}P)^- + H^+ \rightarrow ATP + H_2O$, whereas Walker determined that 3-5 protons are necessary to mechanically drive the recovery of 1 ATP[55]. Both Mitchell's and Walker's concepts are based on an osmotic pH gradient.

A pH gradient is an experimental tool also used to characterise proton-linked MCTs. An artificial acidic environment urges all cell membrane-located proton-linked MCTs to import monocarboxylic acids. On the other hand, proton-linked MCT_1-CAII was characterized as an importing enzyme complex (figure 3), whereas proton-linked MCT_4 (pathway 2) is characterized as an exporting

transporter. The introduced water-free or direct transfer of nascent protons (figure 3) skips the exothermic reaction of the acid; thereby, the environmental pH turns into a non-factor.

Nath further developed Mitchell's and Walker's theory by firstly postulating and later experimentally demonstrating that charge-free succinic acid is wired through mitochondrial ATP synthase[56][57]. The reintroduced intrinsic formation of 3-phosphoglyceric acid strongly supports the stoichiometry of ATP recovery presented by Mitchell and challenges the well-established model based on the pH gradient (figure 2).

The cytosolic lactate flow non-equilibrium

Glycolysis generates 2 H^+ twice to be stored as two sets of 2 ATP. The net chemical formula of the glycolytic flow of protons (H^+) leads to pyruvate:

Glucose → 4 ATP + 2 $pyruvate^-$ + 2 NADH

$C_6H_{12}O_6 = 4\ H^+ + 2\ C_3H_3O_3^- + 2\ H^-$

The glycolytic flow has the physical quantity (acid/time). An enzyme complex catalyses one metabolite at a time, not two metabolites as the net chemical formula suggests. Therefore, a flow is accomplished when the reduced co-enzyme (NADH) is recovered twice to NAD^+ and the process can start again. The GAPDH-LDH complex arrests the co-enzyme (NAD^+/NADH) and thereby determines that the redox unit (2H) is transferred to pyruvate[58]. The biochemical formula of LDH-catalysed recovery of NAD^+ is:

Pyruvate (cytosolic pool) + NADH (glycolytic metabolon) + H^+ (protecity) → (cytosolic) lactate (flow non-equilibrium) + NAD^+ (glycolytic metabolon)

$2\ C_3H_3O_3^- + 2H = 2\ C_3H_5O_3^-$

Pyruvate is the end product of the oxidative branch of glycolysis and thereby the first substrate of the reductive branch (biosynthesis). The hydrolysis of the acid anhydride ATP is a well-established source of energy for driving biosynthesis.

Providing an acid to the GAPDH-LDH complex provides a mathematically infinite concentration of H^+, driving a lactate flow non-equilibrium. Lactate stores energy by discharging the energy entity and the covalent binding of H. Therefore, the net chemical formula of glycolytic flow ends in the first ATP-driven biosynthesis product:

Glucose → - 2 ATP (consuming) + 4 ATP (non-equilibrium) + 2 lactate (non-equilibrium)

$C_6H_{12}O_6$ = - 2 H^+ + 4 H^+ + 2 $C_3H_5O_3^-$

The original EMP pathway was presented in line with the law of the conservation of mass and equilibrium thermodynamics. One molecule of glucose has to be understood to be in equilibrium with two molecules of lactic acid (2 H^+ + 2 lactate)[5]. Chemistry is based on a homogenous pool of all components. In a pool, -2 H^+ and +4 H^+ represent identical mathematical parameters to be equalized by the reactions (basic enzyme kinetics) to +2 H^+. To navigate the review more effectively, the established notion of a pool (closed system) needs to be broadened to include an understanding of a flow (open system). A flow of protons operates along a timeline. E. Rutherford might elucidate the physics of a flow in this manner: "No man ever drinks the same draft (beer) twice, for it is not the same beer and not the same man." Similarly, successful bartenders comprehend that two empty glasses and four beers differ from two beers.

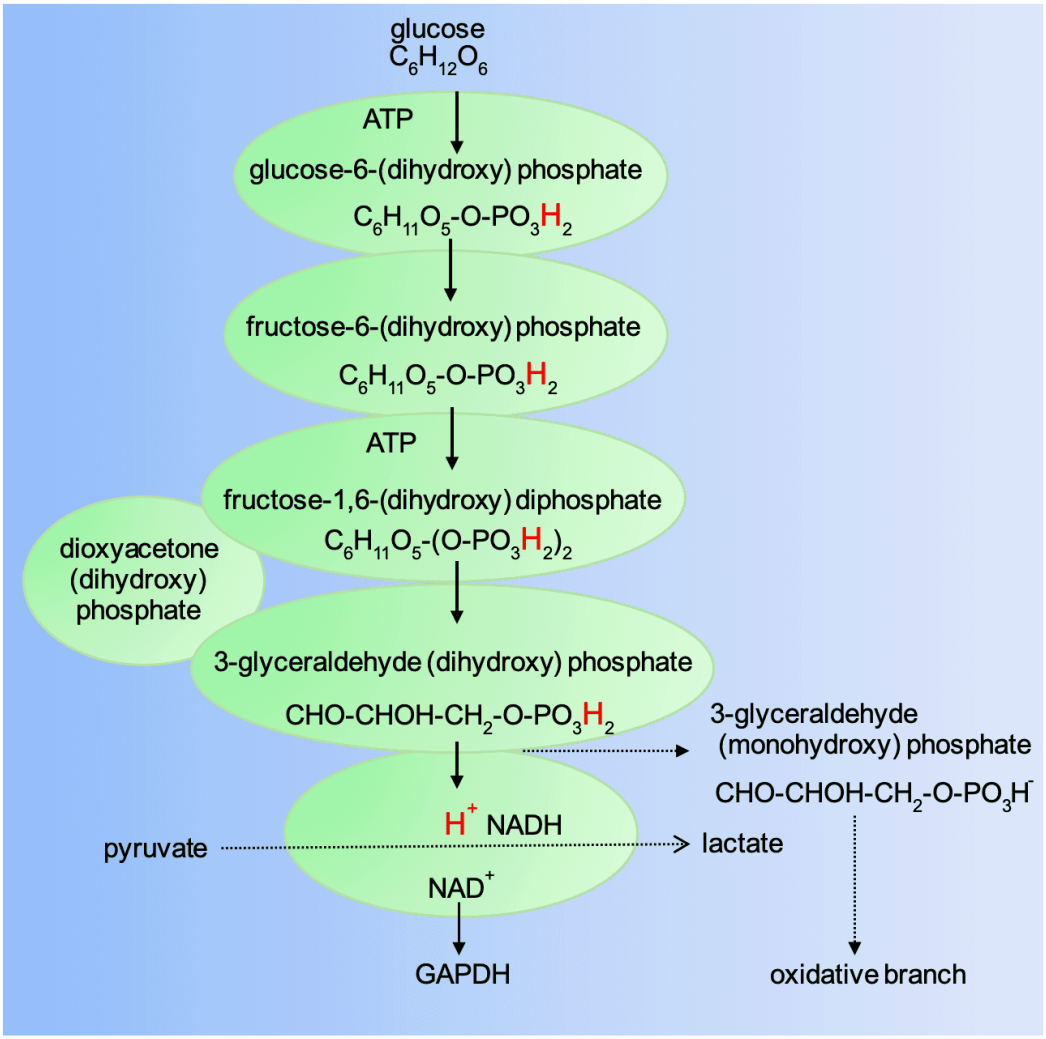
By incorporating the cytosolic lactate flow non-equilibrium as the initial ATP-driven synthesis product, we are on the verge of taking the first step towards bringing Newton's apple back up to the tree. What is lacking, however, is the beginning of the knot: the reductive branch of the glycolytic metabolon.

The first glycolytic metabolon

Although Kurganov and co-workers did not consider hexokinase (HK) as part of the first glycolytic multienzyme complex[8], we have integrated HK in the first glycolytic Proton Transport Chain as follows (Pathway 3):

HK catalysed phosphorylation of glucose and freed 1 proton (H^+). This proton can either associate with glucose-6-(monohydroxy)-phosphate or chaotically emit into the environment. Attaching the energy particle H^+ to glucose-6-(monohydroxy)-phosphate forms the acid glucose-6-(dihydroxy)-phosphate and initiates a Proton Transport Chain. This flow can be directed, with HK forming complexes with either phospho-glucose isomerase (glycolysis) or glucose-6-phosphate dehydrogenase (Pentose Phosphate Pathway). The pH sensitivity of the phospho-glucose isomerase-catalysed reaction supports that the proton (H^+) stays attached as glucose-6-(dihydroxy)-phosphate[59]. The coenzyme of glucose-6-phosphate dehydrogenase ($NADP^+$) is an ideal target to double the energy particle H^+ ($NADPH\text{-}2H^+$) for forcing anabolic processes[60]. Therefore, we count

HK as the first enzyme of a Proton Transport Chain ending at the GAPDH-LDH complex, generating the cytosolic lactate flow non-equilibrium (Pathway 3)



Pathway 3. Reductive branch of the glycolytic flow of protons. We divided the glycolytic metabolon into oxidative and reductive branches. We have replaced reversible reactions with a unidirectional flow, with phosphoric acid substituted by the acid anhydride ATP, and +/-2H replaced by the NAD^+/NADH-H^+ system. The adjustments have been made in line with the law of the conservation of mass. Phosphorylation of glucose forms the acidic intermediate glucose-6-(dihydroxy) phosphate and initiates the Proton Transport Chain. The acidic intermediate 3-glyceraldehyde (dihydroxy)-phosphate provides the proton to drive the reduction of pyruvate to lactate. Recovered NAD^+ returns to GAPDH (oxidative branch). The introduced substrate of the oxidative branch, 3-phosphoglycerate (monohydroxy) phosphate, is given into the cytosolic pool of carboxylates.

doi.org/10.32388/Y9K21X

This review started with the sentence “Every single chemical formula of modern models of glycolysis violates two laws of nature.”. The reaction catalysed by phosphofructokinase (PFK)

liberates the second energy entity (H^+), theorized to combine with pyruvate for lactate biosynthesis. We propose triosephosphate isomerase as the partnering molecule of PFK. The transfer of one H^+ to triosephosphate isomerase is expected to drive the isomerase-catalysed reaction in a unidirectional manner to form 3-glyceraldehyde-(dihydroxy)-phosphate:

Dioxyacetone-(monohydroxy)-phosphate (cytosol) $CH_2OH\text{-}C{=}O\text{-}CH_2\text{-}O\text{-}PO_3H^- + H^+ \rightarrow$

3-Glyceraldehyde-(dihydroxy)-phosphate (reductive branch) $CHO\text{-}CHOH\text{-}CH_2\text{-}O\text{-}PO_3H_2$

The transferred energy particle H^+ is further given to the GAPDH-(NADH)-LDH complex to generate the cytosolic lactate flow non-equilibrium (biosynthesis). 3-Glyceraldehyde-(monohydroxy)-phosphate ($CHO\text{-}CHOH\text{-}CH_2\text{-}O\text{-}PO_3H^-$) is the end product of the reductive branch and is given into the cytosolic pool of carboxylates. The cytosolic pool of 3-glyceraldehyde-(monohydroxy)-phosphate was introduced as the primary substrate of the oxidative branch of the glycolytic metabolon.

Therefore, we suggest a PFK-triosephosphate isomerase complex pumps cytosolic dioxyacetone-(monohydroxy)-phosphate into the reductive branch of the glycolytic metabolon. The energy particle finally fuses with pyruvate to produce the cytosolic lactate flow non-equilibrium.

This represents a provisional framework, quite possibly the initial instance where the distinct pathways of fermentation and glycolysis, cytosolic lactate flow non-equilibrium, and a scientific incorporation of ATP and NADH-H^+ in metabolism are presented. Should we reconsider our assertion that lactate is the primary step in returning Newton's apple to the tree? It is plausible that the initial step in creating an "apple" non-equilibrium with Earth is, perhaps, the introduction of metabolic pumping cytosolic dioxyacetone-(monohydroxy)-phosphate into biosynthesis.

Discussion

The machine is running! A cytosolic lactate flow non-equilibrium or simply the first step of biosynthesis is taken. It has taken a century to integrate ATP and NADH-H^+ in glycolysis in line with the restrictions given by the laws of nature. We will not start to amend assumptions, suggestions, or conclusions taken on the basis of invented models of glycolysis. We will go on sorting statistically relevant data into the concept of *in vivo* metabolism.

The steady state theory is based on a mathematic model showing that the flow ratio of glycolytic intermediates determined in erythrocytes is near the equilibrium that an isolated enzyme catalyses in a closed system (Figure 5). The steady state theory is a modern model of metabolism. The authors neither referred to nor discussed the state of knowledge that was pioneered. The investigated cellular pool of carboxylates was simply set to be glycolytic intermediates[61]. Meyerhof reminded scientists that the glycolytic intermediates are acids[62]. Thus, 3-phosphoglycerate, 2-phosphoglycerate, and phosphor(enol)p yruvate have to be sorted as intermediates of the physically separated biosynthesis pathway[63]. The intermediates of the biosynthesis pathway are rescued from glycolytic degradation. Biosynthesis products are primary substrates of coupled biosynthesis pathways. The lactate/pyruvate flow ratio determined in the liver (7:1) and muscles (12:1) indicates that the flow ratio determines cell function[32][31]. Muscle metabolism generates a steady state lactate/pyruvate flow non-equilibrium designed for mitochondrial consumption of lactic acid (energy on demand), whereas the liver demonstrates a lactate/pyruvate ratio preferring mitochondrial consumption of pyruvic acid. Mitochondrial consumption of pyruvic acid retained one redox unit (2H) in the cytosol for storage of energy as glycogen or lipid. It would be of interest to know if proliferating cells show a near equilibrium between 3-phosphoglycerate and 2-phosphoglycerate.

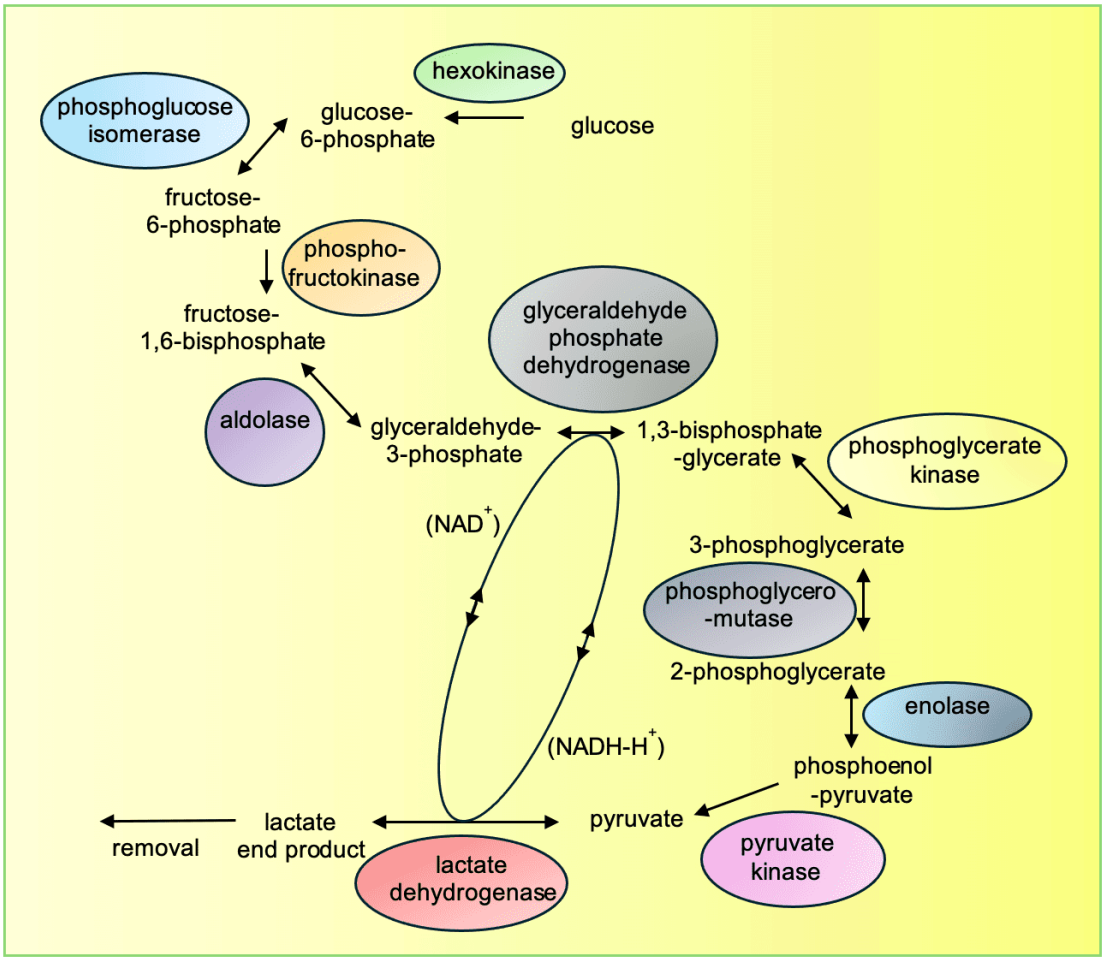


Figure 5. Linear Steady-State Enzyme Chains: In erythrocytes, the reactions catalysed by phosphoglucose isomerase, aldolase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglycerate mutase, enolase, and lactate dehydrogenase are near equilibrium. The reactions catalysed by hexokinase, pyruvate kinase, and lactate removal are set irreversible. Lactate is the end product of linear flow. Glucose ($C_6H_{12}O_6$) is considered to be metabolized to two lactate ($C_3H_5O_3^-$). Two H^+ are not explained.[61].

Sorting glycolytic reactions on the basis of the flow of the energy particle (H^+) divides glycolysis into biosynthesis (reduction) and metabolic burning (oxidation) paths. Meyerhof did not integrate the $NAD^+/NADH\text{-}H^+$ system into glycolysis; Rapoport and co-workers set GAPDH, which formed $NADH\text{-}H^+$, in equilibrium with LDH, which consumed $NADH\text{-}H^+$. Our analysis revealed that glycolytically generated energy is stored as ATP and that the biosynthesis of lactate is driven by the hexokinase and phosphofructokinase-catalysed hydrolysis of ATP (Figure 6).

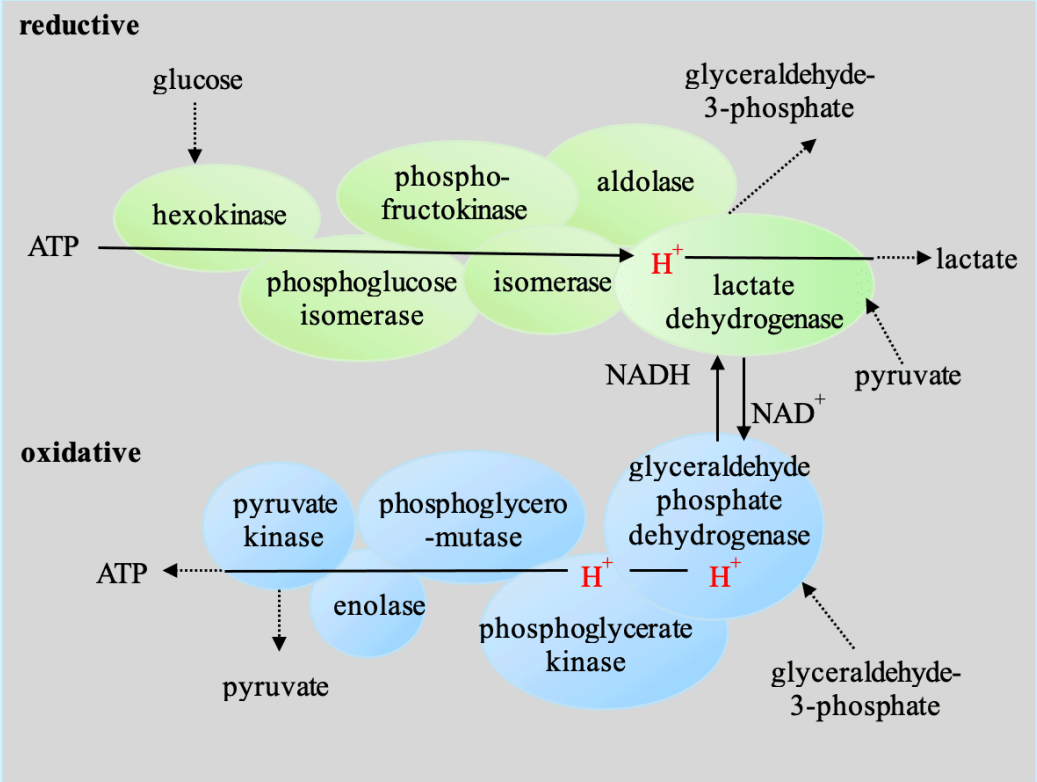


Figure 6. Glycolytic metabolon. The reductive branch of the glycolytic metabolon wires the proton to lactate synthesis. The energy particle discharges and fuses to lactate. The oxidative branch metabolically burns glyceraldehyde-3-phosphate to pyruvate. Gained energy is transformed to temporarily more stable ATP. The glycolytic metabolon physically separates the glycolytic flow of acidic intermediates from the cytosolic pool of carboxylates.

Let us drive the machine. All biological processes are linked to metabolism. Major depression is statistically linked to diminished glycogen storage[64]. Treatment of major depression with sodium serotonin reuptake inhibitors (SSRIs) has the side effects of bleeding and further diminished glycogen storage. Elucidating the mechanisms underlying major depression poses a formidable challenge. Regrettably, to date, neither serotonin, sodium ions, nor glycogen synthesis have been integrated into the two-cell model of metabolism. Yet, the groundwork was laid in 1929: Insulin expedites the cycle from liver to muscle glycogen, while epinephrine accelerates the cycle in the opposite direction[26]. These well-established models of metabolism are built on fundamental principles of equilibrium thermodynamics and enzyme kinetics. Closed homogenous systems in flow equilibrium, by definition, lack specific directions, accelerations, and undissociated acids, strongly suggesting that the

observations published in 1929 could never be effectively incorporated into a functional model of *in vivo* metabolism.

Metabolic pathways sense changes in metabolic messengers and respond by releasing messengers, such as insulin. Importantly, metabolism takes precedence in command, not insulin.

Pullen and co-workers demonstrated that hyperinsulinism during exercise is linked with the cell membrane-located proton-linked MCT_1-catalysed import of pyruvate in β-cells[65]. The authors introduced proton-linked MCTs (as isolated enzymes) transporting carboxylates across membranes in a freely reversible manner. The introduction indicates that the experimental data are evaluated on the basis of basic enzyme kinetics and equilibrium thermodynamics and not as a characterization of proton-linked MCTs as part of a complex unidirectionally acting monocarboxylic acid importing enzyme-complex. In this context, Pullen and co-workers presented statistically significant data without any biological function. Somewhat confusing data, because lactic acid and not pyruvate is deeply linked to exercise.

The two-cell model of metabolism suggests that cytosolic high pyruvate represents an imbalance of the cytosolic pyruvate flow. The imbalance indicates that, in the current configuration of the flow, one redox unit (2H) is retained in the cytosol. As a result, insulin signalling pathways must temporarily adapt the organism to the identified surplus redox unit by storing it as lipid and glycogen. The two-cell model of metabolism allows us to link statistically relevant data with a biological mechanism.

Ho and coworkers determined that the plasma membrane-associated glycolytic metabolon is functionally coupled to a K_{ATP} channel in pancreatic α- and β-cells[66]. Hexokinase IV (glucokinase) is the first enzyme of this glycolytic metabolon, and the hexokinase IV-metabolon-produced ADP opens the K_{ATP} channel, whereas 1,3-diphosphoglyceric acid ATP synthase (a specific PGK isoform) and (enol)(monohydroxy)-phosphopyruvic acid-ATP synthase activity close the channel by recovering the glycolytic ATP flow non-equilibrium pool. The same authors also demonstrated that the glycolytically generated cytosolic ATP pool acts independently from the mitochondrially generated cytosolic ATP pool.

The two-cell model supports the observation of a mitochondrial and a glycolytic ATP pool. The presumed "reverse reaction" involving the release of K^+ is facilitated by the Na^+, K^+-ATPase. Our scientific hypothesis suggests that the Na^+, K^+-ATPase consumes ATP from the mitochondrial-generated pool. Logically, this would mean that utilizing ATP generated by the hexokinase IV-

metabolon to power the Na^+, K^+-ATPase would result in a persistently open K_{ATP} channel and a lifeless state of thermodynamic equilibrium. By asserting that 1 ATP hydrolysis equals 1 H^+, the Na^+, K^+-ATPase-catalysed reaction transforms into a charge-neutral ion exchange:

3 Na^+ (out) = 2 K^+ (in) + 1 H^+ (in).

The stoichiometry implies that the well-established creation of a resting potential by the Na^+, K^+-ATPase has become a perplexing puzzle. As mentioned earlier, sodium ions are not yet integrated into the two-cell model of metabolism.

Now let us explore the opposite direction: epinephrine. Our initial attempt to introduce acceleration into mechanisms involved the recently discovered second Citric Acid Cycle complex (CAC 2.0 complex) [21]. Malic acid flow non-equilibrium was introduced as the metabolic link accelerating ATP recovery by connecting additional engines (CAC 2.0-respiration complexes) to ATP recovery. Briefly summarized, lactic acid is the primary substrate of the lactic acid-consuming CAC 1.1 complex. An oversupply of the fuel (lactic acid) results in incomplete burning, generating a NAD^+-regulated malic acid flow non-equilibrium[7]. Malic acid is the primary substrate of the CAC 2.0-respiration complex. The generated malic acid flow non-equilibrium (biosynthesis) enables CAC 2.0 to consume malic acid. The postulated FAD-regulated product of the incomplete burning of malic acid in muscle is succinic acid[21]. The postulated metabolic pathway of lactic acid to succinic acid comprises the recovery of the mitochondrial ATP flow non-equilibrium to the very limit. The very limit is indicated by the cellular export of succinic acid. Succinic acid is a metabolic messenger of exercise[67][68]. Active muscles export succinic acid through the plasma membrane using the proton-linked MCT_1. Cellular export of the metabolic messenger succinic acid signals that the cytosolic pH limit has been reached. At the current cytosolic pH, the monocarboxylic acid transporter known to be involved in importation reverses its catalytic direction to facilitate the export of the dicarboxylic acid succinic acid.

With the discovery of CAC 2.0, the two-cell model of metabolism comprises the metabolic messengers: glucose, pyruvic acid, lactic acid, and succinic acid. The question arises: How can this metabolic pathway be accelerated by epinephrine when the very limit is already reached?

Costa Rosa and co-workers observed that adrenaline (epinephrine) blocks glucose-6-P-dehydrogenase ($NADP^+$) activity and activates a NADPH-producing 'malic' enzyme[69]. Our concept of *in vivo* metabolism introduced the acid glucose-6-(dihydroxy) phosphate and the anabolic acting coenzyme NADPH-$2H^+$. A rational effect of adrenaline is to block the Pentose Phosphate Pathway at

glucose-6-phosphate dehydrogenase to focus metabolism on glycolysis, whereas increased oxidation of monohydrogen malate to monohydrogen oxaloacetate to `produce´ the coenzyme NADPH rationally blocks acceleration or addition of CAC 2.0-respiration complexes and stimulates anabolic flows. The two-cell model of metabolism sets the catalytic direction of enzyme isoforms in biologic function and interprets the data in the diametrically opposite catalytic direction in muscles: a NADPH-$2H^+$ consuming 'malic' enzyme is activated to reduce cytosolic monohydrogen oxaloacetate and produces monohydrogen malate. In doing so, the adrenaline signalling pathway generates the primary substrate for CAC 2.0 and speeds up the recovery of the mitochondrial ATP flow non-equilibrium.

The two-cell model of metabolism introduces FAD-regulated succinic acid synthesis, supported by experimental data of proton-linked MCT_1 catalysed succinic acid export, but it skips the pathway of monohydrogen succinate transport through the mitochondrial membrane(s). Anions such as monohydrogen succinate or dissociated malic acid, citric acid, phosphoric acid, ADP, and ATP can effortlessly and reversibly traverse membranes based on an understanding coined by equilibrium thermodynamics and homogenized cells, but not *in vivo*.

In vivo, proton-linked MCTs transfer the charge-neutral mono- or dicarboxylic acids through

membranes. Metabolically formed 3-(monohydroxy) phosphoglyceric acid was presented as one source of H^+ turning cytosolic carboxylate ($R\text{-}COO^-$) into a charge-neutral carboxylic acid (R-COOH) transport. Another introduced source of H^+ is ATP.

GTP-driven formation and deformation of succinyl-SCoA emerge as a rational approach for synthesizing a charge-neutral dicarboxylic acid and initiating charge-neutral membrane transfer.

Formation of succinyl-SCoA:

Monohydrogen succinate + H-SCoA + H^+ → succinyl-SCoA + water (H_2O)

Hydrolysis of succinyl-SCoA:

Succinyl-SCoA + H_2O → succinic acid + H-SCoA

Energy as particle H^+ (GTP) is given into the formation of the thioester. The subsequent hydrolysis of the thioester facilitates the charge-neutral transfer of succinic acid through a membrane.

The biological function of formation/hydrolysis of succinyl-SCoA is a well-established part of didactic hypotheses of citrate cycles. Models of citrate cycles propagate the opposite catalytic direction:

succinate + H-SCoA + P + GDP → [succinyl-SCoA] → succinate + GTP + H-SCoA

In enzyme kinetics, identical products and substrates are eliminated from the chemical formula.

~~succinate~~ + ~~HS-CoA~~ + P + GDP → ~~[succinyl-SCoA]~~ → ~~succinate~~ + GTP + ~~HS-CoA~~

Integrating the process into a citrate cycle results in a perpetual motion machine of GTP synthesis. Stoichiometry and Mitchell's chemical formula of ATP formation $(ADP + HO\text{-}P)^- + H^+ \rightarrow ATP + H_2O$ were ignored, allowing the invention of the catalytic direction of [succinyl-SCoA]. Succinic acid carries the energy that mitochondrial ATP synthase transforms into more stable ATP. Over the past 25 years, S. Nath has published 50 manuscripts characterizing the flow through mitochondrial ATP synthase[70][71]. Therefore, a more rational biological function of [succinyl-SCoA] is the GTP-driven membrane transfer of cytosolic monohydrogen succinate into the mitochondrial matrix. The generated mitochondrial succinic acid flow non-equilibrium is a possible target of epinephrine signalling to accelerate mitochondrial ATP synthase in muscles.

Statements and Declarations

Funding

None.

Conflicts of interest

Dirk Roosterman and Graeme S. Cottrell declare that we have no conflict of interest.

References

1. ^*Fara P (1999). "Catch a Falling Apple: Isaac Newton and Myths of Genius". Endeavour. 23 (4): 167–70. doi:10.1016/s0160-9327(99)80040-4.*
2. a, b, c*Thomson W (1879). "The Sorting Demon of Maxwell 1". Nature. 20 (501): 126–126. doi:10.1038/020126a0.*
3. ^*Sheehan D (2020). "Maxwell Zombies: Mulling and Mauling the Second Law of Thermodynamics". Journal of Scientific Exploration. 34 (3): 513–36. doi:10.31275/20201645.*
4. ^*Davis TL (1924). "Neglected Evidence in the History of Phlogiston, Together with Observations on the Doctrine of Forms and the History of Alchemy". Annals of Medical History. 6 (3): 280–87.*

5. a, b, c, d, e, f, g, h, i, j *Meyerhof O (1951). "Mechanisms of Glycolysis and Fermentation". Canadian Journal of Medical Sciences. 29 (2): 63–77. doi:10.1139/cjms51-008.*
6. a, b *Bruijne AW de, Vreeburg H, van Steveninck J (1985). "Alternative-Substrate Inhibition of L-Lactate Transport via the Monocarboxylate-Specific Carrier System in Human Erythrocytes". Biochimica Et Biophysica Acta. 812 (3): 841–44. doi:10.1016/0005-2736(85)90280-9.*
7. a, b, c, d *Roosterman D, Cottrell GS (2021a). "Rethinking the Citric Acid Cycle: Connecting Pyruvate Carboxylase and Citrate Synthase to the Flow of Energy and Material". International Journal of Molecular Sciences. 22 (2): 604. doi:10.3390/ijms22020604.*
8. a, b *Kurganov BI, Sugrobova NP, Mil'man LS (1985). "Supramolecular Organization of Glycolytic Enzymes". Journal of Theoretical Biology. 116 (4): 509–26. doi:10.1016/s0022-5193(85)80086-2.*
9. ^ *Das ML, Koike M, Reed LJ (1961). "ON THE ROLE OF THIAMINE PYROPHOSPHATE IN OXIDATIVE DECARBOXYLATION OF α-KETO ACIDS*". Proceedings of the National Academy of Sciences. 47 (6): 753–59. doi:10.1073/pnas.47.6.753.*
10. ^ *Reed LJ, Hackert ML (1990). "Structure-Function Relationships in Dihydrolipoamide Acyltransferases". The Journal of Biological Chemistry. 265 (16): 8971–74.*
11. a, b *Krebs HA, Johnson WA (1937). "Metabolism of Ketonic Acids in Animal Tissues". The Biochemical Journal. 31 (4): 645–60. doi:10.1042/bj0310645.*
12. a, b *Krebs HA, Salvin E, Johnson WA (1938). "The Formation of Citric and Alpha-Ketoglutaric Acids in the Mammalian Body". The Biochemical Journal. 32 (1): 113–17. doi:10.1042/bj0320113.*
13. ^ *Karpusas M, Branchaud B, Remington SJ (1990). "Proposed Mechanism for the Condensation Reaction of Citrate Synthase: 1.9-A Structure of the Ternary Complex with Oxaloacetate and Carboxymethyl Coenzyme A". Biochemistry. 29 (9): 2213–19.*
14. a, b *Roosterman D, Meyerhof W, Cottrell GS (2018). "Proton Transport Chains in Glucose Metabolism: Mind the Proton". Frontiers in Neuroscience. 12:404. doi:10.3389/fnins.2018.00404.*
15. a, b, c, d *Mitchell P (2011). "Chemiosmotic Coupling in Oxidative and Photosynthetic Phosphorylation. 1966". Biochimica Et Biophysica Acta. 1807 (12): 1507–38. doi:10.1016/j.bbabio.2011.09.018.*
16. a, b *Jorgenson S (1999). 'Tentative Fourth Law of Thermodynamics, Applied to Description of Ecosystem Development - JØRGENSEN - 1999 - Annals of the New York Academy of Sciences - Wiley Online Library'. 1999. https://nyaspubs.onlinelibrary.wiley.com/doi/abs/10.1111/j.1749-6632.1999.tb10438.x.*
17. ^ *Lindskog S (1997). "Structure and Mechanism of Carbonic Anhydrase". Pharmacology & Therapeutics. 74 (1): 1–20. doi:10.1016/s0163-7258(96)00198-2.*

18. ^Becker HM, Klier M, Schüler C, McKenna R, Deitmer JW (2011). "Intramolecular Proton Shuttle Supports Not Only Catalytic but Also Noncatalytic Function of Carbonic Anhydrase II". Proceedings of the National Academy of Sciences. 108 (7): 3071–76. doi:10.1073/pnas.1014293108.

19. a, bRoosterman D, Cottrell GS (2020). "Astrocytes and Neurons Communicate via a Monocarboxylic Acid Shuttle". AIMS Neuroscience. 7 (2): 94–106. doi:10.3934/Neuroscience.2020007.

20. a, b, c, dRoosterman D, Cottrell GS (2021b). "The Two-Cell Model of Glucose Metabolism: A Hypothesis of Schizophrenia". Molecular Psychiatry. 26 (6): 1738–47. doi:10.1038/s41380-020-00980-4.

21. a, b, cRoosterman D, Cottrell GS (2023). "Discovery of a Second Citric Acid Cycle Complex". Heliyon. 9 (5): e15968. doi:10.1016/j.heliyon.2023.e15968.

22. ^Rogatzki MJ, Ferguson BS, Goodwin ML, Gladden LB (2015). "Lactate Is Always the End Product of Glycolysis". Frontiers in Neuroscience. 9 (February). doi:10.3389/fnins.2015.00022.

23. a, bGreen DE (1949). "ENZYMES IN TEAMS". Scientific American. 181 (3): 48–51.

24. ^Krebs HA (1953). 'The Nobel Prize in Physiology or Medicine 1953'. NobelPrize.Org. 1953. https://www.nobelprize.org/prizes/medicine/1953/summary/.

25. a, b, cCori CF (1981). "The Glucose–Lactic Acid Cycle and Gluconeogenesis". In Current Topics in Cellular Regulation, 18:377–87. Elsevier. doi:10.1016/B978-0-12-152818-8.50028-1.

26. a, b, cCori CF, Cori GT (1929). "GLYCOGEN FORMATION IN THE LIVER FROM d- AND l-LACTIC ACID". Journal of Biological Chemistry. 81 (2): 389–403. doi:10.1016/S0021-9258(18)83822-4.

27. a, bLangen P, Hucho F (2008). "Karl Lohmann and the Discovery of ATP". Angewandte Chemie (International Ed. in English). 47 (10): 1824–27. doi:10.1002/anie.200702929.

28. ^Sun RC, Dukhande VV, Zhou Z, Young LEA, Emanuelle S, Brainson CF, Gentry MS (2019). "Nuclear Glycogenolysis Modulates Histone Acetylation in Human Non-Small Cell Lung Cancers". Cell Metabolism. 30 (5): 903-916.e7. doi:10.1016/j.cmet.2019.08.014.

29. ^Gibbs ME (2016). "Role of Glycogenolysis in Memory and Learning: Regulation by Noradrenaline, Serotonin and ATP". Frontiers in Integrative Neuroscience. 9 (January):70. doi:10.3389/fnint.2015.00070.

30. ^Zhang H, Liu J, Yang Z, Zeng L, Wei K, Zhu L, Tang L, et al. (2022). "TCR Activation Directly Stimulates PYGB-Dependent Glycogenolysis to Fuel the Early Recall Response in CD8+ Memory T Cells". Molecular Cell. 82 (16): 3077-3088.e6. doi:10.1016/j.molcel.2022.06.002.

31. a, bSahlin K, Harris RC, Nylind B, Hultman E (1976). "Lactate Content and pH in Muscle Samples Obtained after Dynamic Exercise". Pflügers Archiv. 367 (2): 143–49. doi:10.1007/BF00585150.

32. ^ a, b *Liaw KY, Wei TC, Hsu SC, Lin JK (1985). "Effect of Severe Injury and Critical Illness on High-Energy P hosphates in Human Liver and Muscle". The Journal of Trauma. 25 (7): 628–33. doi:10.1097/00005373 -198507000-00009.*

33. ^ *Tadi M, Allaman I, Lengacher S, Grenningloh G, Magistretti PJ (2015). "Learning-Induced Gene Expres sion in the Hippocampus Reveals a Role of Neuron -Astrocyte Metabolic Coupling in Long Term Memor y". PloS One. 10 (10): e0141568. doi:10.1371/journal.pone.0141568.*

34. ^ *Hebb D (1949). 'The Organization of Behavior; a Neuropsychological Theory.' 1949. https://awspntest. apa.org/record/1950-02200-000.*

35. ^ *Rowland LM, Pradhan S, Korenic S, Wijtenburg SA, Hong LE, Edden RA, Barker PB (2016). "Elevated B rain Lactate in Schizophrenia: A 7 T Magnetic Resonance Spectroscopy Study". Translational Psychiatry. 6 (11): e967. doi:10.1038/tp.2016.239.*

36. ^ *Meyerhof O (1922). "The Nobel Prize in Physiology or Medicine 1922". NobelPrize.Org. 1922. https://w ww.nobelprize.org/prizes/medicine/1922/meyerhof/biographical/.*

37. ^ a, b *Hui S, Ghergurovich JM, Morscher RJ, Jang C, Teng X, Lu W, Esparza LA, et al. (2017). "Glucose Feeds the TCA Cycle via Circulating Lactate". Nature. 551 (7678): 115–18. doi:10.1038/nature24057.*

38. ^ *Hashimoto T, Hussien R, Brooks GA (2006). "Colocalization of MCT1, CD147, and LDH in Mitochondria l Inner Membrane of L6 Muscle Cells: Evidence of a Mitochondrial Lactate Oxidation Complex". America n Journal of Physiology. Endocrinology and Metabolism. 290 (6): E1237-1244. doi:10.1152/ajpendo.005 94.2005.*

39. ^ *Bak LK, Schousboe A (2017). "Misconceptions Regarding Basic Thermodynamics and Enzyme Kinetics Have Led to Erroneous Conclusions Regarding the Metabolic Importance of Lactate Dehydrogenase Isoe nzyme Expression". Journal of Neuroscience Research. 95 (11): 2098–2102. doi:10.1002/jnr.23994.*

40. ^ *Schrödinger E (1944). What Is Life?: With Mind and Matter and Autobiographical Sketches. Canto Class ics. Cambridge: Cambridge University Press. https://doi.org/10.1017/CBO9781107295629.*

41. ^ *Harden A, Young WJ, Martin CJ (1906). "The Alcoholic Ferment of Yeast-Juice". Proceedings of the Roy al Society of London. Series B, Containing Papers of a Biological Character. 77 (519): 405–20. doi:10.109 8/rspb.1906.0029.*

42. ^ *Warburg O (1956). "On the Origin of Cancer Cells". Science (New York, N.Y.). 123 (3191): 309–14. doi:1 0.1126/science.123.3191.309.*

43. ^ a, b, c, d *Kennedy EP, Lehninger AL (1949). "Oxidation of Fatty Acids and Tricarboxylic Acid Cycle Interm ediates by Isolated Rat Liver Mitochondria". The Journal of Biological Chemistry. 179 (2): 957–72.*

44. ^ Nelson DL, Cox MM (2012). Lehninger Principles of Biochemistry. 6th Edition. Macmillian Learning.
45. ^ Prigogine I (1977). "The Nobel Prize in Chemistry 1977". NobelPrize.Org. 1977. https://www.nobelprize.org/prizes/chemistry/1977/summary/.
46. ^ Menard L, Maughan D, Vigoreaux J (2014). "The Structural and Functional Coordination of Glycolytic Enzymes in Muscle: Evidence of a Metabolon?" Biology. 3 (3): 623–44. doi:10.3390/biology3030623.
47. ^ De BK, Kirtley ME (1977). "Interaction of Phosphoglycerate Kinase with Human Erythrocyte Membranes". The Journal of Biological Chemistry. 252 (19): 6715–20.
48. ^ Contreras-Baeza Y, Sandoval PY, Alarcón R, Galaz A, Cortés-Molina F, Alegría K, Baeza-Lehnert F, et al. (2019). "Monocarboxylate Transporter 4 (MCT4) Is a High Affinity Transporter Capable of Exporting Lactate in High-Lactate Microenvironments". The Journal of Biological Chemistry. 294 (52): 20135–47. doi:10.1074/jbc.RA119.009093.
49. ^ Dimmer KS, Friedrich B, Lang F, Deitmer JW, Bröer S (2000). "The Low-Affinity Monocarboxylate Transporter MCT4 Is Adapted to the Export of Lactate in Highly Glycolytic Cells". The Biochemical Journal. 350 Pt 1: 219–27.
50. ^ Meyerhof O (1927). "RECENT INVESTIGATIONS ON THE AEROBIC AND AN-AEROBIC METABOLISM OF CARBOHYDRATES". The Journal of General Physiology. 8 (6): 531–42. doi:10.1085/jgp.8.6.531.
51. ^ Goldberg FW, Kettle JG, Lamont GM, Buttar D, Ting AKT, McGuire TM, Cook CR, et al. (2023). "Discovery of Clinical Candidate AZD0095, a Selective Inhibitor of Monocarboxylate Transporter 4 (MCT4) for Oncology". Journal of Medicinal Chemistry. 66 (1): 384–97. doi:10.1021/acs.jmedchem.2c01342.
52. ^ Li M, Wu C, Yang Y, Zheng M, Yu S, Wang J, Chen L, Li H (2021). "3-Phosphoglycerate Dehydrogenase: A Potential Target for Cancer Treatment". Cellular Oncology (Dordrecht, Netherlands). 44 (3): 541–56. doi:10.1007/s13402-021-00599-9.
53. ^ Sadiqa A, Rasul A, Hassan M, Sultana S, Jabeen F (2022). "Identification of Novel Natural Inhibitors to Human 3-Phosphoglycerate Dehydrogenase (PHGDH) for Cancer Treatment". Molecules (Basel, Switzerland). 27 (18): 6108. doi:10.3390/molecules27186108.
54. ^ Wu YQ, Zhang CS, Xiong J, Cai DQ, Wang CZ, Wang Y, Liu YH, et al. (2023). "Low Glucose Metabolite 3-Phosphoglycerate Switches PHGDH from Serine Synthesis to P53 Activation to Control Cell Fate". Cell Research. 33 (11): 835–50. doi:10.1038/s41422-023-00874-4.
55. ^ Watt IN, Montgomery MG, Runswick MJ, Leslie AGW, Walker JE (2010). "Bioenergetic Cost of Making an Adenosine Triphosphate Molecule in Animal Mitochondria". Proceedings of the National Academy of Sciences of the United States of America. 107 (39): 16823–27. doi:10.1073/pnas.1011099107.

56. ^Nath S (2016). "The Thermodynamic Efficiency of ATP Synthesis in Oxidative Phosphorylation". Biophysical Chemistry. 219 (December):69–74. doi:10.1016/j.bpc.2016.10.002.

57. ^Nath S, Villadsen J (2015). "Oxidative Phosphorylation Revisited". Biotechnology and Bioengineering. 112 (3): 429–37. doi:10.1002/bit.25492.

58. ^Svedružić ZM, Spivey HO (2006). "Interaction between Mammalian Glyceraldehyde-3-Phosphate Dehydrogenase and L-Lactate Dehydrogenase from Heart and Muscle". Proteins. 63 (3): 501–11. doi:10.1002/prot.20862.

59. ^Dyson JE, Noltmann EA (1968). 'The Effect of pH and Temperature on the Kinetic Parameters of Phosphoglucose Isomerase. Participation of Histidine and Lysine in a Proposed Dual Function Mechanism - PubMed'. 1968. https://pubmed.ncbi.nlm.nih.gov/5647261/.

60. ^Mullen AR, Wheaton WW, Jin ES, Chen PH, Sullivan LB, Cheng T, Yang Y, Linehan WM, Chandel NS, De Berardinis RJ (2011). "Reductive Carboxylation Supports Growth in Tumour Cells with Defective Mitochondria". Nature. 481 (7381): 385–88. doi:10.1038/nature10642.

61. a, bRapoport TA, Heinrich R, Jacobasch G, Rapoport S (1974). "A Linear Steady-State Treatment of Enzymatic Chains". European Journal of Biochemistry. 42 (1): 107–20. doi:10.1111/j.1432-1033.1974.tb0332o.x.

62. ^Meyerhof O, Oesper P (1949). "The Enzymatic Equilibria of Phospho(Enol)Pyruvate". The Journal of Biological Chemistry. 179 (3): 1371–85.

63. ^Lardy HA, Ziegler JA (1945). "THE ENZYMATIC SYNTHESIS OF PHOSPHOPYRUVATE FROM PYRUVATE". Journal of Biological Chemistry. 159 (2): 343–51. doi:10.1016/S0021-9258(19)52795-8.

64. ^Maurer-Spurej E, Pittendreigh C, Misri S (2007). "Platelet Serotonin Levels Support Depression Scores for Women with Postpartum Depression". Journal of Psychiatry & Neuroscience: JPN. 32 (1): 23–29.

65. ^Pullen TJ, Sylow L, Sun G, Halestrap AP, Richter EA, Rutter GA (2012). "Overexpression of Monocarboxylate Transporter-1 (SLC16A1) in Mouse Pancreatic β-Cells Leads to Relative Hyperinsulinism during Exercise". Diabetes. 61 (7): 1719–25. doi:10.2337/db11-1531.

66. ^Ho T, Potapenko E, Davis DB, Merrins MJ (2023). "A Plasma Membrane-Associated Glycolytic Metabolon Is Functionally Coupled to KATP Channels in Pancreatic α and β Cells from Humans and Mice". Cell Reports. 42 (4): 112394. doi:10.1016/j.celrep.2023.112394.

67. ^Hochachka PW, Dressendorfer RH (1976). "Succinate Accumulation in Man during Exercise". European Journal of Applied Physiology and Occupational Physiology. 35 (4): 235–42. doi:10.1007/BF00423282.

68. ^ *Reddy A, Bozi LHM, Yaghi OK, Mills EL, Xiao H, Nicholson HE, Paschini M, et al. (2020). "pH-Gated Succinate Secretion Regulates Muscle Remodeling in Response to Exercise". Cell. 183 (1): 62–75.e17. doi:10.1016/j.cell.2020.08.039.*

69. ^ *Costa Rosa LF, Curi R, Murphy C, Newsholme P (1995). "Effect of Adrenaline and Phorbol Myristate Acetate or Bacterial Lipopolysaccharide on Stimulation of Pathways of Macrophage Glucose, Glutamine and O2 Metabolism. Evidence for Cyclic AMP-Dependent Protein Kinase Mediated Inhibition of Glucose-6-Phosphate Dehydrogenase and Activation of NADP+-Dependent "malic" Enzyme". The Biochemical Journal. 310 (Pt 2): 709–14. doi:10.1042/bj3100709.*

70. ^ *Nath S (1998). "A Thermodynamic Principle for the Coupled Bioenergetic Processes of ATP Synthesis". Pure and Applied Chemistry. 70 (3): 639–44. doi:10.1351/pac199870030639.*

71. ^ *Nath S (2024). "Size Matters in Metabolic Scaling: Critical Role of the Thermodynamic Efficiency of ATP Synthesis and Its Dependence on Mitochondrial H+H+ Leak across Mammalian Species". BioSystems. 242 (August):105255. doi:10.1016/j.biosystems.2024.105255.*

Declarations

Funding: No specific funding was received for this work.

Potential competing interests: No potential competing interests to declare.