



ACUTE TOXICITY OF CYANIDE AND SEVERAL IONS CONCLUSIVELY NEGATE ELECTRON TRANSPORT CHAINS AND PROTON PUMPS: *REVOLUTIONARY 'MURBURN' LESSONS FOR TEACHERS AND PHYSICIANS ON THE ROLES OF OXYGEN IN LIFE*

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*Biochemists/biophysicists derive profound mechanistic insights from the experimental data/interpretation of probing the effects of toxic agents. Of all toxic entities known, arsenic and cyanide are the most salient ones in popular/scientific literature. Both are supposed to derail energy metabolism, with the latter being more critical. Low amounts of cyanide deleteriously impact almost instantaneously/totally at the organism level, wreaking havoc on diverse domains of cellular physiology (on powering, coherence, electro-mechanical and sensing-response activities), cutting across the living spectra of organisms. Also, the similar heme-binding triatomic anionic inhibitor of azide shows unusual enhancements and hormetic effects on redox enzyme activity. In this context, the last decade's experimental and theoretical findings lead us to question the long-standing hemeFe-affinity ligand-binding based explanations. Conclusive evidence/arguments against the proton-centric ideas of "electron transport chain, Q-cycle, proton-pumping, proton motive force (chemiosmosis) and rotary ATP-synthesis are now available. The oxygen-centric effective charge separation (ECS) & diffusible reactive species (DRS) based murburn model affords satisfactory rationales (even for the acute/chronic toxicities presented by other diverse ions, which is discussed briefly here). The logic of authorities is also subject to the authority of logic. As the murburn theory has been around for a decade now, it is high time that the CNS (Cell, Nature and Science; also, PNAS / EMBO) of biomedical field took note! Also, the Nobel Committee must come forward to recant several of its recognitions, as the classical perceptions continue to waylay the aspirations and welfare of our younger generations. **Biomed Rev 2025; 36: 1-16***

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1. SOME POISON(S), VOULEZ VOUS?

If one wants to critically understand the underpinnings of life, toxicity studies are the holy grail, and cyanide toxicity is the most intriguing example. Cyanide is a potent and rapid-acting toxic agent, which undoes life-sustaining activities in almost all living beings. This simple hetero-diatomic inorganic anion manifests LD₅₀ at micromolar cellular/physiological concentrations. LD₅₀ is the dosage amount in mg chemical administered per kg of body weight, at which 50% of a subjected test population is killed. This measurement protocol is quite analogous to IC₅₀, the concentration of a molecule that inhibits a given enzyme activity assay by 50% of the maximal value. The analogy is no coincidence, as life is fundamentally dependent on key enzyme functions for enabling growth and vitality.

The lethal dose of cyanide (molecular weight = 26) in humans is around 0.5 to 2.0 mg per kg of body weight of small animals or an oral dose of 50-100 mg could kill any human. Compare this to a small toxic organic molecule like methanol (molecular weight = 32), which has a lethal dose of ~5000 mg per kg of body weight for small animals or an oral dose of at least 50 g is required to kill a man. Arsenic (say arsenite, molecular weight = 122; LD₅₀ of sodium arsenite is ~20 mg/kg) and azide (N₃⁻, molecular weight = 42; LD₅₀ of sodium azide is ~20 mg/kg) are other examples of “popular” (or notorious?) simple inorganic anions that are also toxic at low levels, either over chronic or acute exposures. [The values given are rough factual approximations, derived from the ocean of published research literature and safety datasheets in the field. The reader may also verify such common data with simple queries on the internet.]

In this writing, I shall highlight the key mathematical mechanistic insights these simple anions (and some other cations too!) afford on the most fundamental mechanisms of life. The reader must face the reality of the premise I present here: if you are not concerned with the context of this article, you CANNOT claim that you are interested in biochemical/biophysical mechanistic underpinnings of life, YET! Just because there is some simple arithmetic involved, a biologist should not get discouraged either! Merely drawing directional correlations and not appreciating quantitative relations/logic is a serious disadvantage for any scientist and biology has had more than its elephant's share. If one says that there are four seats in a sedan, it cannot imply that four Asiatic elephants can drive it across from Kerala (south India) all the way to the African savannahs to meet their cousins there! Quite simply, quantitative understandings of life based in chemico-physics

MUST reign supreme in our understanding of life processes. If so, it will be disclosed that classical understanding of redox processes such as respiration (in which O₂ plays a role that does not conform to its physico-chemical properties) is untenable! If we take these properties seriously, it leads to an entirely different understanding of redox metabolism that agrees with experimental data, which together present the revolutionary discovery of how life is energized. Respiration is not based on proton pumping (the classical chemiosmotic “membrane-pump” idea), but on combustion (similar to that in a flame or combustion engine), but then, it only occurs on a nano-sized level (small volume ‘MURed BURNing’; acronym: MUR-BURN). Inhibition of this process by very low concentrations of cyanide (and some other low-molecular poisons) presents a strong and differentiating quantitative argument in favor of this new understanding. Entry of O₂ in the highly reduced environment of the cell (full of 1-electron donating redox complexes) inevitably generates ^{*}O₂⁻ (superoxide radical, formed via a highly exergonic and fast reaction upon 1-electron donation). This effective charge separation (ECS) equilibrium is fundamental, which at low ‘physiological’ concentrations of the latter generates important intermediary compounds (among which ATP is one!) and electromagnetic (EM) radiation (that can be resonantly transferred to other biological processes, a neglected form of energy and information transfer).

2. HOW IS CYANIDE PERCEIVED TO IMPAIR CELLULAR FUNCTION (IN THE CLASSICAL SCHOOL OF THOUGHT)?

The classical membrane-pump theory (CMPT) perception: The longstanding popular perceptions, as evident in textbooks, deem cell membrane-embedded proteins, deemed “cation pumps”, as the primary agents for driving the basic cellular electromechanical mechanism, which powers the cell. Such directional operation of an “intelligent membrane” is deemed to be the foundation of biological intelligence and is also supposed to afford the bioenergetic principle for life, yielding the vast amounts of chemical energy currency of ATP that living organisms must produce daily. In this contextual framework, the ETC or electron transport chain (an ensemble of multi-protein Complexes I through IV and some other associated biomolecules) works in a serial and sequential manner. It delivers electrons from NADH (made from glycolytic pathway and/or Krebs cycle) via multiple stationary proteins’ redox centers and mobile carriers (like coenzyme Q, which purportedly recycles by an elaborate exercise called Q-cycle)

only to reduce oxygen that stays bound at Complex IV (aka cytochrome oxidase), making water. Such an ETC must also work in this deterministic fashion so that matrix-protons are pumped out into the inter-membrane space. In turn, high amounts and gradients of these protons must be present for the Complex V to continuously recycle them for rotary ATP synthesis, when they return to repopulate the matrix! This chemiosmotic rotary ATP-synthesis (CRAS) theory has disconnected oxidative and phosphorylative reaction logic, which is coupled with the mechanical recycling of protons across the membrane in mitochondria (or chloroplasts and prokaryotic cells). The salient attributes of this theory are depicted in left panel of Figure 1.

Cyanide-sponsored inhibition: In this ETC-CRAS mechanistic scenario, the classical perception postulates that cyanide (and other heme-binders like azide) selectively and stoichiometrically binds to the membrane-embedded (or soluble) heme-proteins and disable their activity. Particularly, cyanide inhibits cellular respiration by purportedly disrupting the “electron transport chain (ETC) - Q cycle - proton pumping” mechanism on the inner mitochondrial membrane, by selectively binding to hemeFe of Complex IV and preventing

the transfer of electrons to oxygen (thereby disrupting the formation of water).

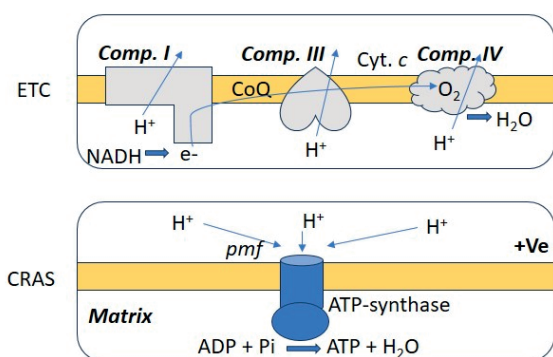
In short, cyanide terminates the classical bioenergetic routine by “the mechanical clogging” of the ETC at the distal end, thereby preventing the accumulation of protons in the intermembrane space for carrying out CRAS by Complex V. By messing up the cellular bioenergetic metabolism, the key physiological systems and processes, cyanide thus immediately arrests or impairs the following human systems: nervous (overall coordination), cardiovascular (circulatory), respiratory (O_2 intake associated with CO_2 purge), musculo-skeletal (movement), excretory-detoxification-acid/base balance (homeostasis), etc. Let us first see the cellular bioenergetics routine.

3. WHERE AND WHEN DOES THE CLASSICAL ETC-CRAS BIOENERGETICS PERCEPTION GO WRONG?

At the very outset and at every instance, it is wrong! Herein, I list only half a dozen blatant anti-realistic (deterministic) issues it overlooks regarding reactants and their environment:

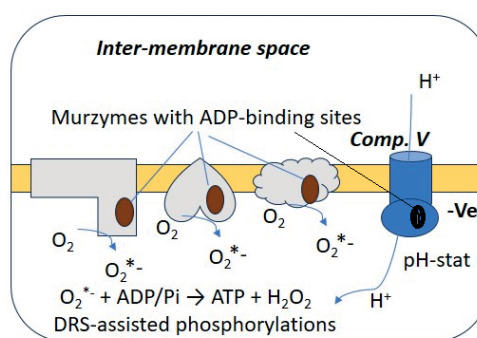
Evolutionary probability: How can there ever be a deterministic flow of electrons or pumping of ions with hundreds

ETC-pmf-CRAS (Deterministic/Electromechanical model)
H-centric, proton pumping, O_2 stays bound to Comp. IV
Diffusible Reactive Species (DRS) toxic/waste byproduct



Electron Transport Chain (ETC), David Keilin (Rudolph Marcus*)
proton motive force (pmf), Peter Mitchell* [* = Nobel Prize]
Chemiosmotic Rotary ATP Synthesis (CRAS), Paul Boyer*
No tangible theory/evidence to support the model!

Murburn (Stochastic/Simple Chemical Engine)
O-centric, Effective Charge Separation, O_2 mobile
Diffusible Reactive Species (DRS) obligatorily needed



Mured (closed) burning, Kelath Murali Manoj
Molecule-Unbound ion-Radical equilibriums
Reactions occurring in proton-limited milieu
Superoxide can make ATP in vitro and in situ!

Figure 1. Schematic of cellular powering: ETC-CRAS (left panel) and murburn models (right panel). The idea of ETC and ion pumps was mooted and advanced by scientists with Cambridge University associations (David Keilin, Robert Dean, Peter Mitchell, Alan Hodgkin, Andrew Huxley, Bernard Katz, Ian Glynn, Daniel Tosteson, Joseph Hoffman, etc.) and they used Rudy Marcus outer-sphere electron theory to justify the ETC. Paul Boyer (UCLA) spun his rotary ATP synthesis on this coterie's support, and his “rotary” model derived inspiration from Howard Berg's functional model for flagellar function. The murburn bioenergetics model was independently conceived by the author and relies on evidenced facts and defensible derivations thereof.

of proteins and other biomolecules spontaneously assembling themselves and orchestrating elaborate processes across three phases? And all that for availing a gambit logic, only to make the phosphoanhydride bond between ADP and inorganic phosphate? When does it sound logical in any segment? Where is the scope or evidence for the evolution of such a system?

Role of oxygen: Next, oxygen is not committed to stay bound and react only at a hemeFe of Complex IV (to receive four electrons and four protons in a hydrophobic membrane!), just to produce water (a relatively abundant molecule!). It is a highly active diatomic gas that can move freely and react with diverse (not just one!) redox active moieties in milieu, to receive electrons in a reducing environment. This is in fact, corroborated by observations.

Protons: Further, to imagine that protons go in and out across a membrane maintained at -200 mV (during active metabolism) is a farce! Particularly, when there is no way that the practically aprotic matrix will have more than finger-countable protons at a given instant! There is no question of specifically binding and pumping protons, when it is 100,000 times smaller than the smallest atom- hydrogen!

Mobile e-carriers and printed circuitry? Furthermore, to seek that a highly hydrophobic and bulky/immobile molecule like CoQ₁₀ would squirm into Complex I and traverse the lipid phase to bind to a specific locus at Complex III as CoQH₂ (that too, when another locus nearby within the same protein must bind oxidized CoQ and an oxidized protein molecule of cytochrome *c* must be bound at yet another locus!) is seeking the impossible! To imagine that several redox centers of unfavorable redox potentials and inter-moiety distances could somehow tunnel electrons is hoping beyond feasibility!

Rotary reversible function? A juicer gives juice and pulp through an aperture at the bottom when a grinding spiral piston is rotated clockwise. Can an anticlockwise rotation of the piston or inversion of the setup or introduction of the juice and pulp from the bottom afford fruit at the top? To top it all, how can a rotary molecule working across three phases auto-assemble?

Mutually exclusive options: It seeks high amounts of protons inside the matrix for the proton pumping ETC to work and low proton concentration inside for *pmf*-aided Complex V to work, at the same instant! (There is no staggered orchestration of the ETC to work at an instant to pump the protons out, lowering the TMP; to be followed by a harvesting of this TMP by Complex V! These activities are supposed to occur continuously and simultaneously!) Impossible!!

4. WHAT IS THE MURBURN PERCEPTION OF BIOLOGICAL INTELLIGENCE AND BIOENERGETICS?

Murburn concept is not a hypothesis (but a given murburn model for a particular process can be treated as one!). It is a forthright deduction from facts we know about cells and the components that make them up. Cells are made of molecules and ions, and murburn concept states that these components can have stochastic interaction/exchange of electrons, moieties and radiations among them. This postulation does not negate selective/specific affinity-based interactions or catalysis thereof. But it allows for important life-sustaining reactions to occur (albeit with significant uncertainty!) beyond the active site of gene-coded proteins. The scheme of reactions and its comparison with classical paradigms is given in Figure 2.

Under this purview, oxygen serves as an effective charge separation (ECS) agent, rapidly generating diffusible reactive species (DRS). Thus, all redox centers in a cell could potentially interact with oxygen! An electron residing at one locus of a molecule could be randomly (and practically instantaneously!) get mobilized to transiently go to another locus nearby, (on the same or on another molecule/ion) and this could be aided by diverse ions. Such processes can be aided by incident radiations or the interacting molecules/ions can also liberate some radiations. Thus, the primary drive for life (electromagnetic force) sets into action, and it involves the chemico-electromagnetic matrix (CEM) as an operational/intelligence principle. Murburn concept postulates that the tree of biological intelligence is rooted in ECS-DRS and stems from CEM. At a cellular level, biological intelligence is a primarily/essentially *EMERGENT* & *SALIENT* manifestation by/from CEM and its PCHEMS (the instantly acting physico-chemical attributes/properties of life, namely- powering, coherence, homeostasis, electromechanical and sensing-response activities, etc.). PCHEMS is the branches/leaves of the tree of biological intelligence. The fruits are the perpetuation mechanisms featured by central dogma. (Therefore, the central dogma is a deterministic fruit born on the tree of the biological intelligence sprouted from murburn!) While the classical perception deems intelligence to be *RESIDENT* in membranes (by virtue of the intelligence being *RESIDENT* in genes that deterministically produce proteins with selective active sites that can topologically identify all molecules and ions, and therefore specifically modulate their conversion/concentrations), murburn perception sees biological intelligence as a paradoxical “set of deterministic outcomes derived from delocalized uncertainty in chaos”.

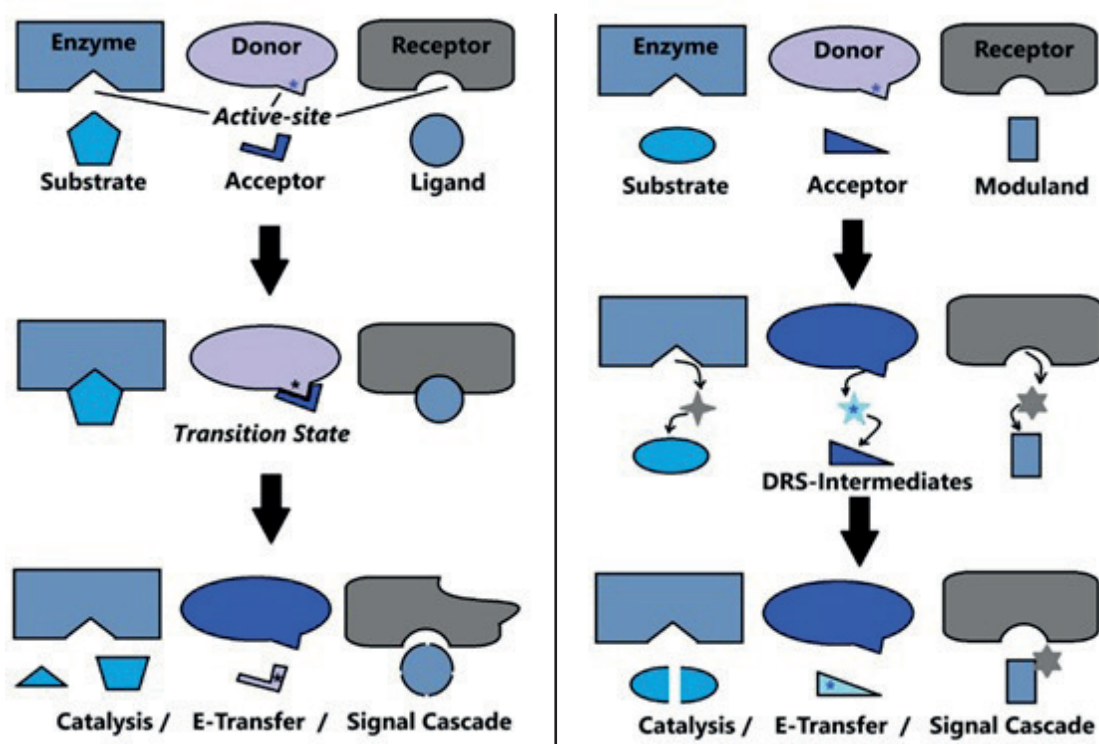


Figure 2. The molecular reaction mechanisms for classical and murburn biological processes. The topological recognition (affinity-based) active-site reactions mediated by different proteins (enzymes, e-donors and receptors) in classical perceptions (left panel) and murburn mechanism (right panel) are shown. Murburn is saliently characterized by the involvement of DRS. Murburn concept does not negate the classical mechanism; but is a larger set of outcomes. It also gives a broader picture and corrects misunderstandings and unrealistic aesthetics owing to narrow perspectives.

That is, murburn deems that controlled chaos also contributes as a quiescent source of deterministic intelligence in biology. This is quite like how one gets pretty deterministic pattern in chaos-ridden fractals like Sierpinski triangle.

At molecular and macroscopic levels, this new perception adds new whorls to our understanding of moiety-modification and electron-transfer catalysis, signal transductions, thermal-electrical-mechanical features, etc. Since the reaction can be mediated via DRS, a protein can catalyze the conversion of “substrates” of diverse dimensions and functional groups, and mediate various types of reactions to give an array of products. This may result in unusual kinetic profiles (unlike the classical hyperbola of Michaelis-Menten), demonstrate variable/non-integral stoichiometries and unusual dose responses. The DRS mediated events could also alter heat content (as the reactions are highly endergonic), affect the electrical property (as the DRS produced may be charged) and modulate the phase property (changing of hydration levels and conformation states of small and larger molecules/ions) of the system. Such

a grass-root level perceptual change affords us a new way to look at cellular bioenergetics and ATP synthesis. In short, murburn purview deems “oxygen-superoxide” one-electron equilibrium as the quintessence and elixir of aerobic life, which dynamically sets the chemical, electrical, mechanical, thermal and radiative energy/forces going.

5. WHAT DOES MURBURN BIOENERGETICS MODEL EXPLAIN THAT ETC-CRAS CAN'T?

I present half a dozen arguments in each of the subsections below (making two dozen in toto!) to argue against the ETC-CRAS model, but which are fully explainable with the murburn model.

Architectural realities: (1) The mitochondrion is not a solid hardwired electronic circuit with a DC voltage supply, akin to what is seen in a laptop! It is a squishy-spongy-fluidic micropot, with a membrane that has proteins randomly strewn around! (2) The mitochondrion is micro-dimensioned and this makes its interior practically aprotic. Same is the case with

other bioenergetic systems like chloroplasts, cyanobacteria and prokaryotes. The highly convoluted lipid folds in these systems further minimize proton/water activity. (3) The mitochondrial bilayered envelope has the internal membrane presenting high invaginations in multiple geometric formats, allowing greater protein loads. (4) The inner mitochondrial membrane is not any specially built proton-impermeable structure. A proton's dimension is in the range of femtometers (the dimension of hydrogen atom is in Angstroms) and it is known that bidirectional traffic of protons occurs across this membrane also (in the millisecond range; but the extent is lower owing to higher protein load). (5) There are no deterministic arrangements or distribution of complexes or other molecules along the cristae to support any purported error-free shuttling of electrons/protons along or across the membrane. The structures-distributions of respirasomes recently unraveled do not support the concept of ETC or proton pumps. (6) Mitochondrial membranes have high amounts of cardiolipin and quinones of various lengths, with the longer CoQ₁₀ being the most preponderant. This is inconsistent with CoQ's role as e-transporter!

Experimental observations: (1) There is little pH gradient observed across the mitochondrial membrane. In fact, it is known that the inter-membrane space (or periplasm) freely/readily equilibrates with the cytoplasm (or external environment)! (2) There have been disagreements about the possibility (Complex IV was not deemed a proton pump earlier!), the working stoichiometry (all proteins had different consensus-enforced stoichiometries in the course of time!) and the nature of ions pumped (Judy Hirst and Leo Sazanov argued if Complex I was a proton or sodium pump!). These show that cross-membrane ion movements are non-specific and of non-fixed stoichiometry. (3) Provision of higher amounts of NADH and oxygen gives higher amounts of DRS and higher amounts of DRS correlates to higher amounts of ATP synthesis and higher TMP (trans-membrane potential). (4) All Complexes I through IV can react with oxygen, produce DRS and bind ADP. (5) Complex V has higher affinity for ATP (compared to ADP) and is an ATPase when isolated/pure. (6) Variable and non-integral stoichiometries are observed of H:P, O:P ratios for various substrates.

Protein structural attributes: (1) Complex I has a highly protruding matrix-arm which has multitudes of redox centers (ETC route?) and the membrane-embedded foot region (the proton pump route?). (2) Complex II has several redox centers (including a heme in the trans-membrane region) and no definite quinone binding locus. (3) Complex III has not adequate redox connectivity of the minimal number of redox centers and

the hemes are solvent accessible. The matrix-ward distended lobes have little roles! (4) Complex IV has no known mechanisms/structural features to prevent the formation or release of DRS. (5) Complex V structure is too flimsy and asymmetric for it to have rotary functionalism. (6) Cytochrome *c*'s heme is solvent accessible and its topology is not conserved.

Theoretical foundations: (1) Some of the reactions of ETC are not thermodynamically viable (spontaneous) at the individual Complexes. For example: Complex I reaction with NADH, wherein CoQ serves as the electron acceptor. (2) Anaerobic ETs are kinetically inefficient for explaining outcomes in intermolecular transfers (and even considering intramolecular events, like in ET between hemes of CcO). (3) Q-cycle at Complex III is a schematically and practically "improbable" explanation. (4) The ETC was supposed to explain the formation of water without the formation of DRS. But the reality is that DRS is noted at high concentrations during ATP synthesis! (5) There is little logical (bimolecular chemico-physical) concurrent connectivity of the redox reaction with the phosphorylation reaction in the CRAS scheme. (6) CRAS seeks unjustifiable conformation changes of several proteins and small molecules.

Murburn obviates the need for vectorial-serial-sequential and precise electron transfer and proton-pumping stoichiometry and insulated (non-existent!) proton/electron circuits and does not seek for rotary ATP synthesis mechanism. The "irreducible complexity" of interlocked complexes and disconnection of redox reaction and phosphorylation reaction is not seen either.

6. ANALYZING THE EXPLANATIONS FOR THE ACUTE TOXICITY OF CYANIDE

We know that there is direct spectroscopic evidence of cyanide binding with heme *a*₃ of CcO and that ATP synthesis stops within minutes of cyanide administration. But what if we have a man knocked out cold first (murdered) by a blow and then hanged on a rope to claim that it was suicide? What if there are primary events (the debilitating ones!) that happen first before the secondary events (of lesser and consequential impact!) which set in later?

Let's see some basic math! For "immediate death", at least 50% of CcO in brain and heart must be inhibited within a few seconds to <2 min, upon ingestion of a 100 mg or such dose of cyanide. Let's calculate the time required for this process with the theoretical foundations given in Box 1.

For a 70 kg human, assuming an LD₅₀ of 3 mg/kg → ~210 mg total HCN (the preponderant species as pK_a is 9.2;

Scenario	Condition	Formula for $t_{1/2}$ (when $[RL] = R_0/2$)
Pseudo-First-Order	$L_0 \gg R_0$	$t_{1/2} = \frac{\ln 2}{k_{on}L_0 + k_{off}}$
Second-Order, Irreversible	$L_0 = R_0, k_{off} = 0$	$t_{1/2} = \frac{1}{k_{on}C_0}$
Second-Order, Irreversible	Any $L_0, R_0, k_{off} = 0$	$t_{1/2} = \frac{1}{k_{on}(L_0 - R_0)} \ln \left(\frac{2L_0 - R_0}{L_0} \right)$
Second-Order, Reversible	General case	No explicit solution. Solve integrated rate equation numerically.

Box 1. The theoretical premises for calculating the time required for half the population of receptor (R) saturation with the ligand (L), under various scenarios. The subscript 0 signifies original/initial concentration. When $L_0 = R_0$, we write C_0 .

molecular weight ≈ 27 g/mol) would be the lethal dose, making ~ 7.8 mmol in total. Let's assume that the density of human body and water are almost the same (which is in fact the case!) and that cyanide needs to be in non-aqueous phase too for causing inhibition (heme a_3 of 1 to 10 μM CcO is lodged in the inner mitochondrial membrane!). So, 7.8 mmol cyanide in 70 liters is ~ 110 μM concentration. Tissue levels of oxygen in physiology ranges from 10 to 30 μM levels in aqueous phases and oxygen is at least 5 times more soluble in lipid phase (whereas the ion of cyanide would not be favored in the lipid phase at all!). These realities would make oxygen very easily and more readily available to heme a_3 of CcO.

Now, let's put these realities in written and compare it with the known thermodynamic and kinetic constants of the interaction of several ligands with CcO.

At initial conditions ($t = 0$), the oxygenated system would be:

$$[E]_{\text{total}} = R_{\text{tot}} = 5 \mu\text{M} \text{ (average)}$$

$$[O_2]_{\text{free}} \approx 30 \mu\text{M}$$

$$[CN]_{\text{free}} \approx 110 \mu\text{M}$$

$$[E-O_2] \approx R_{\text{tot}}, [E-CN] = 0, [E]_{\text{free}} \approx 0$$

With constants of originally published for pure CcO preparation [values from literature advocated later are given in the adjacent large braces; researchers claimed that physiological mitochondria exhibit quite distinct and enhanced binding profiles!]:

$$K_d^{O_2} = 3 \times 10^{-4} \text{ M } [1 \mu\text{M}]$$

$$K_d^{CN} = 6 \times 10^{-4} \text{ M } [0.1 \mu\text{M}]$$

$$k_{on}^{O_2} = 10^8 \text{ M}^{-1}\text{s}^{-1} [10^8 \text{ M}^{-1}\text{s}^{-1}]$$

$$k_{on}^{CN} = 10^2 \text{ M}^{-1}\text{s}^{-1} [10^2 \text{ M}^{-1}\text{s}^{-1}]$$

$$k_{off}^{O_2} = K_d^{O_2} \cdot k_{on}^{O_2} = 3 \times 10^4 \text{ s}^{-1} [10^2 \text{ s}^{-1}]$$

$$k_{off}^{CN} = K_d^{CN} \cdot k_{on}^{CN} = 6 \times 10^{-2} \text{ s}^{-1} [1 \times 10^{-5} \text{ s}^{-1}]$$

1. Let us first see the equilibrium feasibility without competition (with only CN^- and CcO)

The fraction of CcO bound by CN^- (θ_{CN}) at equilibrium with fixed free $[\text{CN}^-]$ is:

$$\theta_{\text{CN}} = \frac{[\text{CN}]_{\text{free}}}{K_d^{CN} + [\text{CN}]_{\text{free}}}$$

$$\theta_{\text{CN}} = \frac{110}{600 + 110} = \frac{110}{710} \approx 0.155 \text{ (or 15.5\%)}$$

So even without O_2 , only 15.5% of CcO can be CN-bound at equilibrium!

2. Considering the presence of oxygen, it is evident that this value will only be lower at equilibrium. For competitive binding,

$$\frac{[E-CN]}{[E]_{\text{total}}} = \frac{[\text{CN}]/K_d^{CN}}{1 + [O_2]/K_d^{O_2} + [\text{CN}]/K_d^{CN}}$$

where $[\text{CN}]$ and $[O_2]$ are free concentrations at equilibrium. Plugging in the initial free concentrations as an upper bound, we get:

$$\frac{[O_2]}{K_d^{O_2}} = \frac{30}{300} = 0.1$$

$$\frac{[CN]}{K_d^{CN}} = \frac{110}{600} \approx 0.1833$$

Or,

$$\theta_{CN,eq} = \frac{0.1833}{1 + 0.1 + 0.1833} = \frac{0.1833}{1.2833} \approx 0.143 \text{ (or 14.3\%)}$$

So, maximum possible (with realistic and reversible binding, $k_{off}^{CN} \neq 0$) equilibrium CN occupancy of CcO in presence of oxygen will be $\approx 14.3\%$ (*only* 0.72 μM CcO of the total 5 μM CcO would be inhibited!). Therefore, the system can never reach 50% CN occupancy, making the concept of $t_{1/2}$ (time required for half saturation) undefined for this parameter set.

If we consider the later values (in large parenthesis), then the competitive scenario would be:

$$\theta_{CN,eq} = \frac{[CN]/K_d^{CN}}{1 + [O_2]/K_d^{O_2} + [CN]/K_d^{CN}}$$

$$\frac{[O_2]}{K_d^{O_2}} = \frac{30 \times 10^{-6}}{1 \times 10^{-6}} = 30$$

$$\frac{[CN]}{K_d^{CN}} = \frac{110 \times 10^{-6}}{0.1 \times 10^{-6}} = 1100$$

$$\theta_{CN,eq} = \frac{1100}{1 + 30 + 1100} = \frac{1100}{1131} \approx 0.973$$

This consideration would definitely allow $> 50\%$ saturation of CcO by cyanide. In this case, let us calculate the f_E for such a physiological regimen below.

Initial free enzyme fraction is:

$$f_E = \frac{1}{1 + [O_2]/K_d^{O_2} + [CN]/K_d^{CN}} = \frac{1}{1131} \approx 8.84 \times 10^{-4}$$

The value is low and both oxygen and cyanide can compete! Effective on rate of CN would be:

$$k_{on,eff}^{CN} = k_{on}^{CN} \cdot f_E$$

$$k_{on,eff}^{CN} \approx 10^2 \cdot 8.84 \times 10^{-4} \approx 0.0884 \text{ M}^{-1}\text{s}^{-1}$$

Then the pseudo-first order rate constant would be:

$$k_{on}[L]_{eff} = k_{on,eff}^{CN} \cdot [CN]_{free} \approx 0.0884 \cdot 1.1 \times 10^{-4} \approx 9.72 \times 10^{-6} \text{ s}^{-1}$$

Assuming that free concentration of ligand cyanide ($\sim 110 \mu\text{M}$) is much higher than the receptor CcO concentration ($\sim 5 \mu\text{M}$) in mitochondria, and if we consider $k_{off}^{CN} = 0$;

$$t_{1/2} \approx \frac{\ln 2}{k_{on}[L]_{eff}} \approx \frac{0.693}{9.72 \times 10^{-6}} \approx 7.13 \times 10^4 \text{ s} \approx 20 \text{ hours!!}$$

Else,

$$k_{obs} = k_{on}[L]_{eff} + k_{off}^{CN} \approx 9.72 \times 10^{-6} + (1 \times 10^{-5}) \approx 1.972 \times 10^{-5} \text{ s}^{-1}$$

In this scenario also,

$$t_{1/2} = \frac{\ln 2}{k_{obs}} \approx \frac{0.693}{1.972 \times 10^{-5}} \approx 3.51 \times 10^4 \text{ s} \approx 10 \text{ hours!!}$$

The difficulty lies in the actual assessment of cyanide concentrations in vivo. Even with “unreal later values” (wherein it was assumed that CN toxicity is and must be explained with the binding-based outcomes), we get a significantly slow process, which would make the equilibrium saturation of CcO-CN an improbable kinetic explanation for CN toxicity.

If we still want to explain the quick death outcome as a result of cyanide binding, we must indulge an argument for non-competitive processes, or yet again unreal higher binding affinity. Else, the physiological steady state (Haldane-Briggs treatment) must be invoked, where oxygen concentration is purportedly lower. If CN is supposed to explain lowering of oxygen consumption, we cannot assume that oxygen concentration would be lowered in mitochondria in physiology! The utilization of oxygen in mitochondria occurs at CcO and this is what CN binding is supposed to involve and explain, in the first place! Availing vain circular logic cannot explain directionality. I shall explain this once more: To speed up CN^- binding to match observed poisoning (minutes), modelers lower $[O_2]$ in calculations (e.g., assume $[O_2] = 1 \mu\text{M}$ instead of $30 \mu\text{M}$). But lowering $[O_2]$ requires oxygen consumption by CcO. If cyanide is already inhibiting CcO, O_2 consumption should stop! This means that O_2 should rise back to high levels, not stay low. Thus, the model uses a pre-inhibition low $[O_2]$ to explain fast inhibition, but inhibition itself prevents the low $[O_2]$ from being maintained. This is a catch-22, in terms of logic. (One cannot have the cake and save it too, in simple literature!!)

Now, if one accounts for the other major highly populated heme proteins like Hb ($\sim 750 \text{ g}$ of tetramer in body at 64500 g/mol gives 0.0465 moles of Hb hemeFe sites) and Mb ($\sim 50 \text{ g}$ of monomer in body at 17000 g/mol gives 0.00294 moles of Mb hemeFe sites), we would have a total approximate amount of ~ 0.0494 non CcO heme sites. In comparison, CcO (assuming mitochondria form ~ 1 to 2% of body volume, a $5 \mu\text{M}$ concentration would give 5 micromoles of CcO hemeFe) would be only at ~ 0.000005 moles, giving a ratio of $[0.0494 / 0.000005]$ or $\sim 10,000:1$ competing non-CcO to CcO hemeFe sites, making availability of CN for binding with CcO a low probability event in immediate time scales. (This is discounting

other abundant heme proteins like the membrane-embedded P450s or soluble peroxidases/catalases in milieu!) Also, the amount of CcO in the body far exceeds the toxic dosage of cyanide, by 100:1 or 1000:1. The 1:1 logic of cyanide-binding based toxicity would still leave behind enough functional heme proteins or/and CcO!

Upon oral consumption, cyanide should take minimally a few hundreds of seconds (at least 2 to 3 minutes) to reach the organs of brain and heart, and even more for reaching an effective concentration of 110 μM in brain/heart cells via diffusion from blood to reach mitochondrial lipid membranes (within the cells therein). Therefore, acute (almost instantaneous!) toxicity documented with its low doses (say, 100 mg level amounts per human) cannot be explained by binding-based perceptions! Also, oxygen would be available at much higher concentration than cyanide at any initial time (as it is more soluble/active in lipid) and is a relatively better ligand for the onward process. So, binding of oxygen should not be inhibited by cyanide at the acute scales of reaching physiologically toxic levels of cyanide, if binding-based rationale were the governing criteria in this context.

Now, let's dive a bit deeper, by comparison with two other inhibiting hemeFe ligands (for CcO), the gaseous carbon monoxide and the ionic azide, with experimental data in the first four columns and derived data in the last three (Table 1).

Discrepancy between K_d and K_i/K_M : In a simple system, K_d is experimentally determined from simple binding studies (like equilibrium dialysis; and theoretically, it can also come from kinetics: $k_{\text{off}}/k_{\text{on}}$) and is equal to the equilibrium (kinetically determined) inhibition constant K_i/K_M . (It must be noted that when we refer to the substrate, we deal with K_d and K_M of the same whereas when we discuss an inhibitor, we refer K_d and K_i of the particular molecule. There are complexities when a substrate can also have a K_i , wherein an excessive amount of substrate may lead to inhibitions.) Also, the classical Michaelis-Menten theory dictates that for a substrate, K_M

must be greater than K_d , as $K_M = K_d + k_2/k_1$. In Table 2, they are wildly different for both the substrate of oxygen and the inhibitor of cyanide.

For oxygen: $K_d = 3 \times 10^{-4} \text{ M}$ (300 μM), but $K_M = <1 \times 10^{-7} \text{ M}$ ($<0.1 \mu\text{M}$).

For cyanide: $K_d = 6 \times 10^{-4} \text{ M}$ (600 μM), but $K_i = 2 \times 10^{-7} \text{ M}$ (0.2 μM).

This 3000-fold difference between the K_d and K_M/K_i for oxygen and cyanide (respectively) is theoretically NOT PERMITTED (impossible!) for simple binding and signifies that the physiological reaction may not be dependent on mere binding-based processes alone but could result from the dynamics DRS in milieu and CcO/other experimental substrate! This is confirmed with the findings in the P450 reaction system wherein oxygen is found to have similar attributes! Murburn provides a simple explanation that the experimental catalysis is mediated via DRS whose instantaneous reaction is sensitive at submicromolar ranges (practically zeroth order at micromolar concentrations)! Therefore, the reaction would be very efficient and would practically give a steady rate for different molecules, which could be desired for routine redox metabolism.

Efficacy parameter: This newly introduced “index” rewards fast recognition/binding (high k_{on}), tight overall receptor-ligand interaction (low K_d or high K_a in the overall sense) and high residence time of the RL intermediate (which could enhance the formation/reaction dynamics of DRS). It can be envisaged that binding would be a function of two concentrations: that of the receptor and the ligand, and the unit of M^{-2} for binding efficacy signifies this aspect. The efficacies of CO (a known superior ligand of hemes!) and CN are vastly different and if binding-based efficiency were the ulterior logic for overall outcomes, we should have CO as the most impacting inhibitor (physiologically) but this is not the case! CO barely makes one unconscious at 40% (some smokers have ~10% CO in their blood!) whereas cyanide is lethal at 2.5%. Cyanide is a lesser efficient ligand in comparison to CO and

Table 1. Experimental and derived thermodynamic and kinetic constants for CcO for oxygen and three simple ligands/inhibitors

Ligands		k_{on} [$\text{M}^{-1}\text{s}^{-1}$]	K_d [M]	K_M or K_i [M]	IC_{50} [M]	$^{\text{a}}k_{\text{off}}$ [s^{-1}/Hz]	$^{\text{b}}\tau$ [s]	$^{\text{c}}\text{Efficacy}$ [M^{-2}]
Substrate O_2		1×10^8	3×10^{-4}	$<1 \times 10^{-7}$	NA	30000	0.00003	1×10^7
1	Carbon monoxide	8×10^4	4×10^{-7}	3×10^{-7}	4×10^{-5}	0.032	333	6.7×10^{13}
2	Cyanide	1×10^2	6×10^{-4}	2×10^{-7}	5×10^{-5}	0.06	16.7	2.8×10^6
3	Azide	1×10^6	6×10^{-5}	3×10^{-5}	7×10^{-7}	0.6	1.7	2.8×10^{10}

$^{\text{a}} = k_{\text{on}} * K_d$; $^{\text{b}} = 1/k_{\text{off}}$; $^{\text{c}} = k_{\text{on}} / (K_d * k_{\text{off}})$ or $(K_a)^2$

azide, as ratified by the binding efficiency index and awareness from other/earlier ligand binding studies with hemes. The fact is that a long-lasting CN-bound complex (high τ or low k_{off}) cannot explain the onset of immediate toxicity with respect to oxygen consumption. It can only explain toxicity after significant exposure time! Surely, the physiological outcome of immediate toxicity must have to do with a pre-existing state, rather than with oxygen utilization per se. The most important but subtle argument is: Oxygen deprivation kills in 4 minutes or so. Cyanide administration kills at much lesser time. So, cyanide must mess something that is more reactive and more needed than oxygen, and this is DROS!

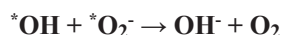
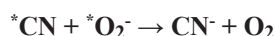
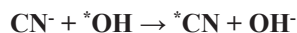
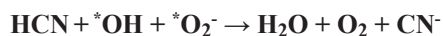
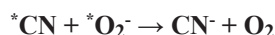
Miscellaneous aspects: The classical view leaves a lot of related questions unanswered and a curiosity driven approach will show us that there is more to the issue than what is apparent.

(1) How are photoreception (visual transduction and the functioning of nerves) in retina and photosynthesis in plants (which is not dependent on Complex IV) acutely affected by cyanide? The pertinent catalyst in these systems is not hemeFe but the common factor of carotenoid! The retina has one of the highest oxygen demands in the body and the photoreceptors and the optic nerve are especially dependent on oxygen but the classical theories do not attribute any role for the diatomic gas. Murburn model directly involves oxygen in the photo-oxidative processes in the two systems. Cyanide inhibits even nitrogen fixation, and these are effectively reasoned by considering that all these physiologies obligatorily involve DRS!

(2) Specific antidotes that work clinically like hydroxocobalamin (scavenges free CN^-) and nitrites (generate methHb to bind CN^-) need not support the idea that free cyanide in tissue is the “inhibitor” acting by binding hemeFe centers. It can also be because free cyanide is lesser available to mediate redox scrambling or DRS disruptions!

(3) Let's say lack of oxygen makes the pyruvate to lactate pathway more active, and this is the cause for lactic “acidosis” found at the later onset of cyanide poisoning. But then, pyruvate ($\text{pK}_a \sim 2.5$) is a stronger acid than lactate ($\text{pK}_a \sim 3.9$) and formation of lactate would incur the usage of protons from the milieu. Then how can it lead to acidosis in cyanide poisoning?! The oxidation of cyanide (or hydrocyanic acid, $\text{pK}_a \sim 9.3$) to cyanic acid (HOCN , $\text{pK}_a \sim 3.4$) with murburn processes is the natural explanation, which is supported with the observation of cyanate in such cases of poisoning. These initial murburn catalytic cascades are bimolecular radical reactions, which are thermodynamically viable and kinetically facile. They regen-

erate cyanide ion and deplete DROS in the proton-deficient mitochondria. The mass-charge balanced relations which make cyanide a highly efficient inhibitor are:



Under acidic conditions, cyanide binding to heme also becomes efficient thereafter, which is what is noted in later phases of higher cyanide poisoning! Cyanide is known to prefer the Fe(III) state of natural proteins and synthetic hemeFe containing molecules, by orders of magnitude (doi: 10.1002/anie.200804116). Therefore, it is difficult to accept that somehow, a reduced form of CcO binds more efficiently with cyanide in vivo owing to some other preexisting physiological conditions.

(4) There are certain plants/organisms (with alternate oxidase, AOX) that are somewhat resistant to CN poisoning but it does not mean that they are immune to cyanide. The diiron centers of AOX in these systems could generate DRS more effectively than their depletion rate by CN. Also, AOX could deplete peroxide formed better.

(5) Cyanide only takes out Complex IV from the ETC. The other Complexes I thru III are still available for function (of which Complexes I & III are purported to be efficient proton pumps!). They could also “pump protons to build *pmf*”, if such a mechanism were to be relevant/operational. (Particularly considering that each complex is supposed to work specifically and independently!)

Such issues are sufficiently addressed by the DRS-based murburn concept, which attributes catalytic roles to cyanide. The latter catalytically converts (without getting used up!) radicals like superoxide and hydroxyl to water, and this reaction would be hyper-efficient in proton-limited regimes. Oxygen is

needed to make the radicals via equilibrium-driven processes (and not for staying wedded to Complex IV, as the classical theory deems!), which electrify cellular activities and catalyze many processes in diverse physiologies. DRS are involved in CEM and PCHEMS, the core of biological intelligence and bioenergetics, and this is why life ceases almost instantaneously with cyanide administration. This is the most essential awareness for mankind for understanding the mechanistic underpinnings of life!

7. THE CURIOUS CASE OF AZIDE

Quite like cyanide, azide is also a strong field, π -acceptor and end-on binding ligand of similar mass/length dimensions that prefers the heme Fe III state (in comparison to the 1e-reduced ferrous species, Fe II) for binding. Azide produces a highly concentration dependent impact on heme-enzyme activity, with higher concentrations (say, millimolar levels) inhibiting and lower concentrations (say, micromolar levels) enhancing the outcomes. We thoroughly investigated this process and found that it was not because of its binding with the proteins. It was due to the reactivity of the azide ion, which is an efficient 1e donor in heme-enzyme mixtures. The presence of azidyl (which is pretty long lived and therefore, can be catalytically viable on its own merit!) and cyanyl radicals in such biological samples have been confirmed with EPR techniques. Therefore, these ions could also affect the outcomes of heme-enzyme processes not just by binding, but also due to their inherent reactivity!

8. THE BIOENERGETIC TOXICITY OF OTHER IONS AND MOLECULES

In vitro, even 1e-active cations (like iron and copper) can modulate the outcomes of such enzyme-catalyzed e-transfer processes in the same manner. Their ability to impact the outcome by virtue of binding can be ruled out at low concentrations, and the murburn reaction-based rationale must be factored in! If the classical ETC/ion-pump model is strictly about selective channels and stoichiometric throughput, it fails to explain the broad-spectrum toxicity of chemically diverse ions. A system based on precise locks-and-keys (selective pumps/channels) and controlled conveyor belts (stoichiometric enzyme complexes) should only be disrupted by agents that fit those specific locks or jam those specific belts. Yet, we see toxicity from diverse species such as:

- (i) Redox-active cationic metals (Fe, Cu, Cr, Mn, etc.),
- (ii) Relatively redox-inert, charge-dense metals (Al^{3+}),
- (iii) Thiol-binders (Hg^{2+} , Cd^{2+} , Pb^{2+}),

- (iv) Phosphate mimics (AsO_4^{3-}),
- (v) Small anionic/acidic inhibitors (H_2S , CN^- , N_3^-),
- (vi) Simple electrolyte imbalances (K^+ , Na^+),
- (vii) organic ions like dinitrophenolate and other molecules.

This diversity is incompatible with a purely mechanistic (deterministic/stoichiometric), selectivity/specificity and throughput-based model. The ETC/ion-pump model functions via selective channels and stoichiometric throughput. Therefore, it fails to explain the broad-spectrum toxicity of chemically diverse inorganic and organic cations and anions. A system evolved and based on precise locks-and-keys (selective/specific pumps/channels) and controlled conveyor belts (stoichiometric enzyme complexes) should only be disrupted by agents that fit those specific locks or jam those specific belts! But this is not the case and the reality agrees with the murburn model's view of mitochondria/cells as a stochastic thermodynamic redox-active basin. Energy transduction is not a series of isolated electron transfers coupled to ions pumps or stoichiometric handoffs along a rigid chain. But it is rather a thermodynamically governed, probabilistic flux of electrons and protons via diffusible radicals (like $\bullet\text{O}_2^-$, $\text{HO}\bullet$) and other redox buffering molecules (CoQ, cytochrome *c*) across redox potential gradients. In the murburn framework, the primary property is the redox potential landscape and the stability of key redox intermediates. Any ion that can perturb this thermodynamic landscape (by catalyzing side reactions, scrambling gradients, or destabilizing critical radicals) would be toxic. Now, the epistemological mapping of the ions and organic molecules to the murburn thermodynamic/redox disruption principle would be:

$\text{Fe}^{2+}/\text{Cu}^+$: Predicted to be supremely toxic as they catalytically amplify the production of diffusible, indiscriminate hydroxyl radicals ($\text{HO}\bullet$) via Fenton chemistry, scrambling the controlled redox gradients essential for murburn's energy transduction.

CN^-/N_3^- : Their toxicity is not about "blocking a specific pump or circuitry in a chain." It's about quenching a critical diffusible reactive species, collapsing the thermodynamic pull of electrons into water. This is not a mechanical jamming of circuitry.

$\text{Pb}^{2+}/\text{Cd}^{2+}/\text{Hg}^{2+}$: Their high affinity for thiolates disrupts the redox-active -SH groups of proteins and small molecules (like glutathione) that maintain the reducing milieu necessary for managing the diffusive radical flux. They poison the thermodynamic basin's "redox buffer."

Al^{3+} (redox-inert): The ions' high charge density can distort local electrostatic fields, potentially altering the diffusion and

interaction probabilities of charged species (protons, electrons, radical ions) within the basin. It's a local thermodynamic perturbant.

AsO_4^{3-} : It short-circuits the phosphorylation logic by forming an unstable anhydride, effectively dissipating the thermodynamic potential that was to be captured in the stable phosphoanhydride bond of ATP.

K^+/Na^+ Imbalances (mM ranges): Even these can be seen as disrupting the ionic strength, hydration scenarios (lattice structures) and which in the murburn view is a bulk thermodynamic consequence of redox reactions, not its primary driver.

Dinitrophenol to carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP) to free fatty acids to uncoupling proteins: These are no trans-membrane proton-shuttlers (the classical Mitchellian "pmf-uncoupler") but they are actually interfacial DRS-modulators disrupting the essential/routine redox traffics. Several other organic and non-ionic molecules that do not have protonation-deprotonation abilities are also effective at this function (discounting the physiologically non-existent *pmf* arguments, as graphically listed in Figure 6 of my group's paper on the uncouplers affecting flagellar motility; please refer my JBSD 2023 publication in this regard).

While the classical (throughput ion-selection and pumping) model asks: "*Does this ion fit into or block a specific machine?*", the murburn (thermodynamic) model asks: "*Does this ion destabilize the diffusive redox equilibrium of the system?*" Only the latter provides a first-principle, unified explanation for the entire spectrum.

Quite simply, the first model assumes that the ion-pumping/channeling/binding mechanisms are selective and highly efficient (to explain for physiological outcomes) but for the pathological outcomes, their actions are to be deemed non-selective and inefficient, to explain for inhibitions. Within experimental setups of mitochondrial or chloroplast ETC, electrons can be input from diverse molecules/ions and the electrons can be drained to diverse molecules/ions. This agrees only with the murburn model. Similarly, the serial ETC was conceived to obviate DRS formation, to keep the system "error-free". But then, DROS are found in the mitochondria and in fact, oxidative ATP-synthesis does not occur without DROS formation. Once again, these realities agree only with the murburn model.

Therefore, from the perspective of explaining the diversity of ionic toxicities with a single, parsimonious principle, the murburn model holds strong logical ground. Its foundation in thermodynamics and probabilistic redox chemistry inherently accounts for why such a wide array of chemical agents can be

toxic (not by acting as selective key-jammers but as general thermodynamic perturbants). It frames toxicity as a fundamental disruption of a pervasive, delicate redox equilibrium, a principle that applies equally to a redox-active transition metal and a charge-dense inert cation (irrespective of dimensions, charge, valencies, hydration states, etc.).

9. HYPOTHESES TESTING OF THE TWO MODELS FOR OXIDATIVE PHOSPHORYLATION

We have two hypotheses:

ETC-CRAS model: Oxidative phosphorylation requires (a) a sealed, insulating membrane to maintain a bulk Δp across itself (in two phases), (b) stoichiometric, electro-mechanically coupled proton pumping by discrete complexes embedded in this bidirectionally selective membrane, and (c) specific electron-circuitry to prevent side reactions (like DROS production).

Murburn model: Oxidative phosphorylation is achieved via DRS chemistry in a membrane-proximate and "proton-deficient" phase. Protons are not cycled in this inherently "leaky" and promiscuous system, but thermodynamically facile radical reactions are stabilized to bring about an otherwise unviable reaction (complexing of two negatively charged moieties).

Evaluation of SCENARIO I [ETC-CRAS model as H_0 vs. Murburn model as H_a]

Let's consider the following premises-

1. Physical scale mismatch: Proton (0.00002 Å) vs. protein/hydrogen atoms (2 Å). The membrane and its proteins are inherently porous to protons on relevant timescales. The concept of a "proton-tight" insulated compartment is physically unrealistic and a protein pumping such small ions is unthinkable.

2. Spatio-temporal disconnect and structural incompatibilities: In Complex I, the redox chemistry (μs in the matrix arm) is structurally and temporally disconnected from the purported mechanical pumping (ms in the membrane foot). A direct, stoichiometric, electro-mechanical coupling (a mechanism is unseen in any other realms!) is physically implausible.

3. Aprotic milieu: Pumping discrete protons from the non-aqueous, protein interior of a membrane is a thermodynamic and kinetic absurdity. Also, there is little pH gradient observed in physiology (almost a three-order gradient was originally mandated under the Mitchellian postulates!) and some organisms function even in alkaline environments.

4. Experimental reality: I/O promiscuity: Electrons can be fed into and drained from the system by a vast array of non-physiological donors/acceptors. This demonstrates a lack of

strict substrate specificity, indicating a system interfacing with a diffusible redox pool. I/O variability: The ratio of proton:ATP or NADH:ATP is different across labs and is non-integral.

5. DROS are Obligatory: oxidative ATP synthesis is mechanistically linked to the formation of DROS, which can drive ATP synthesis in vitro and in situ with simple/viable exergonic reactions. This is a fatal contradiction for a model (classical) conceived to be an error-free, insulated wire, but is a core prediction of a model (murburn) based on diffusive radical chemistry.

6. Effect of inhibitors: A stoichiometric binding-based inhibition (by species such as cyanide binding at Complex IV) would give reduced efficacy (as a result of lowering the contribution of proton pumping by that agent). The murburn model would predict a global disruption due to altered DROS dynamics, depending on the catalytic ability of the inhibiting molecule.

Premises 1, 2, and 3 directly falsify the foundational physical assumptions of H_0 . A model based on a non-existent insulator (premise 1) and an impossible pump mechanism (premises 2 & 3) is not just incomplete; it is fundamentally flawed at the level of physical chemistry. Premise 4 (I/O Promiscuity) is a strong predictive failure of H_0 (which predicts high specificity) and a predictive success of H_a (which expects a diffusible redox pool accessible to many reactants). Premise 5 (DROS are Obligatory) is a direct contradiction of H_0 's core purpose (to avoid free radicals) and a direct support for H_a 's core mechanism. Premise 6 (the core focus of this writing) conclusively rejects H_0 and yet again validates H_a 's relevance.

Verdict: H_0 (ETC-CRAS model) is rejected. H_a (Murburn) is not merely an alternative; it becomes a highly viable hypothesis consistent considering the chemico-physical facts of the system.

Evaluation of SCENARIO II [[Murburn model as H_0 vs. ETC-CRAS model as H_a]]

What criteria could reject H_0 (Murburn)? We would need to demonstrate:

A. A true, bulk-phase chemiosmotic coupling in a perfectly sealed system (with continuously generated and sustained pH gradients) that is abolished by a tiny mechanical leak in the membrane (which would dissipate the *pmf*!). But ongoing oxidative phosphorylation was demonstrated with disrupted mitochondrial membranes also!

B. Demonstration of ATP formation from ADP+Pi at physiological pH without the chemical consumption of protons. (In CRAS, protons are only mechanical rotators of the shaft of

Complex V and demonstration of ATP-formation with an artificially provided pH gradient does not establish this important need! Murburn accepts the ADP+Pi+H⁺ reaction giving ATP!).

C. The complete absence of DROS during coupled phosphorylation and inability of the Complexes to generate or utilize DROS.

D. Absolute substrate specificity for physiological electron donors/acceptors, with no activity from promiscuous redox agents.

E. Stoichiometric, invariant and integral conserved reactants and product ratios.

F. An all-or-nothing inhibition that cannot be explained by the quenching of a critical, high-flux component within a diffusive network. (Inhibition of ATP synthesis at Complex I with an agent like rotenone is not a support of ETC-CRAS!)

Premises A-C: The required conditions for H_a (a sealed insulator, discrete but synchronous proton pumps and staggered harvesting by Complex V) are not seen/achieved as of date and do not appear to be achievable either. Therefore, H_a cannot be considered as an alternate/viable hypothesis. We cannot reject H_0 in favor of an improbable alternative. Premises D-E: These are again not observed but direct observations are consistent with H_0 . Regarding premise F, till date, we have no observations or theoretical consideration (other than biased aesthetics and authority-enforced consensus!) to reject H_0 .

Verdict: H_0 (Murburn) cannot be rejected. In fact, the attempted alternative (H_a) appears to be physically implausible based on first principles. The observable data further questions H_a and corroborates H_0 .

In toto, when the analysis is grounded in the unavoidable physical chemistry of the system: the size of a proton, the porosity of membranes, the distribution of components, the timescales of reactions, and the obligatory role of radicals, etc. The outcome is clear:

1. The ETC-CRAS (throughput/pump) model is conclusively falsified. Its foundational assumptions about insulation and mechanistic pumping are incompatible with the scale and nature of the physical world in which mitochondria operate. It is a conceptually flawed model that does not agree with observed realities. Its "successes" (like explaining inhibitor sites, proton-assisted ATP-synthesis and uncoupling, etc.) are misinterpretations, which are now better explicable under the murburn purview. Its failures (low water activity/proton availability, explaining DROS, promiscuity, ionic toxicity, protein structures with high amounts of redox centers, etc.) are fundamental to understanding the system.

2. The murburn (thermodynamic/diffusive) model is validated as the superior framework that supports the soft-fluid matter of cells. It starts from physically realistic premises (diffusion, porous membranes, redox-radical chemistry, etc.) and directly predicts or accommodates all the observed phenomena: ionic toxicity (unified explanation via thermodynamic/redox perturbation), DROS formation (not a leak, but the essential mechanism), I/O promiscuity/variability (expected in a diffusive redox pool and aids in stochastic flexibility), “coupling” (a probabilistic emergent property of the system’s organization, not a deterministic gear-driven mechanical link).

The murburn model provides the first-principle, parsimonious, and physically coherent explanation for bioenergetics. The classical model, despite its deep entrenchment in textbooks, fails at the level of basic physical plausibility and cannot explain the full spectrum of evidence without self-contradiction. The path forward is to fully embrace the murburn premise as the new null hypothesis and demand that all experimental data (from the structure of Complex I to the effects of cyanide and the impact of various drugs) be re-interpreted through its lens. The burden of proof has decisively shifted and this rigorous reevaluation shows that the murburn model prevails.

10. SO, WHAT DOES OXYGEN DO IN THE MURBURN VIEW OF CELLULAR FUNCTION?

The seamlessly unifying principle of murburn concept elaborates the roles of oxygen and is pictured in the schematics shown in Figure 3. From the same, it would also be clear as to why cyanide practically instantaneously (in a practically diffusion limited kinetics, as the bimolecular radical reactions are highly efficient) derails normal physiology.

Oxygen’s role is ubiquitous and widespread in cells because the $O_2/\bullet O_2^-$ pair is the most abundant and potent tuner of the redox field which is the driver of life; it is involved in practically every process that requires energy transduction. This is the reason that it is acutely needed (to displace the equilibrium to the right, for the formation of superoxide!) and it is the reason cyanide knocks out instantaneously (by fast reaction with the DRS that are present within a few seconds wherever it can reach!).

Thermo-chemical bioenergetics: The common (but yet unrealized primary energy currency) is not ATP; it is the redox gradient maintained by the $O_2/\bullet O_2^-$ couple. Heat production, ATP-synthesis, ion gradients, and mechanical work are different manifestations of this gradient being discharged through different material systems.

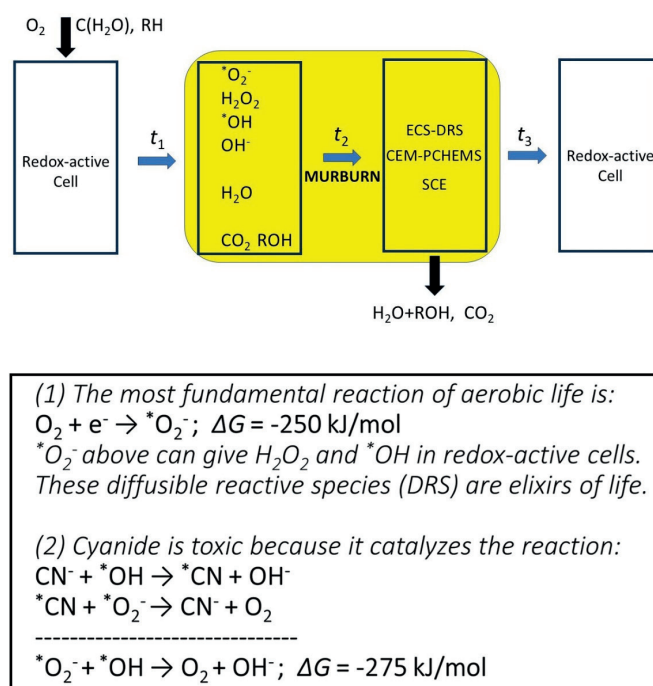


Figure 3. The murburn view of cellular physiological cycling as “simple chemical engine”. The perspective for chemical interactions is shown with $C(H_2O)$ as a simple carbon-based water-soluble molecule which serves as substrate for classical enzymes and RH as lipid-soluble organic xenobiotic molecule (murburn substrate). ECS-DRS mechanisms in the redox active cells break down or oxidize the two molecules into CO_2 and ROH . While the gaseous CO_2 effuses out, ROH is washed out with water. During the whole process, heat is produced, radiations are emitted, electrical activity is noted, etc., all because of the ECS-DRS-CEM-PCHEMS-SCE functionalism afforded by murburn. The inset at the bottom shows that cyanide converts the essential DRS into water and that this is way more viable than the oxygen superoxide equilibrium itself. As a result, as there is not enough lifetime for DRS, the light of life shuts down.

Cellular coherence & homeostasis: The chemo-electromagnetic matrix (CEM, set forth with the primary redox couple!) connects and networks cellular components chemically (electronically) and with radiations. The oxygen-water conversion and physiological cycling based on this logic is the key to cellular homeostasis.

Sensory/mechanical transduction/relay: It is mostly associated with oxygen-superoxide equilibrium and redox-activated hydration (and Lewis-induction driven?) dynamics. Whether in a mitochondrial membrane, a neuron’s axon, a photoreceptor

disc, or a sarcomere phase-change, the coupling between electron transfer (redox change) and proton/ion/water rearrangement (hydration change) is the fundamental step.

11. CONCLUSION

The classical model sees a world of specialized analog devices quite similar to man-made machines: a pump, a wire, a rotary machine, etc. Murburn sees a world of general-purpose redox-hydration chemistry performing different functions based on context and architecture, which enable cells to function as simple chemical engines. Quite simply, the ion-pumping and ETC based explanations become redundant in the context of cyanobacteria, which have neither free protons nor the scope for bidirectional ETCs in the same membrane! Also, erythrocytes (which lack nucleus for genetic governance and mitochondria for adequate ATP production) and plant cells (which lack the pertinent membrane protein pump to maintain Na/K levels) outrightly violate the mandates of the classical model and can be adequately explained with murburn considerations. The world MUST wake up and see

the explanatory power of murburn! Figure 4 shows the picture from a LinkedIn article I penned a few weeks back in the context of this writing, hoping that someone from mainstream would read and appreciate the ideas. This is one of the most important papers I have written and I don't consider it worth adding up a list of references for the work. Quite simply, for whom is the question! Given the importance of such a fundamental work, it should have garnered hundreds and thousands of citations by now! When will physicians and teachers take note? We are talking about the most fundamental pivot of life here... But yet!!! I don't care for the citations anymore (by whom, is the question!). But I do want textbooks to be changed, for the forthcoming generations. For, there is little to be gained by learning redundant ideas on the most important aspects of life and there is a lot at stake for re-interpreting important clinical data.

In 2006, I had questioned the P450cam model of xenobiotic metabolism and disseminated the murburn model in lieu (as a preprint at ARXIV). The recalcitrant trillion-dollar pharmaceutical industry and its prop-up academia's enforced consensus de-

Murburn explanation for cyanide toxicity: Experimental & theoretical aspects

Cyanide does more to inhibit heme enzymes, than merely serving as an active-site ligand

A Parashar, A Venkatachalam, DA Gideon, KM Manoj

Biochemical and biophysical research communications 455 (3-4), 190-193

58

2014

Atypical profiles and modulations of heme-enzymes catalyzed outcomes by low amounts of diverse additives suggest diffusible radicals' obligatory involvement in such redox reactions

KM Manoj, A Parashar, A Venkatachalam, S Goyal, PG Singh, SK Gade, ...

Biochimie 125, 91-111

70

2016

Classical and murburn explanations for acute toxicity of cyanide in aerobic respiration: A personal perspective

KM Manoj, V Soman

Toxicology 432, 152369

41

2020

Acute toxicity of cyanide in aerobic respiration: Theoretical and experimental support for murburn explanation

KM Manoj, S Ramasamy, A Parashar, DA Gideon, V Soman, VD Jacob, ...

Biomolecular concepts 11 (1), 32-56

64

2020

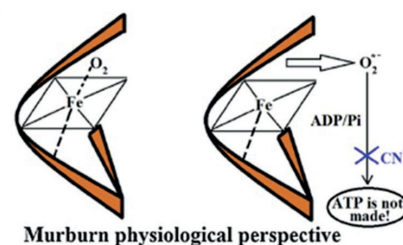
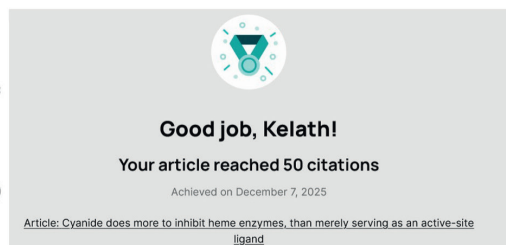
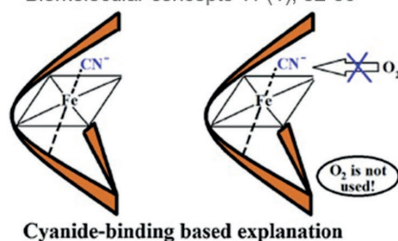


Figure 4. The image accompanying my recent article in LinkedIn projects some of my earlier papers on cyanide-mediated toxicity and their citations (mostly, self-citations!). https://www.linkedin.com/posts/kelath-murali-manoj-968021120_cyanide-toxicity-i-received-a-notification-activity-7403894765731422208-q5j8?utm_source=share&utm_medium=member_desktop&rcm=ACoAAB3nLiABwOamBWR7Y7tW7LdHPYF3Qyvtu-lA

layed my pursuits for a decade, until I could get the details of the new idea published in the mainstream open-access journal, *Frontiers of Pharmacology* in 2016. This was with the support of Stanislav Yanev (who served as a reviewer for that paper!), a long-time associate of George Chaldakov, EiC of *Biomedical Reviews* (BMR). In the same period (2006-2016), my group had also published very insightful mechanistic papers on the impact of ions like cyanide/azide and copper on peroxidase catalysis. In the following year (2017), *Biomedical Reviews* published the first paper conclusively debunking the classical Keilin-Mitchell-Boyer ideas of mitochondrial oxidative phosphorylation (disseminated first as a preprint in ARXIV, yet again!). Immediately thereafter, for unknown reasons, the journal lost its listing in PubMed and SCOPUS indexing. In 2018, another open access journal *Biochemistry Insights* (SAGE) published my detailed analysis of the classical bioenergetic scheme, and immediately thereafter, that journal was shut down! Letters to the Editor sent to many leading journals debating the interpretations of important clinical data (using entrenched/redundant/invalid theories) don't get published or vanish mysteriously, after weeks of publication (without

notifying me or the authors offering rebuttals!). Pre-submission queries to journals on papers based in murburn meet with flat responses, and one even said- "*You should send your work to Biomedical Reviews.*" So, here it is: one of the most important educational piece I have written, with all due respect, to the best open-minded journal I know.

Cell, Nature, Science, PNAS, EMBO.... Where is your sense of purpose? I wrote personal letters to all these journals' editorial teams/leaderships highlighting the important agenda and the prior publications.... All to no avail! What good is a leading element when it is not competitive, meticulous and scrupulous? Scientific communication machinery and leadership must reinvent itself, if scientists need to retain significance in these times of AI taking over. Human passion and integrity must rise to the fore.

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