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Understanding Metabolism: aerobic catabolism, heat production and bioplasma formation in mitochondria and the human body

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Abstract

In a previous work, "Human Experimental Bioenergetics", we reported on the experimental discovery of an electron bioplasma. We then put forth a series of interconnected hypotheses regarding how bioplasma formation and re-energization is gated by the electron transport chain (ETC) component of aerobic catabolism. Following the publication of its second edition, we concluded that these analytical proposals could be further developed to include biochemical and biophysical details that at the time were left unaddressed. We presently focus on these details, such as they concern how aerobic metabolism gates bioplasma formation and re-energization, and controls the production of electromagnetic heat in the mitochondria of the human body. Bioplasma currents are differentiated from other biological electron currents - namely, the ETC flux and the biochemical flux that is stocked up in ATP production. The analysis indicates the existence of two main classes of *free electrons* in the aqueous phase of the body - *modal* electrons of state, to which bioplasma electrons belong, and *supramodal* chemically-active electrons, to which ETC electrons belong. We close by briefly exploring the effects of hypoxia upon bioplasma formation.

Introduction: the context

In the wake of our work in thermodynamics soon to be published, we had to reconsider section 5 of chapter 3 in Part II of our "Human Experimental Bioenergetics" (HEB) [1]. We committed an error of detail in the master equation for the ETC that precluded the full realization of the connections between the ETC cycle and the recruitment and re-energizing of the bioplasma - the latter being one of the core discoveries reported in that work. At bottom, bioplasma mobilization is gated by 2 ETC cycles via the net export of 2 protons (not of 4!, as we had erroneously suggested) that it requires for the production of the water of oxidation. This entailed some degree of detail revision, but without altering the fundamental articulation of bioplasma production and aerobic metabolism. To clarify the full extent of this nexus, we took the occasion to re-write that section 5, which has now been replaced in the second edition of the book. What follows below is, in part, a review of the findings reported in that section 5, plus an enlarged analysis of the connection between bioplasma formation and the biochemical and bioenergetic functions of mitochondria that are central to aerobic catabolism.

This situation prompted the possibility and necessity of quantifying all the different classes of free electrons present in the aqueous phase of the human body and, at the same time, to ascertain which were involved in energy metabolism, and which were not. The population classes may be described as follows:

1) the 4 *chemically-active electrons* donated to 2 molecules of water by 2 ETC cycles;

2) the 38 *metabolically-active electrons* that were ultimately derived from phosphites, and which came to negatively-charge 38 ATP molecules via the coupling of glycolysis to 2 TCA (tricarboxylic acid) cycles, *and* to the oxidative phosphorylation associated with 2 ETC cycles;

3) the 20 to 24 electrons recruited into bioplasma formation from the mitochondrial matrix or, instead, mobilized there by the bioplasma influx, such that either process is gated by 2 ETC cycles via the net export of 2 protons from the matrix;

4) the total number of *chemically-active free electrons* involved in the homeostatic balance of the acid-base and redox cycles of water formation and dissociation;

5) the total number of *free electrons of state* that source the internal temperature and pressure of the system, and are not chemically active.

Our original endeavour in writing that section 5 of HEB was to connect and articulate our experimental data and biophysical model with existing biochemical models of catabolic metabolism, in particular aerobic metabolism. The extensive experimental work reported in HEB firmly laid the foundations to detect and measure in the human body a *bi-laminar* bioplasma composed of weakly-attracted, vertical electron dyads that are horizontally aligned in their direction of flux, and distinct from the equally detected and measured ambipolar radiation (massfree electric energy) emanating from the body. At bottom, the task was finding the knot between biochemical energy metabolism and the functional interrelationship of these two distinct forms of bioelectricity, one massbound and the other massfree.There were many questions of detail raised in this pursuit; to name the most salient ones:

1) Relating to massbound electricity: does the bioplasma originate in mitochondria and similar subcellular structures involved in respiration? Is it generated from zero kinetic energy electrons? Or is it re-energized up to a physiological energy level? What is this level and how can one determine it? If it is re-energized inside mitochondria, how does it exit the latter? And how does it, to begin with, enter it? In other words, how do the released electrons from the matrix return to it - presumably de-energized? What then is the kinetic energy of the electron influx, and how is it de-energized relative to the kinetic energy of the electron outflux? What role does catabolism play in helping import, or importing, via either biochemical reactions or simple membrane diffusion, the electrons which inside the mitochondrial matrix may be recruited into bioplasma formation? How are bioplasma influx and electron recruitment articulated?

2) Relating to massfree electricity: where at a cellular level is the physiological ambipolar energy field emitted? If it depended solely on the capture of solar radiation, it would practically disappear during nighttime, and it doesn't - so, how is it generated? Is its generation coupled to aerobic metabolism? If so, exactly how? Are the phosphorylating enzyme complexes of the ETC involved in ambipolar energy capture and re-radiation?

With the release of the second edition of the HEB, several of these questions have now been more clearly answered in that section 5. But we will revisit them here, and try to address them and those that found no answer then. Our central objective is to tie our

experimental findings - reported in the HEB - with a novel analytical approach to these problems, so as to obtain better definition in our understanding of how bioelectricity functions in the human body.

1. The metabolic source of electrons and protons

In HEB we began by questioning the starting assumption of biochemistry with regard to aerobic catabolism. Even though the master equation for the ETC can be twicked to include a missing net 2 protons on the side of the reagents to balance a net charge of -22 (something biochemists never highlight),

Charge [-6] [-18] [+2] = [+2] [-24]

$$2\ NADH + O_2 + 6\ (H_2PO_4^-) + 6\ ADP + 2H^+ \longrightarrow 2\ NAD^+ + 8\ H_2O + 6\ ATP$$

(4e) (12e$^-$) (6*32=192e$^-$) (8*8=64e$^-$) (6*24=144e$^-$)

it still shows two extra protons on the side of the products that beg the question of where did they come from. Oxidation of the 2 NADH molecules may release 2 protons, but those molecules are supposed to reside *in the cytosol*, and so will the protons they release. So, the above expression is a trick of the hand. Since there is no proton-pump on the mitochondrial outer membrane (MOM), 4 protons must be contributed from the mitochondrial matrix to the *space within* the mitochondrial inner membrane (MIM) and then ejected into the inter-membrane space (IMS) in order to produce 2 out of the 8 water molecules in the above reaction. This is hidden in the way that expression is written. Ultimately, it puts in doubt whether the entire aerobic metabolism really begins with cytosolic NADH (nicotinamide adenine dinucleotide) - which is to say, whether the electrons that enter the ETC are ultimately donated by it. One might object that, even if cytosolic NADH is not their source, they are released from inner membrane NADH to the ETC. But then, how did they get there, to begin with? A good question, indeed.

In the still current model of ETC initiation, it is the mitochondrial form of the glycerol phosphate dehydrogenase (GPDase) enzyme that captures and transfers the electrons shuttled from cytosolic NADH via glycerol phosphate (GP). In doing so, it reduces NAD^+ to NADH on the mitochondrial side, as long as a proton is available there. Subsequently NADH releases a pair of electrons to the ETC and oxidizes back to NAD^+.

For the production of 2 molecules of the water of oxidation by the transfer of 4 electrons down 2 ETC cycles, 4 extra protons must be available. Since the ETC is coupled 1:1 with the citric acid cycle, 2 cycles of the latter can only replenish 2 out of the extra 4 protons. So, a net deficit of 2 protons per 2 ETC/TCA cycles was apparent to us. The argument we presented in that section 5 of HEB was that, in the same way that *covalently-bonded* calcium phosphite is the ultimate source of the phosphate groups of ADP and ATP, it also served to import back into the matrix 2 protons per phosphite, which it released upon hydration.

However, before the proton balance can be restored by phosphite (and whether it can do so or not), a net 2 protons must be available on the mitochondrial side for the termination of 2 ETC cycles. This, we proposed, is the regulatory electrochemical mechanism that - at pH 7.4, and to maintain both the pH and the redox balance - allows for removal of net 2 protons *if* 24 to 25 electrons are *also exported* from the medium of the matrix. The immediate availability of those protons was provided by a proton-to-electron balancing mechanism governing the dissociation and formation of water. The net 2 proton export was a clue to the source of the bioplasma, permitting both the re-energizing of its electrons and, at the limit, the mobilization of 24 electrons from the matrix to form and export 12 dyads of a bioplasma.

Our argument, then, is that the nexus between bioplasma flux and formation (BFF) and aerobic catabolism, operated at the level of the ETC. The latter did not generate BFF per se, but gated it by its own operation. It was an indirect regulatory connection. It was bioplasma flux outgoing from the matrix that revealed the electric battery working inside mitochondria.

2. MIM and MOM pores and channels

In the course of our analysis it became apparent that covalently-bonded calcium phosphite cannot be the ultimate source of all matrix electrons at all times. Yes, per 2 protons that it releases hydrolytically, two electrons are also freed. But 22 to 23 electrons more would have to be released from the matrix to maintain a net pH and redox balance. So, how does the matrix become such a rich cathode? This raised the prospect of a different solution than merely mobilization anew of recruited bioplasma electrons: the

solution must be that the exported electrons must continually return to the mitochondrial matrix in the same way they left - in the form of a bioplasma.

Led to such probable conclusion, we briefly raised the possibility that the bioplasma influx and outflux were performed by subnano *channels* connecting the space between the outer and inner leaflets of the MIM *directly to the cytosol*, which means *across* the MOM in some region where MIM and MOM come into close apposition or adjacency. The bioplasma would flow through these channels, entering the matrix to be re-energized and leaving it once "charged" (unfortunately a very inaccurate expression despite the parallel to a battery). We suggested that the bioplasma influx was *energy-gated* at 508.5 eV, and gated out at the modal energy of 512.79 eV per electron. The *rate of re-energizing* of free electrons into bioplasma dyads was gated by 2 ETC/TCA cycles, at 3 bioplasma dyads per ETC electron.

Could the poorly understood pores on the outer leaflet of the MIM, and the equally poorly understood "apoptotic pores" on the outer leaflet of the MOM and their channels, also provide bioplasma gating at high-impedance? Could they serve as bioplasma flux channels or tunnels?

Pores on the MIM known as mitochondrial permeability transition pores (mPTP), were discovered well over a half-century ago. Yet, *how they work and what they do remains largely undefined* [2]. Their role has mostly been established in mitochondrial dysfunctions leading to inflammatory processes and cell death [3], even though they may well be critical for normal mitochondrial functions. In this respect, recent interest arose from the hypothesis that mPTP may result from Ca^{2+}-dependent conformational changes of F_0F_1-ATPsynthase, and a possible interaction with the adenine nucleotide translocator (ANT) enzyme channel [4]. One open question centers around whether molecular structures afforded by these enzymes can provide high-conductance channels that open at the mPTP. Another, how these pores may serve as low-conductance ion-channels that regulate Ca^{2+}-homeostasis, and import protons and Na^+ from the inter-membrane space into the matrix.

The possibility exists that both F_0F_1-ATPsynthase and ANT constitute separate MIM channels that converge into forming mPTPs, with small mPTPs being involved in low-conductance ion regulation and large mPTPs being associated or coordinated with the appearance of the apoptotic pores that form on the outer leaflet of the MOM [3]. The

body of evidence suggests that apoptotic pores are formed by the BCL-2 family of proteins, BAX and BAK (and to some extent BOK and BID), when they converge to create both the apoptotic pore and i*ts direct channel to the mitochondrial matrix*. But the communication between outer and inner membranes remains envisaged only from the perspective of a permeabilization transition leading to inflammatory responses and cell death.

There are still smaller pores on the outer leaflet of the MOM, known as VDACs, voltage-dependent anion-channels. It is thought they allow the passage of negative ions and small uncharged molecules (up to 5 kDa). Yet, these VDACs do not appear to cluster in particular where the MOM and MIM are adjacent. That rules them out as candidates.

There is no current notion in biochemistry that high-impedance channels could normally exist to conduct electron currents from the matrix to the outer membrane. Yet, the normal abutments of the cristae near the MOM is suggestive of the presence of such channels when MIM and MOM are in close apposition. If they are exclusively dedicated to bioplasma influx and outflux, they will sustain a high-conductance flux and yet form the tiniest of channels. This precludes their identification to either the MIM mPTPs, or the MOM apoptic pore/channels.

It was Paul Boyer who first suggested that the coupling of ATP synthesis and hydrolysis likely involved the rotation of a shaft through the F_0 and F_1 polypeptides. Rotation in one direction would be proton-driven and responsible for the synthesis of ATP and the transfer of protons (back) into the matrix, whereas rotation in the opposite direction would be ATP-driven by its hydrolysis back to ADP. F_1-ATPase (which should better be called F_1-ATPsynthase), is formed by a hexameric stator of alternating alpha and beta subunits, α_3 β_3, surrounding a rotor subunit that is thought to penetrate the intra-MIM F_0 complex, also thought to form a (second) stator. When considered together with the F_0 complex and a peripheral stator stalk that caps the matrix end of the hexameric stator [5], it is denoted as F_0F_1-ATPsynthase. When the rotor rotates in one direction, 2 protons flow down the gradient through channels into the matrix, and ATP is synthesized by the hexameric stator. When free from the F_0 complex and the peripheral stalk, the F_1-ATP synthase complex can rotate in reverse, resulting in ATP hydrolysis - and then can best be described as F_1-ATPase [6]. Still, the evidence for the normal mechanism is inconclusive [7], since, when coupled to the MIM-embedded F_0 complex, it appears to

rotate only in the ATPsynthase direction. If so, this is an unlikely candidate to establish a two-way bioplasma flux. Still, it is possible that bioplasma counter-currents could alternatively flow through the hexameric stator, but they would also require the rotor to turn in opposite directions. Of course, if it did, then the re-importing of protons back to the matrix would be net cancelled by the oscillation. An alternative possibility comes from conformational algorithms that have identified the presence of a cyclically-recurrent internal tunnel - in one of the β subunits of the hexameric stator - "that passes through the central cavity of the enzyme and opens only" [8] in a particular state of the subunit. Its diameter is ~9Å, and it is thought to be involved in the binding and release of P_i.

The size of this tunnel fits the physical requirement for what would be an intermittent bioplasma channel. Calculations based on the aetherometric model of the electron [9-10], indicate that the height of a flux of bioplasma dyads would require a very small lumen diameter on the order of 2Å = 0.2 nm. If the two fluxes, in and out, are gated per dyad, they could be closely apposed on the two surfaces of a dielectric component of the channel protein, across which they would exert an electrodynamic attraction on one another that compressed the separator. If the latter were a 2 carbon structure, one may expect the diameter of a such a channel to reach 6.4 Å = 0.64 nm, making structural identification by even high-power TEM rather doubtful. Furthermore, it would make sense that the gating of bioplasma fluxes across such a channel could be tied in to the flow of "inorganic phosphate", as is suggested is the role of the implicated β subunit that gates the tunnel on and off. One might even speculate that even if the rotor only turned in one direction, the two counter-currents could still flow through the same tunnel with their respective potential driving the rotor, and the two currents being gated by that on/off switching.

However, the foundational problem with this hypothesis is that it is based on conformational modelling that is oblivious to, precisely, the volumetric constraints of the aetherometric model of the electron. For our part, we doubt that such protein modelling could ever be that precise in identifying a 9Å tunnel. If we exclude this possibility, we are left with the ANT channel. Yet, it is strictly conceived as a MIM channel involved in molecular translocation; this implies that its lumen will be much wider than what is needed for a bioplasma flux. Even so, biochemists claim that pore and channel diameters as small

as 4 nm are sufficient for the passage "of small proteins such as cytochrome c" [3]. Conceivably, if the polypeptides are linearized.

In our view, it is, however, even more likely, that the dielectric component of the channel - which serves as flux separator but can also serve as lining of the lumen - may be formed instead by a protein-bound complex of iron oxide akin to a polymer of charged maghemite that would permit a near-π resonance across covalent bonds between ferrous and ferric oxides, in chains capped by water molecules. Such inorganic quasi-polymers could serve as synchronous tracks latching both electrons of a dyad like conveyor belts. They could also serve to magnetically counter-couple the bioplasma dyads, and thus be formative of any recruited dyads at the entry to the outflux channel. Conceivably, the iron oxides may function as prosthetic groups lined up by structural channel proteins (another potential role for the alpha-lipoic acid ligand of the E_2 unit of the phosphorylation complex, or for iron-sulphur proteins?).

The long and short of all this, is that the proposed bioplasma channels have not yet been identified. Ultra-structure tecniques may never reach the required resolution, and analytical detection may well require entirely different volumetric algorithms before possible location(s) can be ascertained.

3. What of the other 6 protons released by the ETC into the inter-membrane space?

We did not overlook the other 6 protons exported from the matrix by the 2 oxidative phosphorylation cycles that generated 6 ATP molecules. Even though we accepted the biochemists' contention that these protons would be returned to the matrix by the F_0F_1-ATPsynthase. At any rate, the problem with this solution was always that each imported pair of protons also added an ATP to the matrix, which not only decreased its ADP:ATP ratio, but partially cancelled the ATP gains of aerobic metabolism released into the IMS. To return the 6 protons back to the matrix would simply cancel 3 out of the 6 ATP produced per 2 ETC cycles. Furthermore, phosphite/phosphate conversion and their metabolism also did not seem capable of further returning these 6 protons - besides the 2 protons each phosphate already did when it supplied half of the 4 protons required by the 2 ETC cycles to form water.

The likely key to the problem of these other 6 protons lies in the function of nicotinamide nucleotide transhydrogenase (NNT). The enzyme was originally isolated in

aerobic bacteria and mitochondria during the 1950's. It was the first enzyme found to utilize the energy of the respiratory chain (and thus consume ATP) without being involved in the oxidative phosphorylation process. However, the molecular mechanism by which it transfers an hydride and translocates a proton still remains unclear [11]. The major function of NNT is the generation of the redox co-factor (nicotinamide adenine dinuleotide phosphate) NAPDH (and *not* NAD^+ per se) while it translocates one proton from the mitochondrial intermembrane space into the matrix:

$$NADH + NADP^+ + H^+_{out} \text{ <— } \mathbf{NNT} \text{ —> } NAD^+ + NAPDH + H^+_{in}$$

The forward reaction (to the right) oxidizes NADH to transfer a hydride (two electrons plus a proton) that reduces $NADP^+$. The reaction is considered to be completely reversible, indicating that NNT can also operate as a NADPH-driven proton pump. In other words, NNT is involved in the regulation of the matrix homeostatic equilibrium between the two pairs of redox factors ($NAPDH/NAPD^+$ and $NADH/NAD^+$). It appears that all antioxidant systems that operate inside mitochondria *downstream* from MnSOD (such as catalase), directly or indirectly depend on the presence of NADPH (*not* NADH) to achieve redox balance. Another such *downstream* antioxidant system is the glutathione peroxidase/reductase system. It also converts peroxide into water by the oxidation of glutathione, while the regeneration of the latter is effectuated by the reductase which consumes NADPH. Thus, the glutathione reductase has a major role in lowering the $NADPH/NAPD^+$ *and* the $NADH/NAD^+$ ratios. Knockdown or loss of NNT has been shown to impair the glutathione system [12].

It is possible, if not likely, that the proton-driven phase of F_1-ATPsynthase could be coupled to NNT's consumption of ATP to further translocate a single proton. Then, 2 cycles of F_1-ATPsynthase activity would generate 2 ATP and import 4 protons to the matrix, and 2 cycles of NNT would consume those 2 ATP and add an extra 2 protons to the matrix for a total of 6. This would be suitable solution that would keep the matrix pool of ATP down and restore the proton balance between the matrix and the IMS *in what concerns those extra 6 protons that were removed from the matrix*. The coupling could be mediated by the elimination of 4 peroxide molecules in 2 rounds of the glutathione peroxidase/reductase system. The only caveat is whether such peroxide production in well

oxygenated cells would be the result of normal metabolism, or itself the result of proton-deficient or depleted matrices (followed by alkalinization and calcification).

4. There are more electrons in water than anyone suspects...

Our foundational thesis that explains the bioplasma electric phenomena we discovered is that the outflux bioplasma current generated by electron re-energizing is gated by the ETC cycle, which in turn is controlled by oxygen concentration and the rate of glycolysis. Inherent to the model is that either captured ambipolar radiation or, in its absence, ambipolar energy re-emitted from "magnetic flipping" of bioplasma clusters, contribute 75% of the energy which ETC electrons need for water formation. Accordingly, even as the ETC cycle gates bioplasma flow, the latter may play a critical role in the completion of the ETC current itself, like a positive feedback mechanism does.

Based on the average ETC production of the water of oxidation in the body, we concluded that its current presented $1.93*10^{20}$ e^- sec^{-1} (equation #334 in HEB) equivalent to 31 A. But, since aerobic catabolism varies rapidy, between (10^7 ATP sec^{-1} $cell^{-1}$) to (10^9 ATP sec^{-1} $cell^{-1}$), the ETC current will vary between $3.906*10^{19}$ e^- sec^{-1} and $3.906*10^{21}$ e^- sec^{-1}. This 100-fold range forms a minimum-to-maximum current range of mitochondrial ETC activity.

If each ETC cycle requires two extra protons (1 net) than immediately available, 2 ETC cycles require 4 protons and 2 net. That means that 24 electrons can be gated out of the matrix for a net 2 proton deficit. The question thus arose as to whether these 24 electrons already included the 4 electrons to be donated to 2 ETC cycles. After all, if there is doubt about cytosolic NADH being the donor of those 4 electrons, the possibility of such an inclusion would put another dent in that hypothesis of biochemists: cytosolic NADH could not donate its proton, but there might also not be any need for it to donate its 2 electrons! If cytosolic NADH contributed them, then the bioplasma was formed at a rate of 6:1, with 12 bioplasma electrons being re-energized and exported per 2 electrons of NADH, or per a single ETC cycle. If cytosolic NADH did not contribute them, then the bioplasma was formed at the rate of 5:1, with 10 bioplasma electrons per single ETC cycle. The result was also a range: some 1.953 to $2.3436*10^{20}$ e^- sec^{-1} if the ETC electrons flowed at $3.906*10^{19}$ e^- sec^{-1}, but 100x greater (up to $2.3436*10^{22}$ e^- sec^{-1}) if they flowed at $3.906*10^{21}$ e^- sec^{-1}. These electron currents have very, very large amperages, but one

should be reminded that these are the sums of all cellular micro-currents in the body at any time. In effect, if we compare this range of bioplasma re-energization currents to the corresponding metabolic range of *total biochemical electron currents* sunk by the body into the formation of 38 ATP molecules from orthophosphates -

$3.7107*10^{20}$ e^- sec^{-1} $body^{-1}$ to $3.7107*10^{22}$ e^- sec^{-1} $body^{-1}$

- we can see how the maximal bioplasma currents are only ~63% of the biochemical ATP-synthesizing currents *for the same catabolic state*.

In HEB, we used as example the ~1483 W of power spent in heating the body when the ΔT towards the environment is 17 °C. In these conditions, the body requires an ETC current of $1.953*10^{21}$ e^- sec^{-1} $body^{-1}$ with a catabolic rate of $5*10^8$ ATP sec^{-1} $cell^{-1}$ that maximally gates a bioplasma current of $1.171*10^{22}$ e^- sec^{-1} $body^{-1}$. The overall power of *the two currents*, which mobilizes a total of $1.3669*10^{22}$ e^- sec^{-1} $body^{-1}$, is 10,382 W. But the total power which the bioplasma current alone requires for both re-energization and its eventual contribution (in the absence of significant ambipolar capture) to the completion of the ETC cycle, is slightly less, at 10,011 W.

At steady-state, the number of electrons recruited into bioplasma dyads must equal (or be close to equal) the number of electrons that return to the matrix in a de-energized state. To determine the concentration of these bioplasma electrons at any time in the body, we took recourse to the ambipolar power-unit which our research uncovered. The latter was based in our novel method to measure the *undisturbed* DC capacitance "of the body" at 31.13 nF. It slightly and non-significantly differed fom our prediction of 28.052 nF. Employing the latter value and the modal electron voltage of 512.79 V, the energy given by $E = C V^2 = 0.0073764$ J corresponded to an electronic charge of:

$$Q = C V = 8.9783*10^{13}\ e^-$$

Had we used the experimental value of 31.13 nF (per ambipolon), the result would be $9.9634*10^{13}$ e^-. Since the maximum measured bioplasma current from the body was 14.38 μA (here, in full agreement with our prediction), the bioplasma unit-power corresponding to the energy $E = C V^2 = 0.0073764$ J resulted:

$$P = V I = C V^2\ sec^{-1} = C V^2\ sec^{-1} = 0.0073764\ W$$

This bioplasma power-unit of 0.00737 W had to be accounted for by biological emission of ambipolar radiation. In fact, it was clocked by an ambipolar power-unit at the 1 PPS rate of the Ambipolar Master Clock (AMC) which we identified:

$$P_{Ambi} = 0.0073764 \text{ J} * 1 \text{ PPS}$$

This suggested one could conceptualize this power-unit as a "power ambipolon" with the aetherometric form of equation #352 in the HEB,

$$P_{Ambi} = E_{Ambi}\, \varepsilon_{MF} = p_e\, W_{Ambi}\, \varepsilon_{MF} = (8.2158*10^{-17} \text{ J})(8.9783*10^{13} \text{ sec}^{-1}) =$$
$$= 0.0073764 \text{ W} = \int = (512.79 \text{ V})^3 = \int = (3.54*10^7 \text{ m sec}^{-1})^3$$

Such a power-unit could provide the energy required to replenish the kinetic energy flux of $8.9783*10^{13}$ e^- sec^{-1}. The *energy concept of the ambipolon* would remain that of a single bio-ambipolon with energy equal to

$$E_{Ambi} = p_e\, W_{Ambi} = 512.79 \text{ eV} = 8.2158*10^{-17} \text{ J}$$

and, therefore, *identical* to the energy of the *modal kineton of each bioplasma electron*. Thus, the concept of the "modal energy ambipolon" directly permitted the understanding of how the "power ambipolon" converted entirely into the equivalent kinetic energy flux of bioplasma electrons

$$P_{Ambi} = p_e\, W_{Ambi}\, \varepsilon_{MF} = (8.9783*10^{13})\, p_e\, (512.79 \text{ V})\, \text{sec}^{-1} = \int =>$$
$$= \int => Q\, V\, \text{sec}^{-1} = C\, V^2\, \text{sec}^{-1} = V\, I = (512.79 \text{ V})\, (8.9783*10^{13}\, e^-\, \text{sec}^{-1}) =$$
$$= (0.0073764 \text{ J})\, 1\text{PPS}$$

But P_{Ambi} does not measure the *total ambipolar power of the body* (anymore than the energy of CV^2 = 0.0073764 J defines *the energy* of an ambipolon). In a first ever, we showed experimentally in HEB that a body can *accumulate charged capacitance over time*, reaching 0.5 mF in 8 minutes - as if unit-capacitances were being added in parallel. Since -

$$P_{Ambi} = (8.9783*10^{13}\, p_e\, W_{Ambi})\, \text{sec}^{-1} = \int => (8.9783*10^{13}\, e^-\, W_v)\, \text{sec}^{-1}$$

- then, when the body must spend 1,483 W in heating, and the power of the current of the total number of bioplasma electrons being re-energized is 10,011 W $body^{-1}$, we obtain the concentration of bioplasma electrons as:

$$(10{,}011\ W\ body^{-1})/(8.2158*10^{-17}\ J\ sec^{-1}/e^{-}) = 1.2185*10^{20}\ e^{-}\ body^{-1}$$

This includes the potential contribution of the bioplasma to the acceleration of the ETC electrons, so that strictly speaking, *the actual concentration of bioplasma electrons undergoing re-energization* is a lesser quantity:

$$[(8{,}899\ W\ body^{-1})/(8.2158*10^{-17}\ J\ sec^{-1}/e^{-}) = 1.083*10^{20}\ e^{-}\ body^{-1}$$

However, the density of the ambipolar energy driving the bioplasma current is not the same as the density of the total massfree energy displaced by this current. The latter includes all the conserved kinetic energy of the bioplasma electrons circulating throughout the body:

$$(1{,}128{,}653.8\ W\ body^{-1})/(8.2158*10^{-17}\ J\ sec^{-1}/e^{-}) = 1.3737*10^{22}\ e^{-}\ body^{-1}$$

Even when they normally generate photons, they tend to stop when they reach the energy level of the de-energized bioplasma (355 modal photons per bioplasma dyad). For a 100 Kg body with 70% water, the total bioplasma electron to water ratio will be high:

$$(1.37368*10^{22}\ e^{-}\ body^{-1})/(3{,}888.85\ H_2O\ mols) = 3.532*10^{18}\ e^{-}/H_2O\ mol$$

Now, in light of our soon to be published work in thermodynamics, it behooves us to ask to what class do these bioplasma electrons belong. It may not be apparent other than to those who will read that work on the physics of heat, but simply put, the critical business of free electrons *of state* and their photons is as follows: the temperature of a liquid (or, for that matter, of a gas or a solid) is entirely due to the energy of *modal* photons that, on a mole of photons per mole of substance, sustain that temperature at thermal equilibrium; whereas the internal barometric pressure of the same liquid is the combined result of the interaction of these modal photons with the tethered energy of a second class, *submodal* and submolal, of photons. These two types of photons are constant ongoing

discharges from *two corresponding classes of free electrons of state*, also modal and submodal, whose energies are linked by a physical mechanism (we will not bother to detail here how these electrokinetic energies are continually regenerated in nonliving systems). At a physiological temperature of 37°C, a body of 100 Kg made of 70% water will contain *modal* electrons of state on the order of $1.21*10^{23}$ e^- $body^{-1}$. It will also contain a smaller population of *submodal* electrons of state, with a much lower energy (see below), at $3.8*10^{22}$ e^- $body^{-1}$. Evidently, the total *bioplasma* quantity of $1.3737*10^{22}$ e^- $body^{-1}$ belongs to the modal category, and its source is given, in turn, by the absorption of $1.3737*10^{22}$ p_{e^o} $body^{-1}$, i.e. biologically generated *energy ambipolons* per body. Thus, as we wrote, in scarcely 9 seconds all the modal free electrons of state in the aqueous phase are recycled through the bioplasma mechanism, so that, aside from the re-energizing mechanism, there is also a net turnover of the bioplasma. To which me must add net thermal and electric losses to the environment, including the ground, that must be matched by renewed electron recruitment.

Though a total bioplasma concentration of $1.3737*10^{22}$ e^- $body^{-1}$ is less than the concentration of the *submodal* or secondary population of free electrons of state, at $9.788*10^{22}$ e^- $body^{-1}$ (for the same 100 Kg model of the body), the latter will have much lower energies of only 447.7 eV (with reference to the modal population, they have a "negative" potential of 65 V), for as long as the internal barometric pressure equals that of one atmosphere. In hypertension, this likely is not the case, as the blood flow is "pressurized" - so that the submodal electron population has a greater kinetic energy. This may well be the most basic root cause of atherosclerosis.

However, we did not treat the ETC current as part of the bioplasma current, even though the 4-electron scheme per 2 ETC cycles (Lehninger's scheme) is highly suggestive of a couple of dyads flowing down the chain's electric gradient. The reason was also discussed in HEB: these *free electrons* are *supramodal* - they have more energy than the bioplasma electrons do, because they belong to the cycle of formation and dissociation (heterolytic and homolytic) of water into its most fundamental components. Their maximal energy is ~526 eV, and seemingly they must reach ~530.75 eV before they can enter into water formation - given that neither the protons or monoatomic oxygen can normally contribute significant energy to the event, only contribute their negligible thermal energy. At 37 °C, 1 Atm and pH=7.4, the number of chemically active free

electrons that are involved in water formation and dissociation cycles in any aqueous phase is $1.67*10^{21}$ e^{-} $body^{-1}$. When the body spends 1483 W in heating, the total ETC current reaches $1.953*10^{21}$ e^{-} sec^{-1} $body^{-1}$. The independently derived numbers are so close that the suggestion is evident: a high metabolism practically involves all the chemically-active electrons that are present in the water phase of the body in the very biochemical production of water (8 water molecules per 2 ETC cycles, 2 of which are the water of oxidation).

For a diagram of all these electron classes, please consult **Table 1**.

By implication, the body must biologically regulate the dissociation of water and re-release of the supramodal electrons as a function of complex equilibria with the water molecules - which will obviously include an account of how much water is ingested by the body, what is its pH and mineral content, and how much water is processed by the kidneys and lost by skin evaporation. The total water produced by the ETC is effectively the result of the conjugation of free radicals (HO• and H•) in low energy states, rather than an acid-base conjugation. Since it has a balance of protons and electrons, we surmise the real pH of the IMS is 6.7, the pH of triple-distilled water.

Table 1

Electron currents and populations in the human body

Current range, e^- sec^{-1} $body^{-1}$	**Type**
$1.935*10^{20}$	Typical average ETC current from production of the water of oxidation
$3.906*10^{19}$ to $3.906*10^{21}$	ETC current
$3.711*10^{20}$ to $3.711*10^{22}$	Total chemical current sunk in the formation of 38 ATP molecules
$1.95\text{-}2.34*10^{20}$ to $1.95\text{-}2.34*10^{22}$	Bioplasma re-energization current

<u>At $5*10^8$ ATP sec^{-1} $cell^{-1}$ (1Atm, 37°C) :</u>

Current, e^- sec^{-1} $body^{-1}$	**Function**
$6.67*10^{19}$	Supramodal ETC-culling current (526V)
$1.759*10^{19}$	Heat-generating supramodal current (526V)
$1.953*10^{21}$	Amplified ETC-water forming current (~18V)
$1.858*10^{22}$	Chemical current sunk into ATP (0.3V)
$1.171*10^{22}$	Bioplasma re-energization current (513V)

Concentration, e^- $body^{-1}$	**Electron group**
$1.083*10^{20}$	Bioplasma electrons undergoing re-energization
$1.2185*10^{20}$	The above plus its ETC contribution
$1.3737*10^{22}$	Total bioplasma electrons
$1.21*10^{23}$	Modal electrons of state (70% water body)
$3.8*10^{22}$	Submodal electrons of state (70% water body)
$1.67*10^{21}$	Supramodal chemically-active free electrons (70% water body)

5. How is the water of oxidation produced and how does it generate heat

One may wonder what is the point of generating water at the end of the redox cycles of the ETC, when 3x more water is obtained from the phosphates in the master reaction. The reason, in a nutshell, is that while both types of water production release heat (or, rather, electromagnetic energy or photons), formation of water via the ETC dwarfs the heat generated when water is formed from the heterolysis of phosphates.

The water contributed by the phosphates is not formed from the most oxidized components (2 electrons, 2 protons and one monoatomic oxygen), but from an acid/base conjugation that is carried on intramolecularly to the phosphates during the reaction. At pH = 7, the water-forming reaction is exothermic, releasing 4 LFOT photons of 3.936 eV (315 nm) each per water molecule (a total of 15.744 eV). The photon energy is high, and their total energy equivalent to ~590 modal photons of 0.02669 eV. But the electrons involved in this reaction as reagents were already covalently bonded and not free, so they have neither the supramodal energy of the chemically-active electrons, nor the the energy of modal electrons of state. Not so with the formation of the ETC water of oxidation: the two electrons that enter the ETC are supramodal free electrons with some 526 eV each. Seemingly, they release the majority of their kinetic energy when they finally reach the energy level required for water formation (530.8 eV). This, however, is just the initial lay of the land. Let us see why, and how a final survey reveals a slightly different territory.

Conventionally, water formation at room temperature and 1 Atm obeys what we have called "a mythical reaction" that has an exothermic Gibbs energy, releasing -237 kJ mol^{-1} equivalent to -2.46 eV per water molecule. But there are different paths to water formation. The path of acid-base conjugation has a greater negative Gibbs energy than this, at -303.465 kJ mol^{-1} = -3.145 eV per water molecule. The free-radical path from primary constituents has a *still greater negative* Gibbs energy, at -422.69 kJ mol^{-1} = -4.38 eV per water molecule.

Conventionally, the (thermal) enthalpy of liquid water is in all cases equal to 285.83 kJ mol^{-1} = 2.962 eV, and it encompasses the chemical energy stored in the covalent bonds of the molecule along with its associated PΔV work. However, in our "Nanometric Functions of bioenergy" (NFOB) [10], we analytically demonstrated how the chemical energy stored in the covalent bonds of water must be more than 10-fold greater than this, at 35.916 eV - because its energy content is not thermal but electric, as if it denoted a

"nonthermal enthalpy" (a contradictory term, since enthalpy comes from the Greek *halpein*, which denotes heat; best thought of as the thermal equivalence of a nonthermal energy).

What we found in NFOB was that acid-base conjugation was indeed an exothermic reaction, but it released 5x more (thermal or photonic) energy than is conventionally accepted: -15.744 eV per water molecule (as groups of 4,800 electrons are involved in resonant ambipolar transmission modes to form 2,400 water molecules). Conversely, we showed that when it proceeded from its primary constituents via the free radical pathway, water formation was endothermic, or properly speaking, *endo-ambipolar or endo-electric*, since it required the absorption of 4.655 to 4.74 eV by each electron involved, or a total of 9.31 to 9.48 eV per water molecule. This meant that, if it were a thermal reaction, it should have had a positive Gibbs free energy of some:

$$2 \text{ electrons } (+449 \text{ to } +457\text{k J mol}^{-1} \text{ per electron}) = +898 \text{ to } +914 \text{ kJ mol}^{-1}$$

and not a negative one of -422 k J mol^{-1}. This is also a far cry from the conventional view, yet analytically it cannot but be the case if the Gibbs equation applies - since it claims that the free energy of the reaction results from subtraction of the product of entropy and temperature from the (thermal) enthalpy of water.

Only the free-radical pathway put into evidence that water formation is not a purely thermal process that can be adequately described by the Gibbs equation. The latter relies on an erroneous concept - that the *thermal enthalpy* of water already encompasses the chemical energy of its covalent bonds. When, in fact, *the covalent energy is electric and not thermal or electromagnetic*, and is a component of the *nonthermal enthalpy* of a substance. Both thermal and electric energies should be accounted for in the reactions of water formation. In our upcoming work on the physics of heat, we show that if we just consider the *thermal* energy of the reaction, the latter is always endothermic, not exothermic.

So, what did life invent that nonliving nature cannot do, with respect to water formation? The concentration of chemically active free electrons in water depends on their energy being supramodal, or greater than the modal kinetic energy of state. They can only donate to water formation the energy that they may have *in excess* of the modal electron energy. However, water formation disrobs them of most of their kinetic energy and, once

formed, water traps them at a much lower energy level than they had when free. That is why water is really not in 1:1 balance with the concentration of chemically active free electrons - since the pathways of water *dissociation*, whether homolytic or heterolytic, are always endothermic, or endoambipolar, that is, it takes energy to dissociate water; it does not dissociate spontaneously, unlike what chemists claim. The two lytic pathways converge in the freeing of the electrons, each being legated a maximal 13.3 eV of energy. But once free, they are again in a position to capture the kinetic energy of the modal state (we will not go into how this comes about, since our work in thermodynamics addresses it in great detail). So, to those 13.3 eV of energy is now added the modal kinetic energy characteristic of free electrons.

In contrast, the pathways of water formation are divergent - the acid-base pathway is exothermic, while the redox pathway is endothermic or endoambipolar. That means that water is far more readily formed from a proton and an hydroxide anion (the base), than from an atomic hydrogen and an hydroxy radical. Accordingly, the primary balance in water is described as between it and its dissociated ions -

$H_2O <=> H^+ + HO^-$

- and it is essentially a function of the proton concentration, so that the hydroxide ions change their partners frequently, whenever the released -15.744 eV per water molecule formed is captured by a neighbouring water molecule to undergo heterolytic dissociation. Rather than just being dissipated as heat, it is stored in water itself and continually passed around.

Yet, the acid-base conjugation is of no biological use unless it occurs intramolecularly (as in the case of hydrolyzing phosphate reactions); it cannot be the underlying mechanism whereby the ETC electrons bring about water formation, since the electron pair in question has already been obtained by the base. In other words, acid-base conjugation does not engage *free* electrons.

However, water is also at all times in redox equilibrium with its ultimate dissociation products:

$H_2O <=> 2H^+ + 2e^- + O$

What aerobic metabolism - common to aerobic bacteria, yeast cells, plants and animals - devised is a way to employ the endoambipolar pathway of water formation from its primary constituents to serve as the means to release far more thermal energy than the

acid-base pathway can. It took advantage of an "endothermic" reaction to give the appearance of an "exothermic" reaction that released heat. But the advantage itself was not a manipulation of the chemical reaction per se.

Originally, we thought that the ETC reaction itself was the means for that release of heat. It seemed possible to write it, at 37 °C and taking into account the minor contributions of the heat content C_pT of each reagent, as

$$[2(e^- + E_{k513} + E_{k13} + h\upsilon_{4.74})] + (0.5\ O + E_{CpT0.033}) + [2(H^+ + E_{CpT0.033})] =>$$
$$=> (H_2O + E_{con35.9} + E_{CpT0.243}) + \{[2(\alpha^{-2} - 2.686\ eV)]\ h\upsilon_{0.027}\}$$

All number subscripts are in eV, including those referring to heat content. The threshold total kinetic energy of the water-forming electrons would be

$$E_{k513} + E_{k13} + h\upsilon_{4.74} = E_{k513} + E_{k17.96} = 530.75\ eV$$

The reaction would release an enormous number of the modal photons $h\upsilon_{0.027}$, close enough to the maximum α^{-2} per electron. The problem with this approach became obvious when considering the ETC current responsible for those 1483 W of body heating: an ETC current of $1.953*10^{21}$ e^- sec^{-1} $body^{-1}$ with a rate of $5*10^8$ ATP sec^{-1} $cell^{-1}$ would generate some 1,025 eV per the above reaction, and 112x greater heat production than the initial heat-producing parameter itself, due to the difference in potential:

$(1.953*10^{21}\ e^-\ sec^{-1}\ body^{-1})\ 530.75\ V = 111.97\ [(1.953*10^{21}\ e^-\ sec^{-1}\ body^{-1})\ 4.74\ V =$
$= 166{,}055\ W\ body^{-1}$

The solution had to be sought elsewhere. Somewhere along the catabolic path a current amplification took place at the cost of the kinetic energy of chemically-active free electrons. In the process, resistive joule heating was generated by a portion of the primary current. Let us put some numbers to this, when the heat released corresponds to a flux with a power of 1483 watts, very slightly more than the heat released by the biological "burning" of a mole of glucose: an initial current of $6.67*10^{19}$ e^- sec^{-1} $body^{-1}$ that culls chemically active electrons with 526 eV is formed upstream of the ETC. The iron-protein arrays inside the matrix may well be responsible for this culling. In the process, the electromagnetic heat equivalent to 1483 W is released, as follows:

$(6.67*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V = 5{,}619.15\ W =$
$= [(4.908*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] + [(1.759*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] =$
$= 4{,}135.93\ W + 1483\ W$

Or, more to the point, the photon heat is released as "joule heating" (across some 188 ohm resistance) when most of the mobilized current is amplified (29.3-fold in the example) at the cost of most of the kinetic energy of the culled electrons (from 526 eV to 13.2 eV):

$(6.67*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V = 5{,}619.15\ W = 4{,}135.93\ W + 1483\ W =$
$= [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 13.218\ V] + [(1.759*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] =$
$= [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 13.218\ V] + (3.467*10^{23}\ h\upsilon_{0.027}\ sec^{-1}\ body^{-1})$

Thus, the kinetic energy of the culled electrons is biochemically distributed to a larger pool of electrons until all have just 13.218 eV of kinetic energy. These are the electrons which are mobilized into the ETC, while 26.4% of the original energy flux was spent in joule heating. The process, then, should be written as the subtraction of a thermal loss:

$[(6.67*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] - [(1.759*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] =$
$= 5{,}619.15\ W - 1483\ W = 4{,}135.93\ W =$
$= [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 13.218\ V] - (3.467*10^{23}\ h\upsilon_{0.027}\ sec^{-1}\ body^{-1})$

To the 13.218 eV of kinetic energy of the ETC-entrant electrons, the ETC will add the biochemical energy of 1.185 eV per electron, while enzymatic ambipolar capture or bioplasma emulation will add the remaining 3.555 eV, for a total of 4.74 eV. Water can be formed when the kinetic energy of each ETC electron reaches

$13.218\ eV + 1.185\ eV + 3.555\ eV = 13.218\ eV + 4.74\ eV = 17.958\ eV$

Thus the next step of the flux is the step that should actually be described as an addition of power or of an influx of energy by:

$[(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 13.218\ V] + [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 4.74\ eV/e^{-}] =$
$= 4{,}135.93\ W + 1483\ W = 5{,}619.15\ W =$
$= (1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 17.958\ V$

In other words, the resulting current at ~18V does *not generate joule heating*, because all of it goes into the production of ETC-water, which traps the electrons and their kinetic energy. Putting it all together we have:

$[(6.67*10^{19}\ e^{-}\ sec^{-1}\ body^{-1})\ 526\ V] - [(\mathbf{1.759*10^{19}\ e^{-}\ sec^{-1}}\ body^{-1})\ 526\ V] + [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 4.74\ eV/e^{-}] =$

$=[(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 13.218\ V] - (3.467*10^{23}\ h\upsilon_{0.027}\ sec^{-1}\ body^{-1}) + [(1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 4.74\ eV/e^{-}] =$

$= 5{,}619.15\ W - 1483\ W + 1483\ W = 5{,}619.15\ W = (1.953*10^{21}\ e^{-}\ sec^{-1}\ body^{-1})\ 17.958\ V =$

$= 9.765*10^{20}\ H_2O\ molecules\ sec^{-1}\ body^{-1}$

Only the current in bold type generates joule heating by photon production; the other two current terms do not, because their power is used to generate water. It is only during current amplification that joule heating is produced.

Such is what we propose as the complete solution. In effect, the 1483 W of power spent in heating the body is in the form of a photon flux released when chemically-active electrons lose nearly all of their kinetic energy (as happens in the resistive or amperian current of a conductor), whereas the 1483 W of power that is added to the ETC electron current is in the form of kinetons, as those ETC electrons are accelerated down the chain.

Inevitabily, if our proposal is sound, there are critical points of intersection with the biochemical understanding. The "burning "of a mole of glucose is said to have a Gibbs free energy of

$\Delta G^{o'}$ = -686 Kcal/mole of glucose = $-2.87*10^{6}$ J mol^{-1} =

= -29.75 eV/glucose molecule

The energy conserved by being stocked in ATP is:

38 ATP * (7 Kcal/mole) = 266 Kcal/mole = $1.11*10^{6}$ J mol^{-1} =

= 11.54 eV/glucose molecule

The remainder of the energy is considered to be released in the form of heat, such that:

(-686 Kcal/mole) - (-420 Kcal/mole) = 266 Kcal/mole

Then, the lost heat energy equals

Q = -420 Kcal/mole = $-1.757*10^{6}$ J mol^{-1} = -18.217 eV/glucose molecule

and constitutes 61.2% of the energy stocked in, and released from, glucose.

We may also calculate the power of glucose heating under the conditions of the recurrent example. With a catabolic rate of $5*10^{8}$ ATP sec^{-1} $cell^{-1}$, the glucose consumption per second becomes:

$(5*10^{8}$ ATP sec^{-1} $cell^{-1})(3.72*10^{13}$ cells $body^{-1})/(38$ ATP per glucose) =
$= 4.895*10^{20}$ glucose molecules sec^{-1} $body^{-1}$

Since one molecule of glucose yields an energy of $4.767*10^{-18}$ J, the power of that glucose consumption is:

$(4.895*10^{20}$ glucose sec^{-1} $body^{-1})$ $(4.767*10^{-18}$ J per glucose) = 2,333.45 W $body^{-1}$

or a consumption of $8.128*10^{-4}$ M glucose per second (one mole every 20 minutes). Since 61.2% of this will be released as heat, it will correspond to a slightly lower power than in our example:

$0.612*$ (2,333.45 W $body^{-1}$) = 1,428 W

From the preceding, one can immediately see that the heat extracted from glucose has the equivalent voltage of 18.2 V and energy of 18.2 eV per molecule, as if it indicated the real voltage and energy of the 17.958 eV electrons donated to water via the ETC. Further, that the implicit confusion between a current that sources joule heating and one that does not, stems from the *merely quantitative equivalence* of the generated flux of photon heat (the result of joule heating) with the *optimally compensating* flux of electron kinetons through the ETC (a flux that generates no joule heating, since it is sunk in water formation) that follows it:

$(3.467*10^{23}$ $h\upsilon_{0.027}$ sec^{-1} $body^{-1}) = [(1.953*10^{21}$ e^{-} sec^{-1} $body^{-1})$ $(4.74$ eV/$e^{-})]$

But they are distinct physical realities and occur at different times in the same process, even if it is the heat production that, in the right conditions (availability of oxygen, nutrients, etc), triggers the ETC kineticization. But, above all, the conventional reductionistic approach is missing the fact that it is not the formation of water that generates heat exothermically, but the stripping of the entirety of the kinetic energy of

26% of the chemically-active electrons that must be input to the ETC. Formation of ETC-water simply compensates for the energy previously lost as heat during that stripping.

Now, of course, one wonders, then, what is, after all the role of the bioplasma in the heat production of the body. First off, the ETC serves to gate the re-energization of the *modal* electrons that form the bioplasma (the result of the role of that net 2 proton deficit in the matrix per 2 ETC cycles), but it is the bioplasma which functions as the fundamental temperature regulator of the body by a constant production of modal photons that sets a baseline. It is also a tremendous store of kinetic energy that can be released as either electromagnetic heat or ambipolons, according to the physiological demand. Furthermore, if there is exhaustion of the chemically active electron population; or if aerobic conditions fail (lack of oxygen, starvation response, etc) and the heat loss is not compensated by the ETC engagement; or if the heat loss is sudden and extreme, then the body is reduced to just the primary process of direct heat production from the kinetic energy stored in the dyad-coupled electrons of the bioplasma. This, of course, is ultimately a finite process. At any rate, heat production from the bioplasma is always ongoing, even when the ETC keeps up with most of the heat losses. If the bioplasma influx is regulated down 4.74 eV from the modal energy, then each dyad typically generates

$2\ (4.74\ \text{eV}/h\upsilon_{0.027}) = 355$ modal photons

during its circulation between exit from the mitochondria and re-entry.

Only the citric acid cycle and FA oxidation can generate NADH. But how can they do so without those electrons and protons coming from somewhere else other than NADH and being present at the right time and place? Locally, in every tissue, there must be ongoing a three-fold process:

1) When it comes to recruiting new electrons, their availability must depend on the balance of acid/base and redox reactions in the aqueous phase of the gelatinous matrix; this means that ultimately their source must be:

1.1. The import of 2 electrons per covalent phosphite molecule.

1.2. The dietary intake of water that already contains a population of chemically active free electrons.

2) When it comes to re-energizing electrons, their availability must depend on the influx of partially de-energized bioplasma dyads through the hypothesized MIM/MOM

tunnels. The bioplasma influx would then be indirectly gated by the ETC gating of the bioplasma outflux. But, at the same time, a voltage-controlled filter must serve to reject dyads whose energy falls below the 508 eV threshold. Likely, then, whenever the influx may be remissive, in charge number or energy, recruitment takes place.

6. Hypoxia, sugars and the insulin system in metabolism and functional disorders

From the preceding one may conclude that the most basic acquired or functional metabolic disorders (such as hypoxia, hypertension, diabetes type II, cardiovascular disorders or heart disease) must be intimately related to the lack of electron availability and a minimal bioplasma flux. One should expect a similar result from a host of genetic deficiencies that affect catabolic enzymes involved aerobic metabolism. Of particular clinical interest to us is the first class of such disorders, a subject that conventional medicine has systematically addressed from a merely topical viewpoint that just seeks to suppress the symptom(s). There is much talk of preventive methods, but a total failure to address the very basic issues of malnutrition, proper water intake and healthy breathing. If biochemists do not really know how body heat is generated, apparently nutritionists and physiologists compound this with a gross underestimate, anywhere from 4x to 9x, of the heat losses from the human body [1]. Likewise, as shown in the HEB, nutritionists and physiologists grossly underestimate the powers the body must mobilize in work tasks, even as this work is averaged throughout the day. It follows that they must grossly underestimate the correct nutritional intake required for the healthy maintenance of the human body. Nowhere is this more evident than in the prescribed guidelines for vitamin intakes - not only in the often ridiculous quantities suggested, but just as well in the total disregard for the quality and chemical specifications of the preparations. It is a critical issue, because intake of vitamins, enzyme co-factors, amino-acids, minerals and ions is one of the essential conditions of aerobic metabolism - besides proper oxygenation. The orthomolecular work of Linus Pauling on the use of large-dose vitamin C to prevent and treat cancer is a prime illustration of this fact [13]. So is the work of Mattias Rath, Pauling's collaborator and continuator, on the etiology of heart disease and atherosclerosis. It remains fundamental to this day. Cholesterol intake per se is not the cause of heart disease or fibrotizing vessels, but systemic deficiencies are - in particular of vitamins B3, C and E, and of L-lysine and L-proline [14]. Few paid attention to it. He also confirmed that

coronary and atherosclerotic deposits can be reversed by vitamin C - which was originally discovered by the Canadian physician George Willis in 1954 [15]! These deposits form where the vascular epithelium presents criss-crossing breaks in continuity, largely due to defective collagen assembly analogous to what is found in scurvy. In fact, Matthias claimed that aetherosclerosis is an early form of scurvy. It is noteworthy that Matthias was one of the first physicians and medical scientists to warn against the introduction of statins in the 1980's as a way to lower blood cholesterol, since these drugs interfere with aerobic metabolism and cause cancer in animal models [16]. Though this remains a contentious issue to this day, we know now that statins promote acquired diabetes and the diabetic evolution of pre-diabetic patients.

From our perspective, the result of atherosclerosis is that the bioplasma flux coursing on the inner surface of blood vessels is interrupted and broken. Then, the released electrons locally contribute to further oxidative damage. In HEB, we also analyzed the formation of proteinaceous deposits - magnetic traps or knots, most often in the lymphatic interstitium - that detach and circularize bioplasma electron segments and block the bioplasma flux. One can conceive of still other bioplasma flux disorders, for example - that could result in the accumulation of electrons in the mitochondrial matrix which fail to be mobilized and exported. This would alkalinize the matrix, which would cease to be able to export protons, and thereby disrupt the battery steady-state cycle of the mitochondrion. It would also trigger the SOD, dismutase, NNT and glutathione systems - both to eliminate ROS and protonate the matrix. Any deficiency in these systems would severely compound the problem.

Clearly, the articulations of the cellular energy system are rather complex. There is, evidently, a close link between proper oxygenation and a healthy nutrition - and it now appears that it must extended to include optimal bioplasma formation and circulation. The link is catabolic - and it highlights the common factors involved in acquired metabolic disorders. Catabolic metabolism in multi-cellular animal systems has 4 basic forms:

i. Glycolytic fermentation - which generates pyruvate from alpha D-glucose or from ketohexose (natural fructose).

ii. Aerobic metabolism - in the presence of oxygen, processes pyruvate through two cycles of oxidative phosphorylation and the tricarboxylic acid cycle (TCA), by coupling phosphorylation to the electron transport chain (ETC).

iii. Lactic fermentation - in hypoxic or anoxic conditions, pyruvate is instead converted into lactic acid.

iv. Fatty acid (FA) oxidation - also generates acetyl-CoA (entry point to the TCA cycle), NADH and $FADH_2$ (flavin adenine dinuleotide, reduced form) from triglycerides if the essential amino acid L-carnitine is available.

Aerobic metabolism has a much greater rate of ATP production than any of the other metabolisms. Healthy, well-oxygenated individuals present a high rate of glycolytic and aerobic metabolism. But many hypertensive individuals also present high metabolic rates. Stress, whether precipitated emotionally, or by fasting, strenuous exercise or a high-fat diet, induces an increased metabolic rate that in most people utilizes FA oxidation. In fact, the lower is oxygen consumption under these situations, the more inert is glycolysis and aerobic metabolism. Ditto for low blood glucose or malnutrition: if glucose is present in blood, then lactic fermentation sets in: cytosolic lactate dehydrogenase comes into play, converting pyruvate into lactic acid by absorbing two protons, while converting NADH back to NAD+. It happens in cancer cells (partial or total switch to lactic fermentation), but it also happens in any hypoxic tissue.

Certain tissues and organs, such as heart muscle and the renal cortex, normally rely on FA oxidation (oxidative lipolysis) to generate hydroxybutyrate, their normal fuel of respiration - just as glucose is the major fuel of the brain. Hydroxybutyrate is then processed into 2 acetyl CoA molecules. Lipolysis of triglycerides from brown adipose tissue together with increased oxygen consumption is the main thermogenesis mechanism of arousal from hibernation in animals. Any accumulation of acetoacetate in blood triggers a decrease in the rate of lipolysis in adipose tissue - and acts therefore in the same direction as insulin .

Hypoxia is likely the major causative factor of low-energy/low-density bioplasma. Hypoxia shuts down oxidative phosphorylation and the ETC, which in turn will apparently bring the bioplasma re-energization to a stop, lest there may be other metabolic links to its production that are yet to be found. Together with deficient iron turnover (role of hemin in blood and of the spleen in clearing red blood cells and reprocessing iron and hemin), it also disturbs the calcium phosphite reactions that ultimately permit the synthesis of ATP. When no water of oxidation is produced, only phosphate-based water is generated by glycolysis.

Insulin's normal function is not simply the control of the glucose level in serum, but also the prevention of lipolysis from adipose tissue and subsequent FA oxidation. If the serum glucose concentration is normal, insulin controls activation of glycolysis and metabolism through the insulin receptor (IR). If the serum glucose level is too high, insulin promotes its uptake by adipose cells, and the synthesis of fat from carbohydrates. In turn, fat acid synthesis stimulates the consumption of oxygen. In the absence of insulin (as in diabetes), blood glucose rises above normal levels and glucose consumption by fatty acid synthesis shuts down. At the same time, lipolysis is turned on, triggering the lipases that initiate FA oxidation - which can only proceed if the carnitine step is viable. A similar process occurs if there is a decreased response to insulin (insulin resistance). However, in either case, if the body is properly oxygenated, the acetyl CoA that results from the FA oxidation is shunted into the TCA cycle, bypassing the ETC.

Natural fructose (from *non-acidic* fruits) is not under the regulation of insulin. By entering "late" into the metabolic cycle of glycolysis, fructose leads to the production of pyruvate, just as glucose does. Sucrose intake almost does the same. Sucrose contains a unit each of glucose and natural fructose, and the liver only breaks it down when there is a need for carbohydrates. Thus sucrose is only indirectly regulated by insulin. Starch is a little different. While its sugars (maltose, maltotriose and dextrin) are not regulated by insulin, the hydrolysis of its sugars (by alpha-amylase, in the mouth and pancreas) results in the production of glucose. So, the intake of natural fructose and sucrose should be preferred over the intake of starch, and all the more so in diabetic and/or obese patients. All that excess starch induces is the growth of white fat cells (rich in saturated fatty acids), which is not the adipose tissue one wants (brown fat cells).

Hypoxia is caused by low concentrations of oxygen in blood, typically the result of poor breathing or poor-oxygen environments. It triggers expression of the hypoxia-inducible factor HIF-1, and the often neglected interaction of HIF-1 with the insulin family of factors - insulin, insulin-like growth factor I (IGF-1) and insulin-like growth factor II (IGF-2) [13]. HIF-1 is a transcription factor that regulates the transcription of genes whose products play critical roles in energy metabolism, erythropoiesis, angiogenesis (including tumor vascularization), cell survival and apoptosis. It consists of two units, alpha and beta. The alpha unit is under oxygen control, for as long as ferrous ion and ascorbate (vitamin C ester) are available. HIF-1 induces genetic expression of proteins that increase

uptake and transport of glucose, convert glucose into pyruvate and, most importantly, lead to lactic acid fermentation.

But HIF-1 can also be activated *independently of hypoxia* or oxygen control. This appears to be a common step in the development of most somatic and leukemic cancers. Critically, this action is mediated by the receptor (IGF-1R) for IGF-1. This receptor can be activated by IGF-1, but just as well by insulin and IGF-2. It has intrinsic tyrosine kinase activity, and is normally involved in controlling uptake of glucose, activation of glycolysis and metabolism via the activation of HIF-1. Activation of IGF-1R is thus required for hypoxia-independent induction of HIF-1alpha. Any deficiency in mitochondrial pyruvate dehydrogenase (PDH) or its Complex I, triggers expression of HIF-1 in normal human (primary) fibroblasts, with attendant increased glycolysis and lactate production [17]. Loss of NNT in hepatoma cell lines appears to have the opposite effect - decreased glycolysis and decreased expression of HIF-1alpha [18]. In this context, we should mention that neither of these studies were carried out in serum-free chemically-defined conditions - a basic approach that in the glorified age of molecular genetics has been haughtily dismissed as unnecessary. But without this sobering caution - that most of the established molecular biology has studied the growth responses to biomolecules, whether in primary populations (which is rare) or in transformed cell lines, *only in the presence of serum-containing media*, or in *cell-free* single-step reaction vessels (indeed, not all chemistry is biochemistry) - many of the observed responses may well have little physiological value.

While lack of oxygen can be considered a "reductive stress", what shifts the metabolism from anaerobic to lactic are the roles of HIF-1 and IGF-1R. This suggests that, in cancer-initiation, the hypoxia-independent IGF axis compounds the effects of hypoxia [13].

Conclusions

Systematic electrophysical and electrochemical experiments detailed in a previous publication (HEB) have solidly demonstrated the existence in humans of a bioplasma composed of dyad electrons each with 512.79 eV of kinetic energy and responsible for the baseline heat generated by the body. They also showed that the body is the source of an electric massfree radiation field that we termed bio-ambipolar. In that prior work, we sought the nexus between bioplasma formation (in a wide sense of the word) and

biochemical catabolism, having zeroed in on the role of the ETC as a gate for bioplasma formation. It led us to wonder whether ETC initiation was really the work of *cytosolic* NADH, in what concerns both donation of a proton or of 2 electrons. Above, we suggested that it likely fails to do both tasks. Instead, we suggest that bioplasma flux is made regularly across both the MIM and the MOM through still unidentified channels, and that this flux is shored up by matrix recruitment of electrons and an oscillating deficit of 1 proton per ETC cycle.

However, unlike biochemists, we have analytically argued that the ETC heat is not released from the formation of water, since the latter is not exothermic when carried out from basic constituents; but rather, that the heat is released as "joule heating" from mobilization of a current of *supramodal* electrons, in the process of current amplification. These supramodal electrons that feed the ETC are distinct from the bioplasma proper, which is formed by a structured dyad flow of *modal* electrons that establish a baseline of internal heat production within what may be strictly regulated bounds. Production of electromagnetic heat beyond these bounds results on de-kineticized dyads dropping off the bioplasma flux chain. There is thus a constant loss of both kinetic energy and electrons from the bioplasma, which is counteracted by a constant re-energization taking place in mitochondria (and perinuclear cisternae), as well as by recruitment of modal electrons from the mitochondrial matrix.

The analytical model indicates that hypoxia is the major shutdown switch of bioplasma formation (re-energization and recruitment). It can temporarily be replaced by FA oxidation, but disjunction of the ETC suggests that either bioplasma formation is tremendously slowed down, or that another key metabolic nexus exists other than what we have focused on. We suggest, however, that if the hypoxia is persistent (anoxia), activation of the hypoxia-independent IGF axis of gene expression (via induction of HIF-1alpha) may initiate the most basic process of adaptive mutagenesis that ultimately leads to malignant transformation of somatic and blood cells. There are no simple solutions to avoid or treat hypertension, heart disease, diabetes and acquired cancer. Their interconnection is complex, and at bottom it lies with what Reich called sympatheticotonia, a fundamental emotional disturbance of the vegetative system that prevents organs from their full development and function. Ultimately, it is what causes blood and tissue hypoxia. Whether compounded or not by malnutrition and

immunotoxicity, it is the basis of most non-hereditary clinical disorders that at present affect the greater mass of human beings.

References

1. Correa P & Correa A (2021) "Human experimental bioenergetics", Akronos Publishing, Concord, Canada.

2. Morciano G et al (2021) "The mitochondrial permeability transition pore: an evolving concept critical for cell life and death", Biol Rev, 96:2489-2521.

3. Flores-Romero H, Dadsena S & García-Sáez A (2023) "Mitochondrial pores at the crossroad between cell death and inflammatory signaling", Molecular Cell, 83:843-856.

4. Bernardi P, Carraro M & Lippe G (2022) "The mitochondrial permeability transition: recent progress and open question", FEBS J, 289:7051-7074.

5. Weber J (2007) "ATP synthase - the structure of the stator stalk", Trends Biochem Sci, 32(2):53-56. See its figure 1.

6. Noji et al (1997) Direct observation of the rotation of F_1-ATPase", Nature, 386:299-302.

7. Kinosita Jr, K et al (1998) "F_1-ATPase: a rotary motor made of a single molecule", Cell, 98:21-24.

8. Sobti M et al (2021) "The six steps of the complete F_1-ATPase rotary catalytic cycle", Nature Comm, 12:4690. See its figure 1.

9. Correa P & Correa A (2011) "The electron mass-energy as a deformable nanometric torus - fine and hyperfine structure and flux topogeometries", Volume III of the Aetherometric Theory of Synchronicity (AToS), Akronos Publishing, Concord, Canada, ABRI monograph AS3-III.4.

10. Correa P & Correa A (2004a) “Nanometric functions of bioenergy”, Akronos Publishing, University of Toronto Press, Concord, Canada.

11. Graf SS et al (2021) "Energy transfer between the nicotinamide nucleotide transhydrogenase and ATP synthase of *Escherichia coli*", Nature Scientific Reports, 11:21234.

12. Rao KNS et al (2020) "Nicotinamide nucleotide transhydrogenase (NNT) regulates mitochondrial ROS and endothelial dysfunction in response to angiotensin II", Redox Biol, 36:101,650.

13. Correa P & Correa A (2010) "A unitarian biochemical and bioenergetic theory of adaptive oncogenesis: from hypoxia and energy starvation (aerobic *and* ambipolar) to the roles of HIF-1, IGF-I, and vitamins C and D", J Biophys Hematol Oncol, 5:1-93.

14. Rath M (2001) "The heart", MR Publishing Inc, Santa Clara, CA.

15. Willis GC, Light AW & Gow WS (1954) "Serial arteriography in atherosclerosis", Can Med Assoc J, 71:562-568.

16. Newman TB & Hulley SB (1996) "Carcinogenicity of lipid-lowering drugs", JAMA, 275:55-60.

17. Glushakova LG et al (2011) "Increased superoxide accumulation in pyruvate dehydrogenase complex deficient fibroblasts", Mol Genet Metab, 104:255-260.

18. Ho H-Y et al (2017) "Nicotinamide nucleotide transhydrogenase (NNT) deficiency dysregulates mitochondrial retrograde signalling and impedes proliferation", Redox Biol, 12:916-928.

Appendix - Brad Marshall's hypothesis of reductive stress

The following commentary was written a propos of an interview of Brad Marshall conducted by Joseph Mercola ("Understanding the role of reductive stress in the development of chronic disease", an interview of Brad Marshall by Dr. Joseph Mercola, 2024, @ media.mercola.com).

I. The core contention of Marshall's reductive stress hypothesis

Marshall's core hypothesis of *reductive stress* is essentially that electron pairs (plus one proton) get trapped in excess NADH localized inside mitochondria, causing a surplus of negative charge to accumulate, and which can only be relieved if pyruvate dehydrogenase (PDH) in Complex I of the respiratory chain churns out superoxide anion (SOA) by transferring those electrons to oxygen itself.

The evidence for PDH producing SOA from pyruvate is scant (2 papers, in 1984 and 2004). What is new and has been recently established - when compared to old biochemistry of 1990's - is that PDH and other dehydrogenases (oxidases) can supply electrons from NADH for the production of peroxide through *reverse* electron transfer (RET). The peroxide is then reduced by catalase into water and oxygen. If it is not removed by catalase, the peroxide inhibits the forward action of PDH. This is one of the routes of *oxidative stress*, when the inhibition remains.

But even if PDH could reversibly generate SOA, the enzyme superoxide dismutase (SOD) will reduce and neutralize it. SOD is regulated by the balance of manganese inside the mitochondrial matrix (the enzyme being referred to as MnSOD) and zinc in the cellular cytosol (ZnSOD). SOD is a well-established inhibitor of cellular proliferation. However, no mention of the roles of SOD or catalase occurs in the Marshall interview. Nor of ascorbate, glutathione and vitamin E as co-factors of their action. *Oxidative stress* occurs when both enzymes fail to neutralize the ROS (reactive oxygen species). But there are still other antioxidant systems involved, such as the glutathione system.

More fundamentally, Marshall's hypothesis suffers from the flawed assumption that *aerobic* metabolism depends on the supply of NAD^+, when the latter is just a co-factor along with others just as important: FAD^+, alpha-lipoic acid, and vitamin B1-derived thiamine pyrophosphate (TPP). But, most important of all is the high partial pressure of oxygen. If it falls below normal, then instead of (forward) PDH activation,

lactate dehydrogenase comes into play, converting pyruvate into lactic acid by absorbing two protons while converting NADH to NAD^+. It happens in cancer cells (switch to lactic fermentation), but it also happens in any hypoxic tissue. So excess NADH, in effect, does *not* cause reductive stress. Rather, under hypoxic conditions, it promotes lactic fermentation as metabolic pathway, for as long as there are protons available. Furthermore, the enzyme nicotinamide nucleotide transferase (NNT) also oxidizes NADH to NAD^+; thereby, any excess of NADH does *not* automatically lead to production of lactic acid.

II. Production and elimination of ROS in metabolism, and the role of NNT

Marshall (p.7) next suggests that NNT can rebalance the excess NADH, because it "uses the superoxide to regenerate NAD^+". And he adds a second hypothesis to his theory - that the more reactive are the oxygen species one produces inside mitochondria, the higher is the metabolic rate. He implies that he is referring to aerobic metabolism, since "one would still be burning oxygen". In his view, PDH generates ROS, and NNT uses it to replace NAD^+ (p. 11).

Marshall's claim is totally incorrect. NNT does not use SOA to regenerate NAD^+. It is true that increased levels of ROS have been found in the tissues and plasma of hypertensive patients. However, it is obvious that its causation is multifactorial and potentially involving not just mechanisms that generate ROS in abnormally high quantity, but more significantly, enzymatic deficiencies - including genetic ones - in SOD, catalase, NNT, glutathione enzymes, and still others implicated in regulating the redox balance. Marshall oversimplifies a rather complex biochemical system - and even more so if one is to take into account the complex roles of the bioplasma. and the ETC.

III. Fatty acids and their metabolism

To buttress his reductive stress hypothesis, Marshall adds a third hypothesis - that NADH excess leads to activation of enzymes called desaturases, that consume NADH to convert (oxidize) saturated fatty acids (like stearic acid) into monounsaturates (like oleic acid). This explains, he argues, why the pattern of low stearic/high oleic acids is found in heart disease.

The first problem with this argument is that the oxidation of stearic acid occurs inside microsomes and the endoplasmic reticulum, and *not* in the mitochondria where the

NADH accumulates in Marshall's hypothesis. Furthermore, the synthesis of monounsaturates is a fall-back mechanism that comes into action *when carbohydrates are abundant and monounsaturates are low*. The process is inactive if there is nutrional intake of oleate and palmitoleate. Thus he cannot be correct when he states that "the more saturated [is the diet], the better".

Moreover, no such oxidative synthesis of linoleic or linolenic polyunsaturates is possible in mammals. Therefore one does need a dietary intake of these fatty acids, which are also low in heart disease and diabetes patients, a well established fact that Mercola disputes. They are definitely not what he abusively calls "the most pernicious metabolic poison in our diet"; certainly not if they are not rancid! In fact, Mercola gets it entirely wrong when he states that high levels of linoleic acid are associated with diabetes (p. 13). Soon after (p. 14), Marshall gently corrects him by indicating that lean breeds of hogs that are not at risk for diabetes have high linoleic acid levels, whereas obese hogs have lower levels because they *reduce* the linoleic acid to oleic or palmitoleic acids. In this respect, it evidently appears that the terms oxidation and reduction (which are inverse reactions) are bandied about freely and erroneously by both Mercola and Marshall. While referring to the lean hogs, Marshal states that they "do not oxidize linoleic acid", that is, "convert all that linoleic acid to monunsaturated fat". However, when one converts linoleic acid to oleic or palmitoleic (the monounsaturates), one is *not oxidizing* it, but *reducing* it. One only *oxidizes* linoleic acid when one converts it into linolenic or arachidonic, since the latter acids have, respectively, one and two more carbon-carbon double bonds (in fact, D6D is a desaturase that *only oxidizes* linoleic to linolenic). Likewise, when stearic acid is converted into oleic acid, one is *oxidizing* stearic acid (by introducing a single C=C double bond). Mercola fares even worse, as when in the same sentence (p. 18) he indicates that linoleic is being "reduced" by the desaturase SD1 because the saturase "oxidizes" it... The nonsense engendered by this total looseness with terms is most distressing.

IV. The build-up of acetyl CoA - and of acyl CoA

What Marshall calls the second part of reductive stress forms his fourth hypothesis - that acetyl CoA builds up, *somehow*, in parallel with NADH build-up. He claims that glucose, fat and amino acids all end up converted into acetyl CoA, and this leads to the acetylation (inactivation) of enzymes, such as "the magical NNT" (p. 8).

First off, *acetyl* CoA is the junction point of oxidative phosphorylation and the TCA cycle, to which also leads the first *end-point* of fatty acid (FA) oxidation - that is, if the latter is *not* to proceed to the production of acetoacetate. Metabolically, acetyl CoA arises from the many sources of pyruvate, including tryacylglycerol (the basis of FA oxidation), but glucose (through glycolysis) does *not* lead directly to acetyl CoA.

Secondly, acetyl CoA is not the same as acyl CoA. The latter emerges *not* as one of the end-points of FA oxidation but as the result of *the initiation step*. It also does not arise from glucose. The main reason *why acyl CoA accumulates* is a blockage of the second step of FA oxidation. In fasting or in high-fat diet, FA oxidation is triggered. But it is blocked if there is either a deficiency of the amino acid L-carnitine (a nutritional deficiency), or of the enzyme carnitine transferase. Either situation blocks FA oxidation at the second initiation step, right after the triacylglycerides are processed by lipases. Marshall does not mention this at all. Yet, it is obvious that, given how the vast majority of people are already deficient in carnitine, the outcome of this situation can only aggravate with fasting (as in weight loss diets). The result is that acetyl CoA cannot be efficiently produced by FA oxidation in the majority of people that fast, let alone accumulate. What accumulates, then, is acyl CoA.

Besides acetyl CoA, FA oxidation generates NADH and $FADH_2$. If these redox factors are high, then mitochondrial FA oxidation is inhibited and it cannot generate acetyl CoA. But this does not necessarily result in accumulation of acyl CoA either.

If acetyl CoA is successfully produced by FA oxidation, then, depending on the availability of OAA (oxaloacetate) to jointly react to generate citrate, it will be processed normally through the TCA cycle, but without activating the ETC or producing ATP. For processing through the TCA cycle it will require, as usual, NAD+, TPP, ascorbic and lipoic acids as co-factors.

However, when the breakdown of fats is the dominant catabolic metabolism, a different situation emerges because the concentration of OAA is no longer high enough to permit production of citrate. The concentration of OAA depends on the availability of carbohydrates, or on their proper utilization. Fasting obviously takes it down. When OAA is insufficient, a real excess of acetyl CoA occurs which has two aspects:

i) if insulin does not downregulate the initiation lipases of FA oxidation, acyl CoA will *also* accumulate; and

ii) instead of entering the TCA cycle, acetyl CoA becomes converted into acetoacetyl CoA, and the latter into acetoacetate, while acetyl CoA is regenerated.

With respect to this second aspect, there is a major feature that Marshall's hypothesis omits: that this acetoacetate can only be rid off in two very different ways. Either it is reduced to hydroxybutyrate by the enzyme hydroxybutyrate dehydrogenase (HBDase), or it is decarboxylated into acetone. The switch depends entirely on the availability of NADH. This is a critical switch that, on its own, directly contradicts Marshall's whole theory of reductive stress. For, if there is an excess of NADH and a reliance on FA oxidation under low OAA concentration (essentially low blood sugar), then NADH is oxidized to NAD^+ (which is old hat - see, for example, Stryer L (1991) "Biochemistry", WH Freeman & Co, San Francisco, CA, p. 393) so that hydroxybutyrate is produced. Thus, any accumulation of acetyl CoA in healthy FA metabolism leads to the removal of excess NADH by the hydroxybutyrate pathway (even as it regenerates the acetyl CoA). Furthermore, any accumulation of acetoacetate in blood triggers a decrease in the rate of lipolysis in adipose tissue. When HBDase fails, acetoacetate accumulates in blood. As the pH of the latter becomes acidic, the acetoacetate becomes converted to acetone, with the evolution of carbon dioxide. This is the prevalent situation in diabetes patients, acetone being easily smelled on their breath. If acetone is produced from acetoacetate, then NADH is not oxidized and now may accumulate in the mitochondrial matrix - while acetone is exported to the blood. We should note that even though acetoacetate and hydroxybutyrate are commonly called ketones, hydroxybutyrate is not a ketone (as p. 15 of the interview claims) but a fatty acid.

V. Final thoughts

All the preceding remarks underlie the fact that regulation of the NADH/NAD+, NADPH/NADP+ redox pairs is a complex matter that cannot be mechanistically reduced to a simple causation such as "acumulation of NADH is the culprit because it traps electrons". Marshall's hypothesis is deeply flawed and at times confused. As we have seen above, the confusion emerges at critical points. Yet another example occurs when Marshall defends that a protein diet ("branched chain aminoacids") is not necessarily bad (as Mercola supposed was the case) because it raises the glycine serum levels and, *somehow*,

coincides with the "unhealthy" presence of acylglycines in urine which, according to his theory, result from "a build-up of acetyl groups, and that is the acetyl CoA".

Irrespective of the flaws in Marshall's theory, it is always good to eat protein gels, whether animal or vegetal, because they come from bouillons that break down protein into free amino acids that are immediately absorbed by the gut, and also because they are rich in L-proline and L-glycine. But such foods critically lack, for example, L-lysine and L-arginine entirely, which must be supplemented from other sources. Moreover, it is not good to encourage the sluggishness of the body in deploying its anabolic enzymes to break down proteins. As we have seen above, complete FA oxidation (that ends in the production of hydroxybutyrate) is a normal metabolism that can be hijacked in metabolic disorders. FA oxidation can be altered at several points according to the nature of the underlying disorder, which is often acquired by particular nutritionally-unhealthy behaviours. Such alterations can result in various and distinct accumulations - of acyl CoA, acetyl CoA, acetoacetate and acetone.