(Biological Oxidation by Amirova M.F.)

ENERGY METABOLISM

Metabolic pathways are sort into catabolic & anabolic. Catabolic means breakdown of molecules, while anabolic means synthesis of compounds more complex than previous ones. In catabolic reactions the energy is released, ergo they are called exergonic, while in anabolic reactions the energy is consumed, these reactions go with investment of energy. So they are called endergonic reactions. Any pathway has its own committed first step which is closely regulated. The other reactions are also regulated but not so seriously as the committed step. & in general, all the pathways are regulated.

Hydrogen with electron in it is the main energy carrier in living organisms. In green part of plants, inside chlorophylls plastids absorb the light energy in quanta form & this way raise their energetic level. This appears in jump of electron from lowest level in hydrogen to highest one. After it this hydrogen becomes active & can participate in synthesis reactions, mainly in synthesis of glucose & other organic compounds. When glucose enters the body cells, this energy of sunlight cumulated in glucose & other organic compounds is extracted by mitochondria. In mitochondria occurs process opposite to plastids: the electron of hydrogen jumping on lower level of orbital, liberates energy previously cumulated. This liberated energy is trapped in ATP form. The energy is transferred from sunlight to hydrogen for cumulation in organic compound bonds. After ingestion by human, this energy is liberated in mitochondria. All the energy liberated from nutrient hydrogens, is further transferred to ATP bonds or dissipated in form of heat. Heat is used to maintain a body temperature, while ATP – as an energy currency in cell reactions, mechanical work (contraction).
Nutrients serve for organism as fuel for engine. Fuel combines with oxidizer & releases an energy. Breakdown products are thrown out to environment. The identical process happens in the cell: the nutrient undergoes combustion reaction with

participation of O2. Energy released is cumulated in ATP form, while waste products are thrown outside, mainly by kidney (waste water) & lungs (CO2).

ATP, produced after breakdown of carbs, fats & proteins, is further used for muscular work, heating of body & any other demands. Food carbs, proteins & fats of food are alien to the body, which is why they are first degraded to simple building blocks & only after that used for package of body own carbs, proteins & fats, which are inherent in the body. ATP serves as energy link between anabolic & catabolic reactions: ATP produced in catabolism, is utilized in anabolism

To maintain the structure & functions of living organism, constant energy supply is needed. This energy is derived during oxidation of food intermediates. Each gram of carb or protein oxidation gives 4 kCal of energy, while fat gives 9 kCal.

There is a concept ***basal metabolism***. This is a quantity of energy needed for maintenance of vital functions. Actually, this is an energy that the body expends in order to survive in the rest. In the rest occur: respiration, blood circulation, cellular & glandular activities, temperature maintenance but no any muscle activity. Reaction are mainly reversible. A converted to B & B converted to A. The direction of reaction depends of free energy present in reactants (substrates). Free energy simply means energy of molecule utilizable in the reaction. Change in free energy is called Gibb's free energy delta G. If molecule loses an energy in the reaction (the electrons jump to lower level orbital), such reaction may go spontaneously.

Delta G may be zero, positive & negative.

Delta G= 0 means that the reactants are in equilibrium, both directions: A to B & B to A are possible & identical. There is not losed or gained the energy in such reaction.

Exergonic reaction is a reaction with lose of energy by substrate. After reaction, the free energy of substrate is less that before reaction. These reactions are called catabolic. Delta G is negative.

Example of exergonic reaction is reaction of ATP hydrolysis. ATP at hydrolysis releases approximately 7.3 kCal of energy, ergo this reaction is exergonic.

All exergonic reactions of body may be classified as follows: to main stages of catabolism. I – breakdown in the CIT, II. Conversion bulky building blocks to simple

intermediates that can enter general pathways. III – general pathways. During digestion in GIT proteins are degraded to amino acids, lipids – to fatty acids & glycerol, & polysaccharides – to simple carbs, mainly glucose. Approximately 1% of energy cumulated in food is released in this stage.

About 20% of substrate energy, no more is released in this stage. This stage comprises conversion reactions, in which all compounds are degraded to acetyl-CoA or any intermediate of TCA cycle, the main combustion process of the body fuel substrates. TCA is the final process giving approximately 80% of energy for body. A plenty amount of ATP is created in these reactions. This is process of acetyl-CoA oxidation with formation many NADH2 & FADH2. NADH2 & FADH2 are the carriers of reducing equivalents (H+) that provide synthesis of ATP.

Anabolic reactions, which are endergonic (utilizing energy), are used to synthesis of our body compounds, actually proteins, fats & carbs inherent in the body. After these reactions the energy of product raised & is higher than energy of substrate (in initial state).

Delta G is positive: since the reaction is energy requiring, it does not proceed spontaneously.

In the cell, there are high energy compounds which provide the reactions with energy needed. One of them is ATP, a reference molecule. All the other compounds are compared with ATP. If the energy of bond breakdown is higher than energy released under ATP hydrolysis (7.3 kCal) is considered as high energy compound. Such molecules can pass their energy for synthesis of ATP & participate in substrate level phosphorylation. Compounds liberating less than 7.3 kCal of energy are referred to as low energy compounds.

Phosphoenol pyruvate, 1,3-bisphospho glycerate CP belong to high energy compounds liberating 14, 12, 10 kCal of energy respectively. ADP, Glucose- 6- ph, Fr-6-ph refers to as low energy compounds.

When terminal phosphoric group is released from ATP, energy liberated is used to meet cell demands. ADP indicates a low energy state of cell, thus cell strive to

replenish ATP. When ATP in a cell is high, we replenish the stocks; when ATP is low, we run out of stock.

ATP possess 2 anhydride bonds, ADP – only 1, while AMP – no one.

The role of ATP in the cell: ATP is provider of energy for all possible processes.

ATP is produced in oxidation reactions. Oxidation always proceeds simultaneously with reduction. That's why they are called in a couple: oxidation-reduction reactions. Oxidation means lose of H or e, or addition of O to molecule. Reduction is opposite process: addition of e, gain of H or lose of O. It can be presented by the example of Fe. Losing e, II valence Fe is oxidized to III valence. Opposite process is called reduction of Fe.

Redox system works in a couple: A loses e, & along with e it loses part of energy present in molecule, thus it is oxidized after reaction. B gained e, & along with e it gains some energy & raised its energy state, ergo it is reduced after reaction

Main power stations for the cells are mitochondria. Here, main oxidation reactions occur. O2 absorbed by breathing, enters mitochondria for these oxidation reactions & is used as oxidizer, that is why this process of energy production in mitochondria is called aerobic respiration, or ETC.

Brief review on mitochondria. Structure. Small smooth outer membrane covers a large inner membrane which is folded into cristaes. Enzymes of ETC are embedded into inner membrane of mitochondria. The intermembrane space occurs between inner & outer membranes.

Tissue respiration, or ETC is a sequence of reactions in which energy-rich hydrogen is taken out from substrate & its energy is released & stored in ATP. This proceeds by complexes I, II, III, IV & V. Complex I is FMN-containing, c. II – FAD-containing, c. III comprises cytochromes b & C1, c. IV – cyt. A & a3. V complex is ATP synthase. There are 2 relatively mobile compounds of ETC: CoQ & Cyt C.

During tissue respiration, the free energy of compounds is lowered (lose of energy occur) Redox potential of complexes from I up to IV is raising, what means is raising the ability to accept e. The less potential has complex I, what means is has less ability to accept electron: as soon as it accepts e, it passes it to next compound of ETC. But,

the most strong oxidizer is O2, it has the highest redox potential, i.e. highest ability to accept e. O2 is not a component of ETC, it is last acceptor from last (aa3) component.

The components (enzymes) of ETC are arranged in the inner membrane in direction from lowest redox potential (complex I) to highest (comp. aa3), which is why the electron flow in this direction. More negative, or low redox potential signifies less ability to accept but greater ability to lose electros & vice versa.

First complex, FMN containing, is called ***NADH2-DH***. II comp. FAD containing is mainly represented by ***Succinate DH*** (***SDH).*** Both these complexes pass their electrons to CoQ, after which electrons move to Comp.III (cyt b-C1). Then cyt C1 transmits e to cyt C and eventually – Cyt aa3. Cytochromes comprise Fe which is reduced after acceptance of electron becoming II valence. In 3 points of ETC, namely comp.I, III & IV energy release is so high, that they serve to pump protons into intermembrane space: 4H+ - by comp.I, 4H+ - by comp.III, & 2H+ - by comp.IV.O2 accepts electrons from ETC & turns to water. 4 electrons & 2H+ for this required. Electrons are taken out from ETC, while H+ - from matrix.ATP synthase, the last complex of ETC, uses H+ for synthesis of ATP.

Complexes of ETC are named: first 2: ***NADH2-DH & Succinate DH*** , as we already know. III is ***cyt—reductase***. CoQ & Cyt C are relatively mobile components of ETC. Complexes I & II are the first accepting protons from substrates. They pass electrons to CoQ. ETC starts mainly with comp.I (NADH2-DH), but very rare only – with SDH (comp.II).

Each carrier accepts electrons from previous component & donates them to subsequent one releasing this way the electron energy. Moving from one complex to next, e releases quanta of energy used for pumping protons from matrix to intermembrane space.

Fifth complex (ATP synthase) combines ADP with Pi to create ATP at the expense of energy of proton, that re-enter matrix from intermembrane space through comp. V. Term “oxidative phosphorylation” signifies reaction ADP+ Pi =ATP. This is the goal product obtained by oxidation of nutrients.

Part of energy not trapped in ATP anhydride bonds, is dissipated as heat to maintain T of warm-blooded.

There are also iron-sulfur proteins embedded in ETC at points between FMN of comp.I & CoQ, & cyt b & cyt C1 of complex III. They possess Fe with variable valence & Sulfur atom.

Let's be acquainted how ETC components work. NADH2 is the first donor of first electron pair to FMN containing comp.I, First of all, proceeds reduction of NAD to NADH2 under the action of different enzyme, mainly from I & II general pathways(TCA). Donated electrons convert NAD to NADH2.

Pyruvate, Oxaloacetate, alpha-KG, fatty acids are the substrates donating e to NAD.

NAD accepts 2 electrons & 1 proton, the second H+ stays in the medium, which is why reduced NAD is more correctly signified as $NAD^+ + H^+$. NADH2 is designed only to simplify the way of writing.

One of substrates providing NAD with e is lactate. Hs pass to structure of NAD forming NADH2.

Accepted by NAD electrons pass to active site of I complex, namely FMN.

Donated by succinate electrons are not so rich in energy to donate to NAD (Comp.I), ergo succinate donates to FAD of complex II instead.

Both I & II complexes pass their e to CoQ. CoQ accepted 1e turns to semiquinone, accepted second e, becomes completely reduced ubiquinone, or ubiquinol. Picture shows pass of e & protons separately.

Keto group of ubiquinone turns into alcohol group.

CoQ transfers electrons to cytochromes. Since cyt possess Fe, transporting e only, CoQ transmits only electrons on the structure of cyt, but not H+. H+ is pumped into intermembrane space. Thanks to quick work of cyt, 4 e reach O simultaneously & lead to formation of water molecule. Oxygen active species are formed when less than 4 e reach O2 in ETC.

Any cytochrom has its own specific porphyrin ring with specific side chains in it.

Heme b is component of Hb, heme C – of cyt C, heme a – of a& a3.

3 steps in ETC pumping H+ into intermembrane space are: Comp.I (NADH – reductase), Comp III (Cyt C-reductase), & Comp. IV (cyt oxidase). I & III complexes pump each 4 H+s, while IV comp. pumps only 2.

Synthesis of ATP in ETC was explained by Mitchel in chemiosmotic theory. Pumped protons strive to return into matrix, & they return through comp.V activating it.

H+ gathered in intermembrane space, lower here pH, ergo here pH is lower than in matrix. This difference in pH between inner & outer surfaces of inner membrane is called gradient of pH. By the way, electrical gradient of H+ is also due to cumulation of H+ in the intermembrane space. These 2 gradients are forces that make possible synthesis of ATP in the mitochondria. But ATP can be synthesized not only by oxidative phosphorylation in ETC. Substrate level phosphorylation also occur. For example, in glycolysis 2 ATPs are synthesized without oxidation in complexes of ETC, but directly during oxidation of appropriate substrate, which is why such ATP synthesis is called substrate level phosphorylation.

Electrical gradient (cumulation of H+ inside of INN. Membrane) activates the movement of H+ into the matrix through the pores created for this purpose: ATP synthase.

As synthesis of ATP is coupled with oxidation in ETC, process is called oxidative phosphorylation.

Hydrogens flux through ATPase channel forces activation of this enzyme with subsequent combination ADP with Pi. Enzyme helps attachment of Pi by ADP.

Proton pumping ends with ATP synthesis on H-ATPase . ATPase contains

*protruding into the matrix F1 subunit and

*Subunit Fo buried into the inner membrane.

Thanks to H+, the electrical gradient pH gradient is created. When H+ strive to move into the matrix, they can not find another place to cross membrane but ATPase. When passing through ATPase, H+s loose a lot of energy, which is used for activation of ATPase & its rotation. Rotated, ATPase brings ADP & Pi closer, thus catalyzing ATP production.

When electrons enter ETC through NADH2 (complex I), they pass to O_2 PUMPING SUFFICIENT protons into intermembrane space, thus producing 2.5 ATPs, while entering through FADH2 (complex II), electrons energy released promote synthesis of only 1.5 ATPs.

There is also ATP/ADP transporter in inner membrane of mitochondria. It transports ATP outward of mitochondria in exchange for ADP entered mitochondrial matrix. This transporter protein serves for entry of ADP & Pi resulting from ATP hydrolysis inward the matrix.

However in the nature there are the compounds that inhibit production of ATP in ETC.

Malonate, Carboxin, Rotenone etc. belong to them. Malonate inhibits ETC inhibiting succinate protons entry to the chain. **Carboxin** prevents movement of protons from SDH (complex II) to CoQ.

Amobarbital, Piericidin A, Rotenon interfere with the transport of protons from complex I to CoQ.

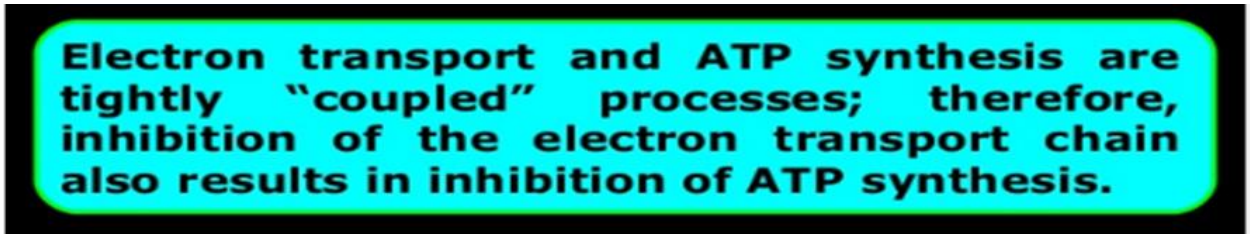
Antimycin A stops action of complex III,

& Hydrogen sulfide, carbon monoxide, Cyanide ions shut down Complex IV.

Uncouplers & oligomycin stop the work of complex V (ATPase synthase)

Rotenone inhibits pumping of protons into the intermembrane space by complex I, antimycin – by complex III, and Cyanide with Carbon monoxide – by complex IV. All these points are main energy producers in the ETC, because energy of electron is released mainly in these 3 complexes, which is why the ATP production stops after their action.

62.



Oligomycin binds to ATP-synthase closing H+ channel, thus preventing re-entry of H+s into the matrix, blocking oxidative phosphorylation by ATPase,

while atractyloside interferes with action of ATP/ADP translocase providing ATPase with ADP & Pi. After its action intramitochondrial ADP is depleted, & ATP production cessation occur.

UNCOUPLERS

Uncoupling means a leak of H+ from intermembrane space into matrix with simultaneous dissipation of energy in form of heat, ergo ATP can not be produced. Valinomycine is such ionophore.

Thyroxine & long chain fatty acids belong to these compounds.

Uncoupler serves as channel to re-enter H+ into matrix, so there is no need to pass through ATP synthase. Energy capture in the ATP form stops.

In inner embrane has been found uncoupling protein 1, thermogenine. It uncouples oxidation & phosphorylation in brown adipocytes of newborn babies & hibernating animals leading to heat production instead of ATP. Synthetic uncoupler 2,4-dinitrophenol & overdoses of salycilates, namely aspirin lead to fever. Mechanism of action is the same: they allow entry of H+ bypassing ATP synthase.

Both absence of ATP & overproduction of heat are dangerous & may lead to death.

ATP in norm is produced thanks to electrical gradient of H+ and pH difference on the inner membrane. Dissipating gradient of H+, uncouplers block phosphorylation after oxidation.

About 2,4-dinitrophenol:

it was used in medicine as drug to lose weight. A small lipophilic molecule, it easily diffused through inner membrane, allowing protons to re-enter matrix without production of ATP. This change in the body led to lack of energy and hence lose of weight. But multiple side effects made it reasonable that they have been banned.