Ray Peat's Newsletter

False symbolisms are corrected by semantic shocks. R.F. Creegan

Copyright 2014 **Raymond Peat P.O. Box 5764 Eugene OR 97405** **July 2014**

Approaches to renewal

Several groups have recently become interested in identifying the substances circulating in the blood that regulate cell renewal, using the old techniques of transferring plasma from a young animal to an old one, and vice versa, and of grafting a young animal to an old one, so that they share blood circulation. This is called parabiosis. Plasma extracted from a young animal delays or reverses some of the changes produced by aging.

Although there had been almost no work of this sort in the last 50 years, before that, the techniques were developing for 100 years. Paul Bert (a pioneer in high altitude research and oxygen toxicity to animals), in 1864 described his method of grafting two rats together. Although later investigators such as Alexis Carrell sewed blood vessels together in their experiments with joined animals, Bert found that common blood vessels were formed spontaneously in the joined animals.

The early parabiosis research was done without knowledge of "tissue typing," and others in subsequent years also had successful grafts even when the animals weren't littermates. One experiment that I read about in the 1960s described joining a two month old rat to a two year old rat (at the end of the normal rat life expectancy), with the pair living until the older rat was more than 1500 days old, which was considered to be the equivalent of 150 years for a human. (The question of why genetically different animals could be successfully joined is very important, and I think relates to the mechanism of aging.)

Alexis Carrell believed that cells were intrinsically immortal, and that something in the body fluids causes aging. Cells from a chicken heart that he put into tissue culture in 1912 were still growing and "beating" 20 years later when the laboratory discontinued the experiment. Several other researchers in the first half of the 20th century demonstrated effective anti-aging methods, but after the second world war there was a tremendous change in the nature of science, beginning in the U.S., with the government's massive intervention in research funding.

Before the war, Germany, Japan, and the U.S. had been funding secret research in chemical and germ warfare, and with the Manhattan Project's successful production of the atomic bomb, the flow of government funds began going into "basic science" which happened to be essential to progress in chemical, biological, and radiological warfare. Large numbers of the physicists and mathematicians who had worked on the bomb project suddenly found that they were interested in "molecular biology," which was now being generously funded by the government.

Many of them probably believed that the government's motives had become purely humanitarian, despite the continuing military-political use of atomic energy. The government was controlling the cultural context, even before the creation of the CIA's Congress for Cultural Freedom, for example attacking biologists who studied the inheritance of acquired traits, denouncing Lamarckism as "ideological," while Mendelism was promoted as objective and non-ideological. (C.C. Lindegren discussed this in his book Cold War in Biology.) Biological fields, epigenetics, environmental causes of cancer and aging, and other traditional themes disappeared from science and were replaced by reductionist molecular biology in the second half of the 20th century.

E.V. Jensen who had been working on chemical warfare, was funded by the government to identify the "estrogen receptor," leading to the termination of the research that had been

illuminating the relation of estrogen's metabolic effects to inflammation, cancer, aging, anxiety, and depression. Leonard Hayflick "discovered" (by faulty cell culture methods) that cells other than germ cells are not able to divide more than 50 times before dying, "proving" that Carrell's theories were false and that there are no stem cells. Until the cloning of Dolly the sheep in 1996, most biologists still sided with Hayflick, against stem cell research. Then the genetic engineers began to see stem cell research as a logical and profitable application of molecular biology.

I think the reason for the recent sudden renewal of interest in the antiaging substances in the body fluids is the growing realization that stem cell research, in the sense of "reprogramming" and transferring cells to renew tissues, has been on the wrong track. In January, Haruko Obokata published an article in Nature describing a technique for quickly making stem stems by stressing them, which she had described in 2011 in her dissertation. Although the idea of stress inducing "stemness" in cells was more than 50 years old, her publication of the simple technique in a major journal produced an intense reaction among the people working with stem cells, as if its claimed simplicity was an insult or a blasphemy.

Although the editors of Nature announced on July 2 that the articles describing her technique were being withdrawn (and she might be forced to withdraw even her dissertation), her work is part of a current, a spreading recognition that the body is always epigenetically reprogramming itself, and that no molecular biologists are needed to produce stem cells, and that it is signals of some sort, probably carried in the body fluids, that create stemness and appropriate reprogramming; the problem that needs to be answered is why the new cells are unable to mature and to survive, without becoming fibrotic tissue or tumors.

Parabiosis obviously involves the exchange of stem cells, but transferring even cell free plasma involves the exchange of some genetic information, in the form of microvesicles (very small cell organelles) released by cells into the blood and lymphatic vessels. These particles carry proteins and genetic material between cells, and are apparently an important part of ordinary developmental, "immune," and repair processes. They are apparently intercellular messengers that coordinate oxidation and reduction processes of cells (Larson, et al., 2014), and the control of oxidation and reduction in cells is fundamental for cell renewal and functioning and survival.

Cells depend on the movement of electrons from a fuel (a reductant or electron source) such as glucose to an oxidant, which is usually oxygen. The productive use of this energy can be disturbed by the presence of abnormal oxidants or reductants, or by an imbalance of the normal oxidants and reductants. If the use of oxygen is blocked (for example by cyanide or absence of oxygen), the electrons from fuel are captured by (i.e., reduce) inappropriate oxidants, such as iron, which can then activate oxygen, causing it to damage cell structures. Antioxidants such as vitamin E and vitamin C can intercept the reduced iron, breaking the dangerous sequence of reduction and oxidation; their original state is restored by accepting electrons derived from fuel, via NADH and glutathione, GSH. Fructose, tagatose (a sugar very similar to fructose), and aspirin protect from free radical damage by binding iron; caffeine also protects against oxidative damage from iron, possibly by a different mechanism.

The fact that NADH and GSH have a role in preventing damaging oxidation has led to their use for therapy. This is sometimes explained with the idea that the "most powerful antioxidants" are the best. In fact, even normal antioxidants can have harmful effects if they are present in the wrong concentration; NADH, GSH, and ascorbic acid can react directly with iron, producing the free radical damage that, in the right concentration in the right place, they would prevent.

The antioxidant mystique leads many researchers to believe that a reduced state of the cytoplasm (represented by higher NADH and GSH, lower NAD+ and GSSG) is generally protective, and that a shift toward oxidized cytoplasm and higher GSSG is the result of oxidative damage or stress. In fact, in healthy cells the level of NAD+ is hundreds of times higher than NADH, and in injured cells or cancer cells, the concentrations approach equality, corresponding

to a relative failure of mitochondrial respiration. In the region of the cytoplasm where proteins are synthesized, the ratio of the oxidized glutathione, GSSG, to the reduced GSH, has to be very high, to allow the new proteins to be folded properly.

This same mystique has affected the way ascorbic acid is understood. Most nutrition tables show a very low ascorbate content for meats, but when a muscle tissue is cultured, it releases a large amount of ascorbic acid, as the cell metabolizes. The oxidized form of ascorbic acid, dehydroascorbic acid, is relatively hydrophobic, and is present at a much higher concentration inside cells than the reduced form. This oxidized form of vitamin C is itself an oxidant, but it participates in the vitamin's protective effects, especially by oxidizing glutathione in the endoplasmic reticulum to the GSSG form, which is also an oxidant. A liver toxin such as ethyl alcohol increases the NADH/NAD+ ratio, while fructose, restoring a more oxidized ratio, protects the liver (Khan and O'Brien, 1995). In the mitochondrion, direct oxidation of GSH, with GSSG as a catalyst, contributes to maintaining the right balance of oxidants and reductants throughout the cell.

The free radical theory of aging, combined with the antioxidant mystique, has led many researchers to think that the degenerative diseases, and old age itself, involve an excessively oxidized state of the cells. This view fits into many other widely held beliefs about the nature of cells.

Oxygen got its name from its ability to produce acids; a Lewis acid is electrophilic, attracting electrons, and oxygen's actions in a cell create an electrophilic condition, proportional to that cell's rate of oxidative metabolism. The brain, with its extremely high rate of oxygen consumption, causes the head to be slightly electropositive or anodal relative to the abdomen or the feet, with their lower oxidative rate. When a cell is damaged or stressed, it becomes less electropositive, and less acidic. Relative to functioning cells, an injured cell is negative or cathodal, and likely to be reductive, rather than oxidative. This used to be called the "injury potential." Conventionally, it's called the "membrane potential."

The ideology of a cytoplasmic membrane controlling cell physiology contributes to the way most researchers think about cell oxidation-reduction, and about the meaning of those for cell adaptation and aging. The essence of the membrane doctrine is that there is physical-chemical chaos on both sides of the membrane, and that random movements of molecules, combined with barriers and pumps, are responsible for the cell's interactions with surrounding fluids.

This assumption of randomness, a molecular chaos controlled by genetically defined machinery, is the basic ideology of reductionist molecular biology, and a similar assumption is a dominant ideology in physics and other aspects of the culture, with roots in Plato's doctrine. To a platonist, argument from evidence means nothing, and--judging by the reaction to Gilbert Ling's and A.S. Troshin's work over the last 50 years, evidence is similarly harmless to the ideology of the biological-medical establishment.

When a cell is stressed, the shift to a more reducing state coordinates changes of protein synthesis, turnover, and activity, to compensate for the stress, according to its situation and resources. This shift, toward a higher ratio of GSH/GSSG, occurs in intestinal cells that are multiplying, in cancer cells, especially during metastasis, and in regenerating liver cells, and is often seen in germinating seeds; in non-dividing cells, and in hibernating squirrels or estivating toads, the balance is more oxidized. (In some situations, measurement of total sulfhydryl groups, including free cysteine and reduced protein cysteine groups, is a more meaningful measurement.) A slight shift toward oxidation is seen in liver cells influenced by DHEA, caffeine, thyroid hormone (T3), and aspirin. T3, like insulin, increases oxidative metabolism, and the GSH/GSSG which is less oxidized stimulates the formation of those hormones, leading to restoration of the more oxidized state.

The proper degree of oxidation regulates the folding of newly synthesized proteins and the stability of functioning proteins, the amount of water held by the cell, the way fuels are metabolized, and the sensitivity of cells' ability to respond to their environment.

A slight shift towards reduction in the cell's redox balance, when the cell's use of oxygen is limited, can cause a large shift in the ratio of lactate to pyruvate; the production of lactic acid takes excess electrons out of the system, helping to maintain homeostasis. However, the increased lactic acid has systemic effects, displacing carbon dioxide, and spreading its reductive influence as a sort of hormone. Diabetes, dementia, and cancer have in common the metabolic problem that Warburg described, the tendency to produce lactic acid even when oxygen is available. The failure of the proper use of oxidation contributes to the diversion of electrons into random "oxidative damage," but a deficiency of "antioxidants" isn't the primary problem, it's the failure to maintain the slightly oxidized balance of NAD+/NADH and GSSG/GSH in the cell.

The shift of the cell towards excess NADH activates (inflammatory) prostaglandin production (Rathaus, et al., 1986), and a shift of the balance towards increased GSH activates the basic inflammatory signal, NFkappaB (Hayashi, et al., 1993).†† The obesity of the "metabolic syndrome" is corrected by shifting the balance away from NADH toward NAD+ (Hwang, et al., 2009).

Things that activate cells' antioxidant defense system, including an excess of oxygen in their environment, increase the reduction of glutathione, and consequently ascorbate/dehydroascorbate and vitamin E, with generally protective effects. However, shifting of the oxidative balance because of failure of oxidative metabolism has complexly harmful effects. Challenges of various sorts increase the reduced state of cells, "antioxidant defenses" follow, and oxidative damage may accumulate; the damage to the organism from remaining too long in a reduced state has generally been neglected by researchers, but a few individuals are rethinking the meaning of the age-related diseases (Naviaux, 2012).

In the cornea, the concentrations of NADH and NAD+ can be measured by their light absorption and fluorescence. When the cells are deprived of oxygen, by keeping the eyelids closed for several hours during sleep or wearing impermeable contact lenses, the cells become more reduced, with an increase of NADH, and their water content increases. A slight swelling of the cornea changes the focus slightly, but at a certain point the swelling can cause opacity of the cornea, with an increase of lactic acid formation and decrease of carbon dioxide. Increased carbon dioxide protects against swelling (Sarroca, et al., 1997), and excess oxygen, probably by displacing carbon dioxide (as lactate does) and activating the cells' "antioxidant" defenses, can also increase the corneal damage (Doughty, 1995). Treating the cornea with a solution containing GSSG, the oxidized form of glutatione, improves the cornea's barrier function (Araie, et al., 1988).

The stressed cornea is a convenient model for any failing organ, for studying the effects of stress, shock, and aging, and the regulation of cell water is a universal feature of life; the balance of reduction and oxidation probably affects the cell's hydration before other metabolic changes are obvious. In the various degenerative diseases, the failure of oxidative metabolism shows up as increased lactate production, a shift away from glucose oxidation to fat and amino acid oxidation, and cell swelling. Lactate production itself is an indication of a more reduced state of cells. An excessively reduced state of glutathione has been measured in some degenerative diseases--ALS (Tohgi, et al., 1999), and Alzheimer's disease (Russell, et al., 1999; Kim, et al., 2000).

Nighttime is intrinsically stressful, with progressive damage such as calcium loss from bones occurring mainly during the night. The reduced state of glutathione has a daily cycle, with the reductive peaks occurring late at night and around dawn, when cortisol is reaching its daily peak. Cortisol tends to support the oxidized state of GSSG/GSH, so the reductive peak would probably be more extreme without its compensatory influence. In old age, as the protective oxidative hormones T3 and DHEA are decreasing, the daily cycles of reduction and oxidation become more exaggerated.

Nitric oxide is an important factor in shock, whether produced by sepsis, hemorrhage, excessive vagal stimulation, or physical or psychological trauma. In excess, it interferes with energy production (as ATP) and use (Cr kinase),

decreases nerve conduction and muscle contraction, and increases inflammation and cell swelling. The degenerative processes of aging share some of the essential features of shock, and nitric oxide excess is one of the central mechanisms of shock. Reduced glutathione activates nitric oxide synthesis.

Although some of the people doing the recent experiments with parabiosis and plasma transfer between young and old animals have proposed that a certain protein in the young plasma might be responsible for the antiaging effects, I think some of the known differences between the plasma of healthy young animals and that of animals in shock or in old age are likely to be useful for understanding the aging process and treating the degenerative diseases.

George Crile found that circulating the blood of a healthy animal into the brain of a traumatized animal prevented the shock that would have been produced by the trauma, and previous experimenters knew that overstimulation of the parasympathetic nervous system could produce shock, with systemic failure of oxidative metabolism. Tourniquette experiments showed that substances released into the blood from oxygen-deprived tissues could cause shock. Young blood provides a high ratio of glucose to fatty acids, and of carbon dioxide to lactic acid, along with adrenal and thyroid hormones, sustaining oxidation. Blood from an old or shocked animal generally contains a high ratio of fatty acids to glucose, of polyunsaturated fats to saturated fats, of lactic acid to carbon dioxide, and increased amounts of pituitary hormones, and is more likely to contain increased amounts of nitric oxide, prostaglandins, endotoxin, and inflammatory mediators of many sorts.

The microvesicles shed by cells into the circulation during stress can guide the development of stem cells (Quesenberry, et al., 2014; Candelario and Steinberg, 2014), and metabolic conditions can influence the composition of the microvesicles.

Many of the anti-adaptive features of old blood can be reduced. Long daylight hours and high altitude (the high altitude "lactate paradox" is an example of a cellular oxidative increase caused by lower oxygen pressure) shift the balance of some of the factors, and others can be improved by modifying the diet, and supplementing with things such as the protective steroids, thyroid hormone, aspirin, niacinamide (which can increase the oxidized state of NADH/NAD+), and caffeine. Providing the things needed for producing cellular energy while blocking some of the maladaptive factors approaches the problem of aging in a fundamental and holistic way.

REFERENCES

Exp Eye Res. 1974 Jul;19(1):1-10. **Disulfide stimulation of fluid transport and effect on ATP level in rabbit corneal endothelium.** Anderson EI, Fischbarg J, Spector A.

Invest Ophthalmol Vis Sci. 1988 Dec;29(12):1884-7. **Effect of oxidized glutathione on the barrier function of the corneal endothelium.** Araie M(1), Shirasawa E, Hikita M.

Am J Physiol Cell Physiol. 2005 Nov;289(5):C1220-8. **Temporal changes of multiple redox couples from proliferation to growth arrest in IEC-6 intestinal epithelial cells.** Attene-Ramos MS(1), Kitiphongspattana K, Ishii-Schrade K, Gaskins HR.

Trends Mol Med. 2014 Jul;20(7):368-374. **The role of extracellular vesicles in the progression of neurodegenerative disease and cancer.** Candelario KM, Steindler DA.

Biochemistry. 2013 Sep 24;52(38):6712-23. **Redox modulation of endothelial nitric oxide synthase by glutaredoxin-1 through reversible oxidative post-translational modification.** Chen CA, De Pascali F, Basye A, Hemann C, Zweier JL. "We demonstrate that Grx1 in the presence of glutathione (GSH) (1 mM) reverses GSSG-mediated eNOS S-glutathionylation with restoration of NO synthase activity...."

Free Radic Res. 2011 Nov;45(11-12):1245-66. **Redox biology of the intestine.** Circu ML, Aw TY.

Semin Cell Dev Biol.+ 2012 Sep;23(7):729-37. **Intestinal redox biology and oxidative stress.** Circu ML(1), Aw TY.

Ophthalmic Physiol Opt. 1995 Nov;15(6):585-99. **Evaluation of the effects of saline versus bicarbonate-containing mixed salts solutions on rabbit corneal epithelium in vitro.** Doughty MJ.

Arch Biochem Biophys. 1995 Feb 1;316(2):699-706. **Regulation of hepatic nitric oxide synthase by reactive oxygen intermediates and glutathione.** Duval DL1, Sieg DJ, Billings RE.

J Biol Chem. 1993 May 25;268(15):11380-8. **Oxidoreductive regulation of nuclear factor kappa B. Involvement of a cellular reducing catalyst thioredoxin.** Hayashi T, Ueno Y, Okamoto T.

Diabetes. 2009 Apr;58(4):965-74. **Pharmacological stimulation of NADH oxidation ameliorates obesity and related phenotypes in mice.** Hwang JH(1), Kim DW, Jo EJ, Kim YK, Jo YS, Park JH, Yoo SK, Park MK, Kwak TH, Kho YL, Han J, Choi HS, Lee SH, Kim JM, Lee I, Kyung T, Jang C, Chung J, Kweon GR, Shong M.

Biochim Biophys Acta. 1995 Nov 9;1269(2):153-61. **Modulating hypoxia-induced hepatocyte injury by affecting intracellular redox state.** Khan S, O'Brien PJ.

Antioxid Redox Signal. 2000 Fall;2(3):485-9. **Protein disulfide isomerase in Alzheimer disease.** Kim HT, Russell RL, Raina AK, Harris PL, Siedlak SL, Zhu X, Petersen RB, Shimohama S, Smith MA, Perry G.

Exp Pathol. 1985;27(1):41-8. **Redox status of cultured fibroblasts. Possible relations with specific catabolic rates of proteoglycans.** Kittlick PD, Babin W. "The NAD/NADH ratio is reduced when the lactate concentration increases at pH 7.4 with and without hypoxia."

Free Radic Biol Med. 2014 Apr 18;73C:214-228. **Circulating membrane-derived microvesicles in redox biology.** Larson MC, Hillery CA, Hogg N.

Mech Ageing Dev. 1993 Aug 15;70(3):177-99. **Maximum life span in vertebrates: relationship with liver antioxidant enzymes, glutathione system, ascorbate, urate, sensitivity to peroxidation, true malondialdehyde, in vivo H2O2, and basal and maximum aerobic capacity**. Lopez-Torres M(1), Perez-Campo R, Rojas C, Cadenas S, Barja G.

J Pharmacol Exp Ther. 2012 Sep;342(3):608-18. **Oxidative shielding or oxidative stress?** Naviaux RK.

FASEB J. 2001 Oct;15(12):2131-9. **Induction of mild intracellular redox imbalance inhibits proliferation of CaCo-2 cells.** Noda T, Iwakiri R, Fujimoto K, Aw TY. "Low concentrations of diamide plus 1,3-bis(2 chloroethyl)-1-nitrosourea (BCNU) increased intracellular GSSG and decreased GSH and the GSH:GSSG ratio. These changes occurred within 30 min, which preceded a decrease in thymidine incorporation at 6 and 24 h. ROS formation was not detected under these conditions. This suppression of cell proliferative activity was attenuated by N-acetyl cysteine, in parallel with restoration of the intracellular GSH redox status."

Front Oncol. 2014 Apr 4;4:56. **Marrow Hematopoietic Stem Cells Revisited: They Exist in a Continuum and are Not Defined by Standard Purification Approaches; Then There are the Microvesicles.** Quesenberry PJ, Goldberg L, Aliotta J, Dooner M.

Arch Biochem Biophys. 1999 Oct 15;370(2):236-9. **Increased neuronal glucose-6-phosphate dehydrogenase and sulfhydryl levels indicate reductive compensation to oxidative stress in Alzheimer disease.** Russell RL, Siedlak SL, Raina AK, Bautista JM, Smith MA, Perry G.

CLAO J. 1997 Oct;23(4):282-5. **The effects of low pH and CO2 on bovine corneal lactate production.** Sarroca A(1), Lin S, Bonanno JA.

Neurosci Lett. 1999 Feb 5;260(3):204-6. **Increase in oxidized NO products and reduction in oxidized glutathione in cerebrospinal fluid from patients with sporadic form of amyotrophic lateral sclerosis.** Tohgi H(1), Abe T, Yamazaki K, Murata T, Ishizaki E, Isobe C.

****