Spiraling complexity, robustness, and fragility in biology

As biologists delve deeper into genomics, gene regulation and subcellular interactions, the networks that regulate cell function can sometimes appear wildly and gratuitously elaborate, since it is known that enormous complexity is not necessary for basic cellular survival and function. Minimal cellular life is thought to require about ~300 genes, yet even E. coli have ~4000 genes. Gene knockout studies of E.coli confirm that about 90% of its genes are not individually essential for viability in the laboratory. Recall that in our idealized allometry wind tunnel laboratory, almost the entire 150,000 element 777 "aeronome" can be ***simultaneously*** knocked out without exhibiting a phenotype. These observations are clues that the reason for the "excess" complexity is not merely redundancy, but the presence of complex regulatory networks that effect robustness but not minimal functionality.

Many knockouts of mouse genes also appear to generate no phenotype until the animal is somehow perturbed. For example, a gene product used during development of an organ system can be knocked out, but the organ system still develops via compensating networks. However, damage to the organ later in life can unmask a regeneration-defective phenotype in a stressed, adult environment.

Laboratory environments are highly controlled and may not expose E.coli to wide ranges of temperatures and gas and nutrient concentrations. For example, well-fed E. Coli do not make any of the sensors, actuators (flagella) or signal transduction elements involved in chemotaxis, and thus simultaneous knockouts of these more than 50 genes are not lethal in an ideal laboratory setting.

E. coli can grow anaerobically, but prefer aerobic metabolism and will attempt to control their O_2 environment by aerotaxing to an intermediate oxygen tension. Too low and they are anaerobic and thus metabolically inefficient, and too high and they suffer excessive free radical stress, although they can survive at a wide range of O_2 tensions.

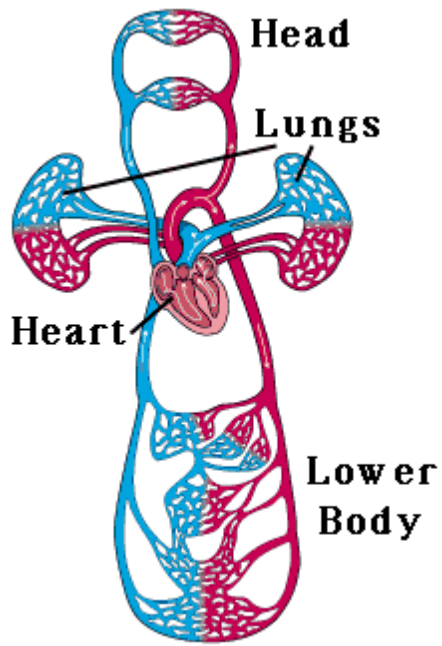
The E. coli chemotaxis system has some remarkable design features, from the modularity and dynamics of the sensors and signal transduction network, to the construction and action of the flagella, to the stochastic search algorithm implemented by "run and tumble" to overcome the inherent limitations in gradient sensing at such small scales. It has been correctly remarked that nothing like this system exists in engineering. Only in bacteria has this ***particular*** combination of methods been combined in this particular way, and it will be some time before nanotechnology allows engineers to build micron-size swimming robots. In contrast, ***all*** of the known system level organizational principles appear to correspond to standard engineering practice. Such similarities throughout biology to engineering control systems have been noted elsewhere.

Variations in the environment require complex regulation.

All large animals are strict aerobes. For us, too much or too little O_2 spells disaster. So our reliance on this essential nutrient obligates us to use complex multi-level feedback control mechanisms to assure not only appropriate sufficiency of O_2 matched to tissue need but protection from O_2 toxicity. Coordinate, multi-layer, distributed, multi-timescaled networks maintain precise internal, local O_2 concentrations despite variations in both supply and demand.

At the gross anatomic level our circulatory system is composed of two distinct vessel types, one for picking up oxygen and one for delivering oxygen. In fish the gills interface with the circulation for picking up oxygen. (The frog circulatory system is an intermediate evolutionary step between the fish and mammalian circuitry.) But mammals have evolved a bilateral circulation, because the lung requires a separate circulatory loop. The low-pressure pulmonary circulation receives blood from the right ventricle and delivers oxygenated blood back to the left atrium. The high- pressure systemic circulation delivers blood to all organs starting with left ventricular delivery into the aorta.

The cardiac output is distributed variously to the organ systems depending on oxygen and nutrient need. (Cardiac output is the (heart rate per minute)x(the volume delivered to the circulation with one contraction of the heart). A normal adult cardiac output is 5-6 L.) For example though about 20% of a 5 L cardiac output is delivered to skeletal muscle when we are at rest, a well-trained athlete can generate over 20 L of cardiac output during peak performance and most of that output will be redirected to the skeletal muscle.



See: Weibel ER: The Pathway for Oxygen. Harvard University Press, Cambridge MA, 1984

For illustrations: http://gened.emc.maricopa.edu/bio/bio181/BIOBK/BioBookcircSYS.html

O_2 Regulation

Huge physiologic changes are set into motion when O_2 is limited (altitude, trauma, anemia, exercise). At the systems level, brain respiratory centers are stimulated via signals from peripheral O_2 sensor cells in the carotid. Blood vessels competent to dilate, mostly venous capacitance beds, relax to increase local blood supply of some vital organs.

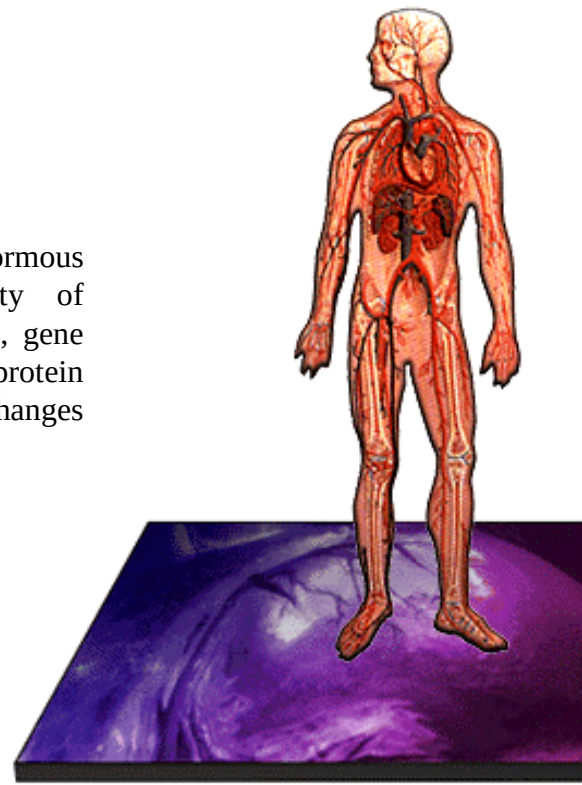
Short-term responses to hypoxia at the cellular level include translational arrest (for energy conservation), and upregulation of glycolytic pathways. More long-term solutions to increase O_2-carrying capacity are also set into motion including elaboration of growth factors that promote proliferation of blood vessels (vascular endothelial growth factor), and production of red blood cells (erythropoietin). Many of these physiologic responses are coordinated through the transcription factor hypoxia-inducible factor 1 or HIF-1.

(See Semenza GL: J Clin Invest 106:809, 2000).

HIF-1 partners determine the specificity of HIF-1 responses in specific cell types. The vascular endothelial growth factor response is a good illustration of the robust yet fragile nature of complex physiologic regulation. VEGF levels go up and down in time and space throughout life, depending on the need for new vessel growth, making us robust to injury, changes in altitude, and to a variety of disease processes. But halving or doubling its amount at the right time and place is lethal to an embryo during development.

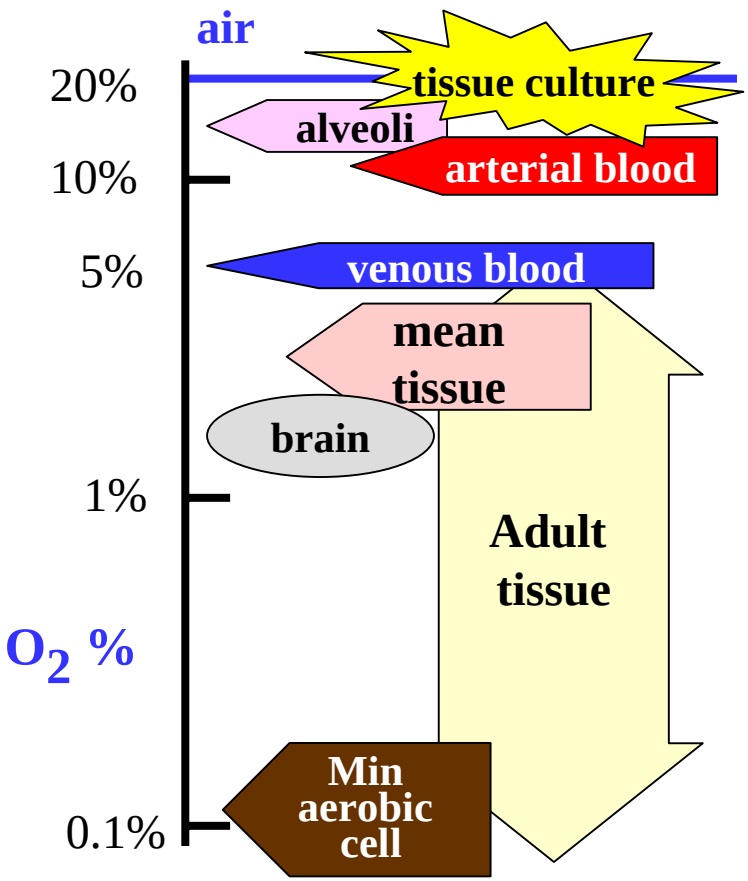
(See Miquerol et al: Development 127:3941, 2000.)

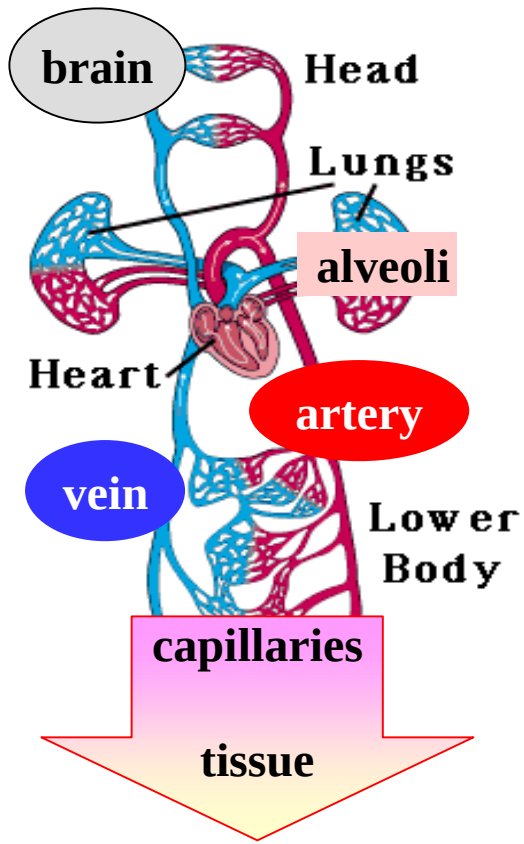
This discussion ignores enormous features of the complexity of diffusion, cell shape changes, gene expression changes, protein conformation and function changes all induced by varying O_2.



Physiological O_2 Levels

High oxygen levels are also toxic to cells, which has large implications for how cells are studied in the laboratory environment. High oxygen in the usual tissue culture setting is imposed by doing experiments in room air (21%), unless special precautions are taken to surround cultivated cells with a more physiologic (lower) oxygen environment. For reference, the mean oxygen tensions in the normal physiologic environment vs. those used in traditional laboratory cultivation of cells are depicted below. Note the logarithmic scale.





Oxygen tensions in vivo vs. in vitro. Oxygen tension is commonly measured clinically in blood using microelectrodes. The highest oxygen levels in the circulation are in arterial blood (12%), whereas venous blood is about 5.3% oxygen. Using the same technology, oxygen tensions can be measured in solid tissues experimentally, and the mean oxygen tension of adult tissues is about 3% (See Guyton AC and Hall JE: Textbook of Medical Physiology, WB Saunders Co., Philadelphia, 1996). Organ systems and subsystems vary widely in their oxygen tensions, depending on supply and demand for oxygen, such that mean brain oxygen tension in mammals is about 1.5% whereas mean skeletal muscle oxygen tension approximates venous levels.

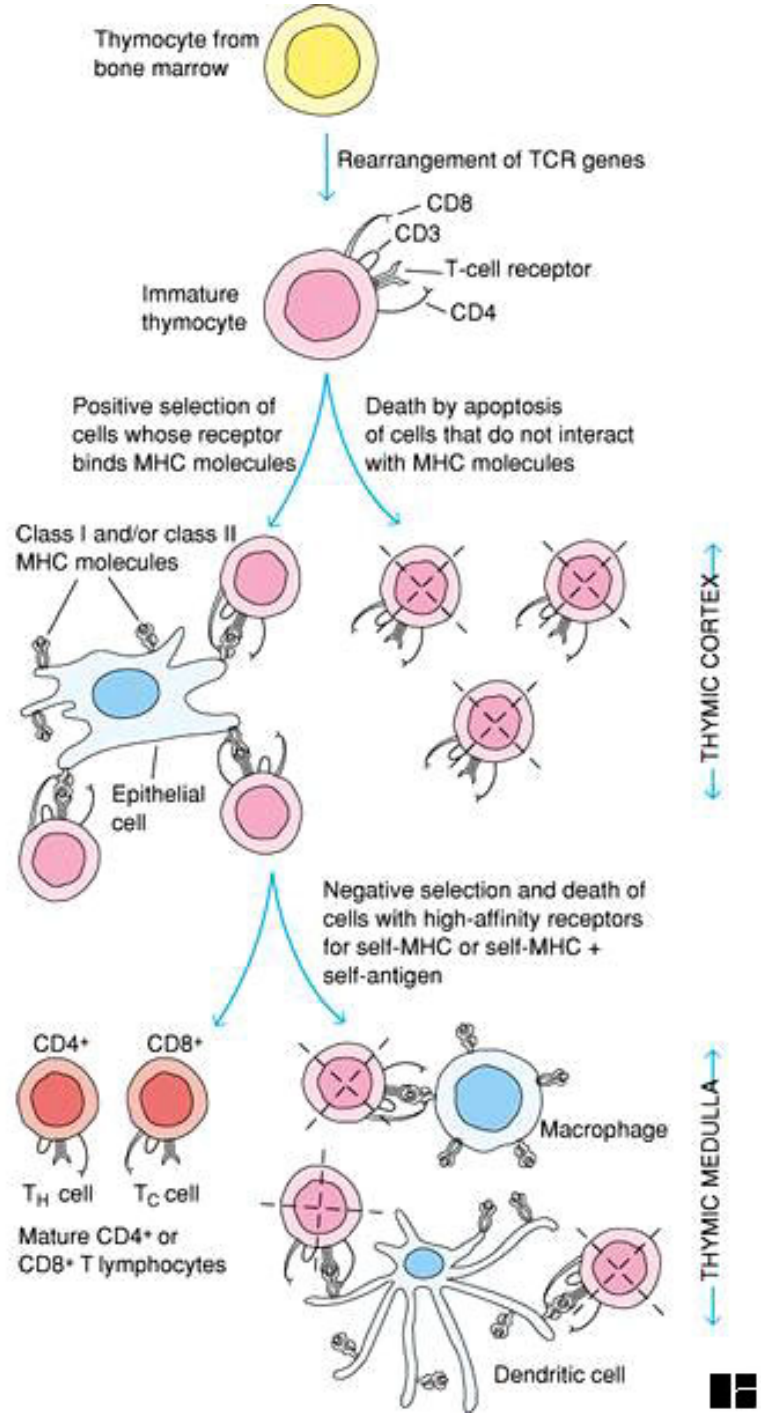
The immune system and spiraling complexity

The efficiency and specialization facilitated by the complex regulatory machinery elaborated to allow fine tuning of internal gases, temperature, pressures, nutrients also makes us an attractive host for parasites. Our incredibly complex immune system evolved as a barrier against microbes, with the daunting task of recognizing and dealing with a vast assortment of "foreign" as well as "self" gene products. Through complicated, and not yet fully understood processes, one major arm of the immune system (T cells) is educated as exquisite sensors. During development, T cells sample antigen profiles, efficiently presented to them in a precise, specific context. The subsequent reactivity of the T cells determines whether they are allowed to develop and proceed on to the periphery (positive selection) or whether they are inaccurate sensors and must self-destruct (negative selection). The widely dispersed population must not react to (tolerates) "self" while maintaining flexibility to respond to "non-self" challenges. Specific T cell clones can expand explosively when stimulated by the appropriate trigger. This proliferation is ultimately balanced by T cell death such that the immune reaction does not become immune over-reaction.

The systemic response is usually inspiringly robust, resulting in lethality to harmful microbes without significant damage to the host. Layers of feedback loops insure that this difficult balance is maintained, and T cells also interact with and signal other immune effectors (communication protocols), independently enormously complex networks.

For further explanation and illustrations see: http://www.whfreeman.com/immunology/CH12/kuby12.htm

Also: Germain RN: The art of the probable: system control in the adaptive immune system. Science 293:240-245, 2001.



Autoimmune disease

Autoimmunity is a prominent illustration of the fragility (in some cases cascading failure) that is the consequence of an exceedingly complex immune system.

Examples of Autoimmune Diseases:
(Listed by the Main Target Organ)

Nervous System:
Multiple sclerosis
Myasthenia gravis
Autoimmune neuropathies
such as Guillain-Barré
Autoimmune uveitis

Blood:
Autoimmune hemolytic anemia
Pernicious anemia
Grave's Disease
Autoimmune thrombocytopenia

Blood Vessels:
Temporal arteritis
Anti-phospholipid syndrome
Vasculitides such as Wegener's granulomatosis

Skin:
Psoriasis
Dermatitis herpetiformis
Pemphigus vulgaris
Vitiligo

Gastrointestinal System:
Crohn's Disease
Ulcerative colitis
Primary biliary cirrhosis
Autoimmune hepatitis

Endocrine Glands:
Type 1 or immune-mediated diabetes mellitus
Grave's Disease
Hashimoto's thyroiditis
Autoimmune oophoritis and orchitis
Temporal arteritis
Autoimmune disease of the adrenal gland

Multiple Organs :
Rheumatoid arthritis
Systemic lupus erythematosus
Scleroderma
Polymyositis, dermatomyositis
Spondyloarthropathies such as ankylosing spondylitis
Sjogren's syndrome

For a primer on autoimmune diseases see: http://www.niaid.nih.gov/publications/autoimmune

Cascading failures

One example (among many) autoimmune diseases is primary biliary cirrhosis (PBC) in which self-reactive lymphocytes somehow slipped through the surveillance of the white cell education program. PBC patients elaborate antibody against a particular mitochondrial protein in bile ducts of the liver. The organ specificity of this and many other autoimmune diseases is indirect evidence that a single antigen, among the huge number of possibilities, initiates the disease process. Autoimmune damage to this superficially miniscule component of the human body can lead to multiorgan failure, illustrative of and dictated by the interconnectedness necessary for normal, but complex, physiologic functioning. Autoimmune damage to conduit ducts causes bile accumulation in the liver and bile acids damage liver cells (hepatocytes).

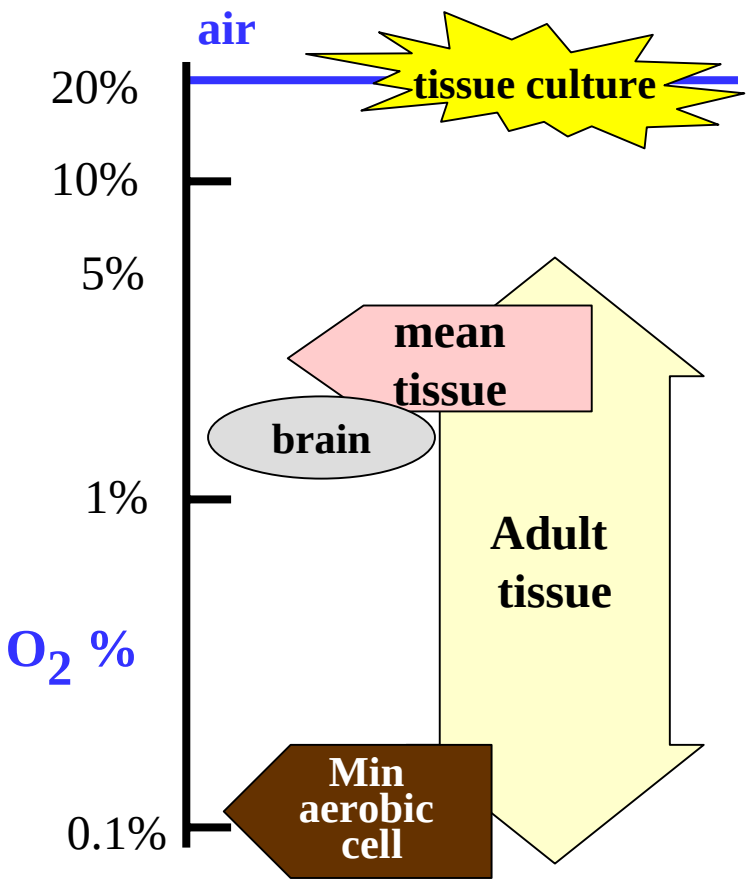
Damaged hepatocytes do not clear hormones well, and these circulating hormones mediate increased pressures in the liver circulation (and decreased pressures elsewhere). Portal (liver) hypertension is transmitted to connected venous systems that can rupture (bleeding esophageal varices), and pressure-induced distortion of the spleen traps platelets and white cells in the induced tortuous circulation. Damaged hepatocytes do not produce sufficient amounts of blood clotting proteins, and blood loss after trauma is exaggerated. Ultimately virtually every organ of the body, unable to capitalize on the usual homeostatic feedback interactions with the liver, fails: Brain dysfunction occurs because toxic proteins cannot be cleared by the liver. Kidney failure occurs when blood supply to the kidney is constricted under the influence of hormones circulating in excess because they are not metabolized in the liver.

Medical interventions for this disease, drug therapy and transplantation (with its obligate immunosuppression), demonstrate yet more layers of regulatory networks and new fragilities. Metabolism of many drugs is dependent on the P450 enzyme family, which is thought to have evolved to allow metabolism of varied plant foods. These enzymes do metabolize xenobiotics, including poisonous plant components and drugs, using oxygen to attack an enormous range of molecules. Genetic variation in P450's leads to considerable interindividual variation in drug handling and side effects. This variability is of significant enough concern to prompt large-scale efforts directed at diagnosis of genetic profiles (microarrays to identify mutations in P450 genes--pharmacogenomics) for individualizing drug therapies. Not surprisingly, failure of the P450 network balance can lead to accumulation of toxins including carcinogens. Polypharmacy—a common clinical necessity--results in even more unpredictable interactions because P450 activity is modulated by drugs themselves. In other words, some portion of the population may negate the effectiveness of a beneficial drug by taking another drug for the same problem.

Medicine's high technology liver transplantation is now standard therapy for PBC, but commits the patient to lifelong (polydrug) immunosuppression. The patient is now delicately balanced: Immunosuppression must be sufficient to quash the elaborate immune mechanism that developed to recognize a foreign invader, but too much immunosuppression allows foreign infectious agents and tumors to go unchecked. The fragilities of transplantation are fatal infections and cancers.

For background on P450 enzymes see:
http://www.ama-assn.org/special/hiv/newsline/briefing/cytochro.htm

Implications for tissue cultures

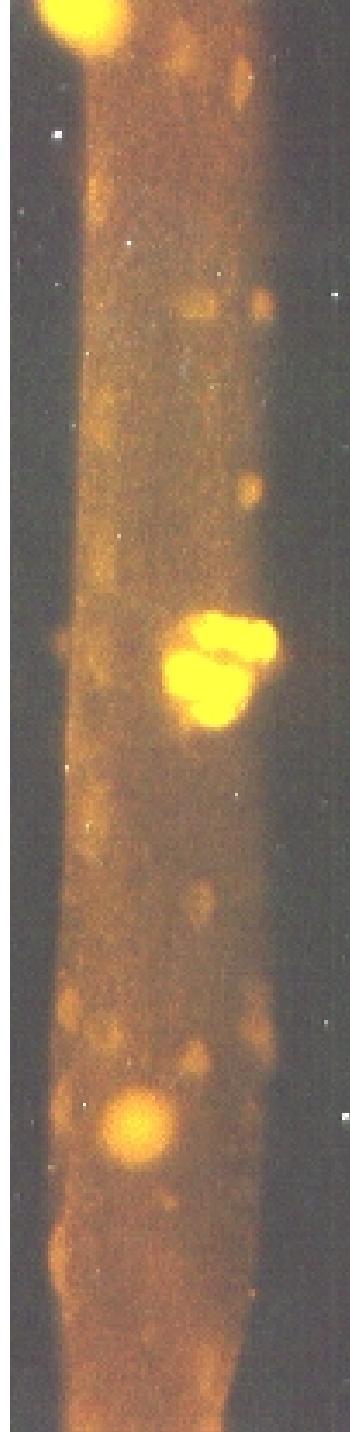


Note that normal adult tissue oxygen tension varies widely spatially within the body, but that all the complex regulatory machinery described earlier aims to hold that level as constant as possible over time. Standard systems engineering practice as well as clinical experience suggests that such tight regulation is usually not accidental, and that deviations from normal levels then becomes an important signal of damage or disease. Thus cells in culture in room air oxygen may be damaged by reactive oxygen species generated in high oxygen conditions. In addition, though, reactive oxygen species generated in this setting are specific signaling molecules that affect virtually all biologic processes including apoptosis, proliferation, generalized and specific transcriptional responses, and cellular senescence.

The remaining slides show some evidence for the significance of this observation for tissue cultures.

Proliferation of satellite stem cells is higher in 6% vs. 20% oxygen conditions in vitro. Satellite stem cells are the resident adult muscle stem cell population. Regeneration of this stem cell population can be studied in vitro by cultivating the cells on the parent single murine muscle fibers. (In the figure, satellite cell nuclei are bright orange, adherent to the cultured muscle fiber.) Proliferation of cells can be quantified by exposing them to the thymidine analog bromodeoxyuridine (BrdU), which is taken up only by cells synthesizing DNA in anticipation of mitosis. Cells that take up BrdU can then be identified by immunohistochemistry using an antibody directed against BrdU. In this study, BrdU was added to cultured fibers/satellite stem cells for 12 hour intervals, then the fibers were fixed and stained for reactivity to anti-BrdU antibody. The data are expressed as the number of labeled satellite cell nuclei per unit length of fiber. In general proliferation of satellite cells was enhanced by culture in oxygen levels closer to normal physiologic oxygen tensions vs. usual 20% O2 culture conditions. (See also J Cell Physiol 189:189-96, 2000).

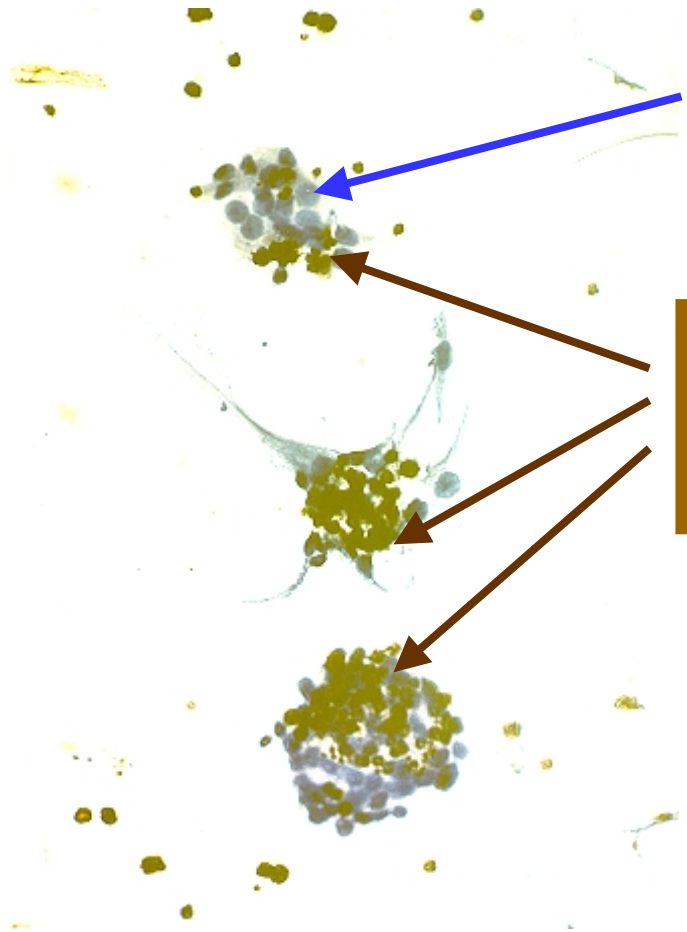
	20%	6%	p
0-12 hrs	0	(rare)	
12-24 hrs	0.6	1.6	.0001
24-36 hrs	0.4	1.2	.056
36-48 hrs	2.8	4.0	.20
48-60 hrs	2.0	6.0	.009



Central nervous system (CNS) stem cell apoptosis is decreased in culture by lowered oxygen conditions. Human CNS stem cells (Clonetics, San Diego CA) were cultured in either 2% (physiologic) or 20% oxygen conditions for several weeks. The cells were fixed and analyzed by TUNEL staining (Boehringer-Mannheim Inc.) which marks cells undergoing apoptosis. After about two weeks in culture, a large number of CNS stem cells grown in 20% oxygen were TUNEL-positive, whereas those maintained in lower oxygen were less likely to be labeled (Csete M, unpublished results).

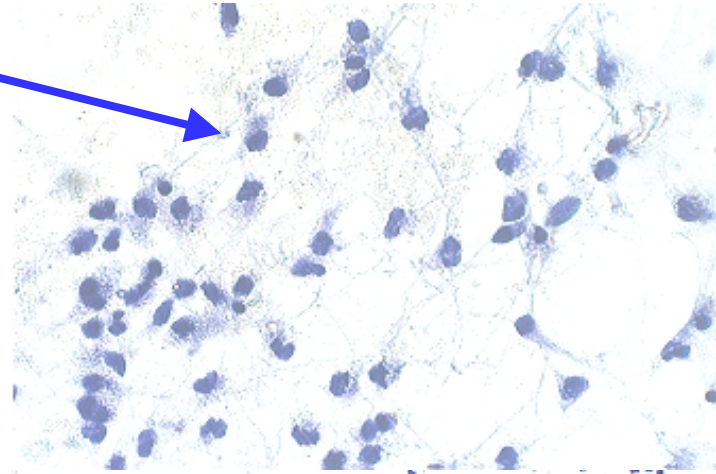
20% oxygen

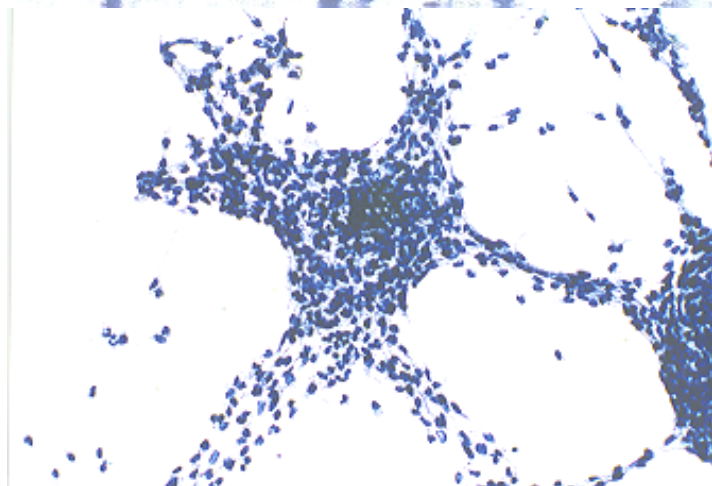
2% oxygen



Unlabeled cells are blue.

TUNEL-positive cells are brown. (apoptosis)

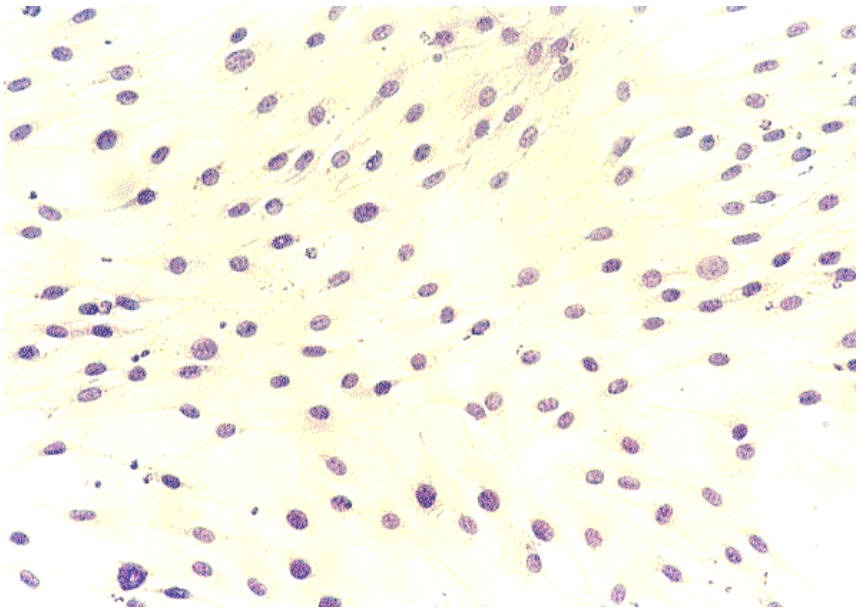




Note: there are no visible TUNEL-positive cells in 2% oxygen.

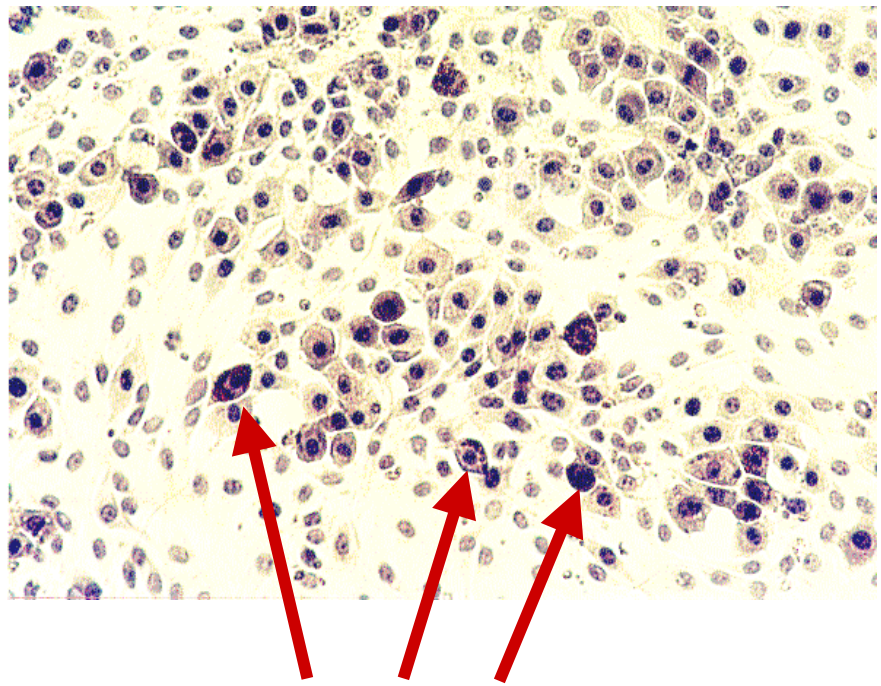
Fat differentiation in stem cell lines is decreased by culture in lower, physiologic (vs. 20%) oxygen conditions. The 3T3 mesenchymal stem cell line can differentiate into muscle or fat cells in culture, and is commonly used to study differentiation in these and other lineages. In these studies, fat differentiation was encouraged by the addition of insulin, dexamethasone, and isobutylmethylxanthine to the cultures. When the cells were maintained in 20% oxygen, adipogenesis was easily induced (block-like, reddish cells are differentiated fat cells) whereas much less fat cell differentiation occurs when cells are maintained in 2-6% oxygen conditions. (See also J Cell Physiol 189:189-96).

Low O_2



Few differentiated fat cells.

20% O_2



Many differentiated fat cells.